Advanced Dietary Fibre Technology

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Dedication

This book is dedicated to Angela Kennedy, Business Director of Megazyme International, Ireland Limited and ICC National Delegate for Ireland. Angela organised and planned the conference 'Dietary Fibre – 2000' from which the seeds of this book were planted. The manner in which Angela accomplished the various tasks was exceptional, and those scientists who were fortunate enough to attend will remember the conference as a first-class event, both scientifically and socially.

Contents

Preface

Although the laxative effects of dietary roughage have been known for more than 2000 years, it is only during the past 25 years that roughage – now termed 'dietary fibre' – has been accorded the scientific importance that it deserves. We are indeed indebted to T.L. Cleave, D.P. Burkitt, H. Trowell, A.R.P. Walker, J. and W. Kellogg, D.A.T. Southgate, M.A. Eastwood and the many others investigators who contributed to the science and the popularisation of dietary fibre as a vital constituent of the diet.

To many people, dietary fibre is considered a nutrient along with protein, fat, carbohydrate, minerals and vitamins in the diet. The contributors to this volume, from 26 countries, have pushed dietary fibre research forward to a new plateau, discussing: (1) the analytical methodology associated with the measurement of dietary fibre, including standards for the enzymes used in the measurement of fibre and a new updated definition/description; (2) plant breeding to improve the dietary fibre content of foods; (3) the various sources of dietary fibre and the food products that lend themselves to supplementation with dietary fibre. There are also chapters on: (4) resistant starch, which should be considered as fibre; (5) the metabolism of dietary fibre; (6) enzymes and the processing of fibre; (7) new products which do not measure as dietary fibre in the accepted methods, but which act as fibre physiologically; and finally (8) the nutrition of dietary fibre and how it impacts on population in several countries.

Recently, following a year-and-a-half of deliberations with many scientific societies and individual scientists, the Board of Directors of the American Association of Cereal Chemists (AACC) approved a new definition/description of dietary fibre. The definition includes several foods which do not measure as dietary fibre in the AOAC method, and also attaches physiological effects aside from laxative properties. The AACC's approved definition is 'Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.' This is just one instance of the timeliness of this book. Another example is the approval of AOAC methods for inulin and oligofructose and, polydextrose, with the AOAC currently considering methods for Fibersol-2 (a resistant maltodextrin) and galacto-oligosaccharides. These all come under the umbrella of the newer definition of dietary fibre.

We would like to thank the supporters of the conference, who helped in many ways to make the conference successful. A special thanks goes out to An Taoiseach (Prime Minister) Mr Bertie Ahern, T.D., who took time from his busy schedule to welcome the delegates and tell them of the importance of nutrition, foods and dietary fibre to the health concerns of the community. We thank the staff of Megazyme International Ireland for their efficient running of all aspects of the meeting 'Dietary Fibre – 2000'. We also thank the contributors to this conference for their excellent papers, and the other participants in the conference for their

enlightening participation in the question and answer discussions of the presentations. We also thank the scientists from the many countries who presented their talks in poster sessions. These posters were of the highest quality and were very well attended.

Finally, we would like particularly to thank the Official Scientific Associations that sponsored the conference, and the companies that financially supported the conference. These associations and companies are listed below.

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Part 1

Nutrition and Diet for a Healthy Lifestyle

1 Nutrition and Diet for Healthy Lifestyles in Europe

Michael J. Gibney

1.1 The regulatory background in public health nutrition in the EU

The European Union is the largest trading bloc in the world, with a population of 350 million that will shortly expand to half a billion with the accession of four new member states. Whereas each member state is independent, each has acceded some of that independence to the structures of the EU. These structures are regulated and governed by the Treaty of Rome and its many revisions which have allowed the EU to evolve from an initial six-member economic community to the present 15-member political union with its own common currency. In order to understand the issue of nutrition in the EU, it is necessary to have some understanding of this political dimension to the EU. Thus until recently, the EU had little interest in health issues which were regarded as an issue of 'subsidiarity', i.e. the business of the member states themselves. The EU could concern themselves with issues of health only in so far as it influenced the movement of goods or people. For example, the EU developed a series of recommended dietary allowances (termed Population Reference Intakes) in 1994. This was solely to establish a standard for nutrition labelling purposes. However, in 1995, the Maastricht Treaty revised the remit of the EU and gave it considerably more power to act in the realm of public health. Since then, there have been dramatic shifts in the agenda of public health nutrition in the EU, and further shifts can be anticipated. The EU's White Paper on Food Safety envisages a comprehensive food and nutrition policy for the EU and the development of EU dietary guidelines.

1.2 Food intake patterns in the EU

The member states of the EU differ in very many respects, by orders of magnitude more than for example do the constituent states, territories or provinces of such countries as the USA, Australia or Canada. They differ linguistically, culturally, socially, politically and, of course, gastronomically. The divergent patterns of food intake in the EU means that whereas it may be possible to derive dietary guidelines in terms of nutrients, it will be extremely difficult to do so for food-based dietary guidelines. Table 1.1 summarises the intakes of fat (% energy), saturated fatty acids (SFA; % energy), dietary fibre and fruit and vegetables in selected EU member states, while Fig. 1.1 compares the mean values across the range of upper and lower quantiles (quartiles or tertiles) for the intakes of SFA, fibre, fruit and vegetables and folic acid against the range of recommended levels of intake (Becker 1999; De Henauw & De Backer 1999; Graça 1999; Haraldsdóttir 1999; Hermann-Knuz & Thamm 1999; Koenig & Elmadfa 1999; Löwik *et al*. 1999a; Mischandreas & Kafatos 1999; Serra-Majem *et al*. 1999; Valsta 1999; Wearne & Day 1999; Gibney 2000). These data need to be *cautiously* interpreted, since the methodologies used to collect them vary across all member states, and since such vari-

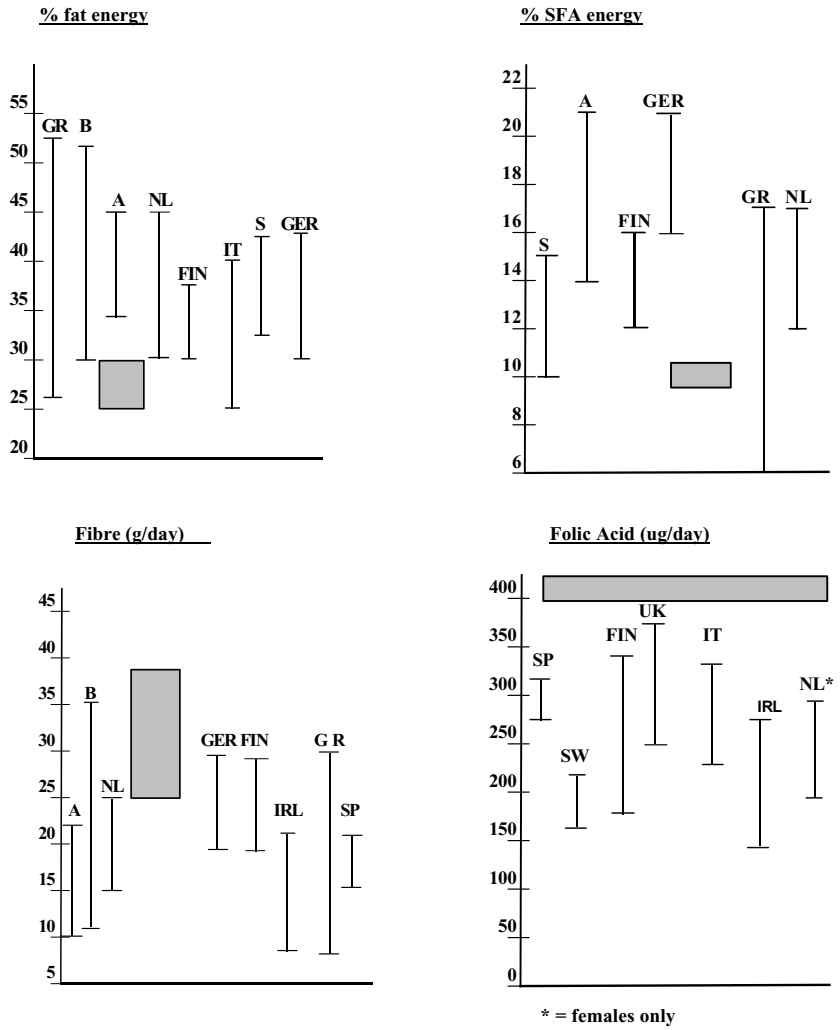

Fig. 1.1 Range of intakes (mean of highest to mean of lowest quartiles/tertiles) of % fat energy, % saturated fat energy, fibre (g/day) and folic acid (µg/day) in relation to range of member state recommendations (shaded boxes) for these nutrients.

ations in design can influence estimated nutrient intake. However, they can be used to illustrate two key points. The first is that there is a clear North–South divide with respect to % energy from saturates and that, by and large, nutrient intakes fall short of dietary guidelines for optimal nutrient intake. The issue of dietary fat intake is explored further in Table 1.2, which clearly demonstrates a North–South divide in terms of fatty acid categories expressed as a percentage of dietary energy or a percentage of dietary fatty acids. However, this table also shows that when upper or lower quantiles of percentage fat energy are compared, the composition of dietary fat does not differ with each geographic unit. In effect, be it North or South EU, shifts in total fat intake are not associated with shifts in dietary fat composition. Both these observations of short-falls in meeting the established dietary guidelines and the

Table 1.1 Patterns of intakes of fat, saturated fatty acids (SFA), fibre and fruit and vegetables in selected European Union (EU) member states.

Country	Reference	% Energy from		Fibre (g/day)	Fruit and vegetables (g/day)
		Total fat	Saturated fatty acids		
Austria	a	39	17	18	257
Belgium	b	42	18	29	361
Denmark	c	37	16	20	395
Finland	d	34	14	22	433
Germany	e	41	18	22	296
Greece	f	40	12	18	455
Ireland	g	35	–	21	257
Netherlands	h	38	14	21	250
Portugal	i	29	9	26	NA
Spain	j	38	13	17	480
Sweden	k	37	16	17	265
UK	m	38	16	22	247

NA, not applicable.

(a) Koenig & Elmadfa (1999)
(b) De Henauw & De Backer (1999)
(c) Haraldsdóttir (1999)
(d) Valsta (1999)
(e) Hermann-Knuz & Thamm (1999)
(f) Mischandreas & Kafatos (1999)
(g) Gibney (2000)
(h) Löwik *et al*. (1999a)
(i) Graça (1999)
(j) L. Serra-Majem *et al*. (1999)
(k) Becker (1999)
(m) Wearne & Day (1999)

inability to change dietary fat composition as dietary fat levels fall, have significant policy implications.

1.3 Nutrition policy issues in the EU

1.3.1 *The structures of food policy*

The recent EU White Paper on Food Safety calls for the establishment of a comprehensive nutrition policy in the EU, and for the development of dietary guidelines for the EU. A forerunner to this has been a project funded by the Commission of the EU which is examining the full breadth of the issues involved through four working parties. One examines the relationship between health and nutrients, the second is examining the relationships between nutrients and food, the third focuses on the food-people issue, and the fourth on people and policies. Full details are available at www.eurodiet.uoc.gr. The deliberations of this Eurodiet project will be embraced by the French Presidency of the EU when her Minister for Health chairs the Council of Ministers for Health of the EU for the latter part of 2000. It is anticipated that this will lead to an EU Council of Ministers resolution, which will empower the

Table 1.2 Dietary fat composition expressed as (a) percentage of energy or (b) as dietary fatty acid categories and percentage (w/w) in upper and lower quartiles/tertiles of percentage energy from fat in typical southern and northern European Union (EU) states.

	Low-fat diets			High-fat diets		
	SFA	MUFA	PUFA	SFA	MUFA	PUFA
(a) Dietary fatty acids as % of energy						
Southern						
Spain[a]	11	15	4	14	19	5
Greece[b]	8	12	4	15	28	6
Portugal[c]	6	10	4	11	15	6
Northern						
Finland[d]	11	8	4	18	14	5
Germany[e]	14	12	5	21	17	6
Netherlands[f]	12	11	5	17	17	9
(b) Dietary fatty acids as a % of total dietary fat						
Southern						
Spain[a]	37	50	13	37	50	13
Greece[b]	34	51	15	30	50	13
Portugal[c]	32	48	20	35	47	18
Northern						
Finland[d]	47	37	17	48	38	15
Germany[e]	45	40	15	46	39	14
Netherlands[f]	41	39	19	40	40	20

(a) Serra-Majem *et al*. (1999)
(b) Mischandreas & Kafatos (1999)
(c) Graça, (1999)
(d) Valsta, (1999)
(e) Hermann-Knuz, & Thamm (1999)
(f) Löwik *et al*. (1999b)

Commission to have a much greater involvement in public health nutrition and which will lead to a stronger remit for the forthcoming European Food Safety Agency. At that point, public health nutrition issues in the EU will have a very strong centralised dimension.

1.3.2 *General issues of EU food and nutrition policies*

Food policy is a complex area. Whereas in the past it was largely producer-driven, in the future it will be more consumer-driven. It embraces policy issues in agriculture, the environment, rural development, trade and many others, including health. The latter has three main components: microbiology, toxicology and nutrition. Both microbiology and toxicology are heavily represented in food law, with nutrition very poorly represented in EU legislation. It may well be that this reflects the evolution of public health as a competency of Brussels, but it must also reflect the difficulties that are faced in translating policy into law in a geo-political entity that is so culturally and gastronomically variable. The basic objective of this policy is to shift a less than desirable pattern of nutrient intake into a more desirable pattern, to reduce chronic disease. This can be achieved in two ways: (1) change the pattern of food consumed; or (2) change the properties of foods consumed.

1.3.3 *Changing the pattern of foods consumed in the EU*

The manipulation of price or of supply is one route to modifying the patterns of food consumption. This route may have some role in future EU policy options, but it will be limited since it may infringe on some of the dogmas of trade liberalisation which the World Trade Organization are charged to defend. Most likely, efforts to change the food supply will be by stimulating the demand for food supply, i.e. demand-driven food policy. This in turn raises a number of questions for research by the nutrition community, namely a more detailed understanding of food consumption patterns and a more detailed understanding of consumer attitudes and beliefs regarding healthy eating.

Toward a greater understanding of the complexities of food and nutrient relationships

In terms of altering the population pattern of food intake, four options are available for target foods:

(1) Alter the % of the population consuming the food.
(2) Alter the frequency of consumption among consumers.
(3) Alter the intake at eating occasions.
(4) Change to a comparable alternative.

These have been applied to the task of identifying the best dietary option for increasing fibre intake in Irish women (Gibney 1999). The exercise is entirely illustrative. Although vegetables and potatoes are important sources of fibre in the Irish diet, together contributing some 28% of total intake, they are not considered in this exercise because: (1) the percentage of the population consuming them is maximum; (2) the portion sizes cannot be increased; and (3) the frequency of consumption will be difficult to increase. Table 1.3 illustrates the options and outcomes. Bread fulfils some of the criteria outlined above for potatoes and vegetables, but unlike these foods there exists the option of a comparable alternative. A 50% switch to wholemeal bread intake would increase fibre intake by 2.5 g/day. The present level of fibre in the population intake of breakfast cereals is low (2.25 g/100 g), and the percentage of consumers among the target group is low. If there was a shift to selecting higher-fibre varieties of cereals and a 50% increase in fibre intake, the overall impact would be 1.5 g/day of extra fibre. For both pulses and for fruits, a trebling of eating frequency would increase fibre intake by 3.6 g/day for each group. The accuracy of these calculations is not the issue here since the exercise is entirely illustrative. The exercise serves to show that in identifying foods which could be the focus of public health nutrition programmes, the present and past approach has not been very scholarly, quite probably because the areas of food–food, food–nutrient and nutrient–nutrient relationships have been poorly regarded as worthy research topics. Analysis of Dutch food intake data shows a poor relationship between what is conventionally deemed to be the desirable food choice patterns and desirable patterns of nutrient intake (Löwik *et al.* 1999b).

The problem – in itself extremely complex – is exacerbated by the wide variety of food choice patterns in the EU, and this will certainly tax the minds of those wishing to set EU food-based dietary guidelines. This is illustrated in Table 1.4, which compares the intakes of potatoes among consumers of potatoes in eight EU states where intakes are ranked according to quantile (quantile/tertile) of dietary fibre intake. The food group 'potatoes' is deliberately

Table 1.3 An outline of approaches to selecting target foods for increasing fibre intake in Irish women[(a)].

Food group	Percentage of consumers	Main option for change	Degree of change	Change in fibre intake (g/day)
Bread	98	A switch to whole-meal bread	50% increase	+2.5
Breakfast cereals	38	(i) Switch to higher-fibre varieties (ii) increase % consumers	(i) from 2.3 to 10 g/100 g (ii) 50% increase	+1.5
Pulses	77	Increase frequency of intake	From one to three times/week	+3.6
Fruit	80	Increase frequency of intake	From one to three times/day	+3.6

(a) Gibney (1999)

chosen because it is unlikely to have a different meaning in different countries – a problem that frequently bedevils such comparisons. Quite clearly in four countries, those with higher fibre intakes have higher potato intakes (Belgium, Germany, Greece and the UK). This is not so for the remaining countries in the comparison. This very simple example illustrates the problem that is faced in the EU in trying to derive centralised food-based dietary guidelines,

Table 1.4 Mean daily intakes of potatoes among consumers only in selected European Union (EU) member states classified according to quartiles or tertiles of dietary fibre intake.

	Reference	Lower tertile/quartile Potato intake* (g/day)	Upper tertile/quartile Potato intake* (g/day)
Belgium	a	216	460
Germany	b	113	212
Greece	c	116	178
UK	d	76	104
Ireland	e	217	207
Netherlands	f	132	157
Spain	g	77	75
Sweden	h	116	95

* Note that absolute values are partly determined by the duration of the surveys concerned which range from 1 to 7 days

(a) De Henauw & De Backer (1999)
(b) Hermann-Knuz & Thamm (1999)
(c) Mischandreas & Kafatos (1999)
(d) Wearne & Day (1999)
(e) Flynn & Kearney (1999)
(f) Lowik *et al*. (1999)
(g) Serra-Majem *et al*. (1999)
(h) Becker (1999)

and it highlights the wisdom of the UN agencies who have advised that these should always be devised using local data with the local cultural context.

Toward a greater understanding of consumer attitudes to food, nutrition and health

The Institute of European Food Studies (www.iefs.org/publications.html) has conducted several pan-EU surveys to ascertain consumer attitudes to food, nutrition and health using face-to-face interviews of demographically representative samples of 1000 adults in each member state of the EU. The results show that EU consumers rank 'Trying to eat healthy' as a priority in selecting foods (5th from 15 possible factors). They are also able to define 'Healthy eating' correctly when asked to do so in their own words (i.e. 'less fat' stated by 48%, 'more fruit and vegetables' by 41%, and 'balance and variety' by 40%). The barriers identified to healthy eating were both internal (i.e. 'cannot give up my favourite foods' at 23% and 'lacking willpower' by 18%) and external (i.e. 'irregular working hours' and 'busy lifestyle' cited by 41%). Remarkably, some 71% either agreed or agreed strongly with the statement: 'I do not need to make changes to my diet as my diet is already healthy enough'. The results of such research are essential in formulating public health nutrition programmes because in their absence, policy-makers are often driven by perceived wisdom of consumer attitudes to food, nutrition and health.

Of the results of the study, one that has attracted most attention has been the widely held belief of individual consumers that they do not need to make changes to their diet as it is already healthy enough. Given the data in Fig. 1.1, it would appear that consumers are wrong in thinking this way, since patterns of population intakes fall quite short of established dietary guidelines. There are, however, two caveats to this possible consumer misconception. The first is that the guidelines established may not be readily attainable, since they are based on epidemiological theory rather than observed acceptable food consumption patterns. The second is that the actual nutrient intake patterns of those who believe their diet to be optimal are not known, and may in fact be relatively good, i.e. in the overall context of food choice patterns as opposed to a theoretically derived dietary guideline. A recently completed survey in Ireland with 1000 adults in the Republic of Ireland and 500 adults in Northern Ireland examined not only food intake over seven days, but also compiled many other profiles including consumer attitudes to their diet (Gibney 2000). Table 1.5 compares dietary fat energy by age in those agreeing or strongly agreeing and those disagreeing or strongly disagreeing

Table 1.5 Dietary fat energy (%) across age groups in subjects strongly agreeing, tending to agree, tending to disagree and strongly disagreeing with the statement; 'I do not need to make changes to my diet as my diet is already healthy enough'[7].

	Age group (years)		
	18–36	37–51	52–64
	% fat energy		
Strongly agreeing	37.6	36.9	34.0
Tending to agree	37.5	36.6	34.9
Tending to disagree	38.3	38.5	35.3
Strongly disagreeing	39.1	38.5	37.6

with the statement: ' I do not need to make changes to my diet as my diet is already healthy enough'. The results show an age-related decline in percentage dietary fat energy across all attitudinal groups, and also show that for all age-groups, most notably for the 52- to 64-year-olds, a tendency to believe their diet is healthy as it stands, is borne out by a very strong tendency toward lower fat energy percentage. Future analysis of this new and very large database will reveal much more about the relationship between attitudes and beliefs about nutrition and actual nutrition habits, and that in turn will improve the capacity to deliver more accurately acceptable public health nutrition messages to specific target sub-populations.

Changing the nutritional and functional properties of foods consumed

Given the findings cited in the proceeding section that: (1) EU consumers value healthy eating; (2) that they understand the principles of healthy eating; and (3) that time/lifestyle pressures for choosing a healthy diet are the main obstacles encountered, it is not surprising that the food industry are seeking to combine enhanced nutritional quality of foods with quality and convenience. In this context, the unique situation of the EU will also pose problems and challenges for policy-makers. An example of the problem is shown in Table 1.2, where it is evident that patterns of fatty acid intake do not vary with actual dietary fat intake. If patterns of fat intake are to change, then this is quite likely going to need some technological intervention. The nutritional properties of foods can be altered by technological processes, by micronutrient enrichment and by biotechnology, including genetically modified (GM) foods. The latter are poorly regarded by EU consumers and by their political servants, and this has led to measures ranging from a moratorium on the release of GM foods to calls for labelling. The addition of micronutrients to foods is regulated very differently across the EU, with no success in terms of harmonisation of national legislation. In some countries the laws are quite liberal while in others the sale of foods enriched with micronutrients is very rigidly controlled. At present, the EU Scientific Committee for Food is examining the concept of 'safe upper levels' for micronutrient intake. The issue is not quite academic, as is illustrated in Table 1.6 using recent Irish data (Gibney 2000). In women, intakes of iron, vitamin E and vitamin C are very heavily skewed toward higher intakes due to the use of supplements. For men, this is

Table 1.6 Mean, median and percentiles (2.5th, 5.0th, 95.0th, 97.5th) for intakes of iron, zinc, vitamin E and vitamin C in adult Irish males and females[7].

			Percentiles			
	Mean	Median	2.5th	5.0th	95.0th	97.5th
Iron (mg/day)						
Men	14.8	13.9	7.0	7.6	26.0	28.9
Women	13.7	10.0	5.3	5.9	25.4	45.6
Vitamin E (mg/day)						
Men	11.2	6.1	2.0	2.4	18.2	27.7
Women	11.0	6.1	1.7	2.0	23.7	38.5
Vitamin C (mg/day)						
Men	118	75	23	27	263	599
Women	111	69	21	26	292	590

true for vitamin E and C. Unregulated use of food fortification could lead to exposure to high levels of some micronutrients, although this has not proven to create any public health issues in the US, where both supplement use and food fortification are very widespread.

The functional nutritional properties of food can be created or enhanced giving rise to functional foods. Examples of these from the ILSI Europe Programme on functional foods include genetic modification, extraction of nutrients from unconventional sources, fructose oligosaccharides, new nutrient delivery systems, fat replacers and bio-active peptides (Diplock *et al*. 1999). Many functional foods fall into the broader category of novel foods which are regulated at EU level. However, EU legislation is very restrictive about health claims in respect of foods, novel or otherwise, which is in marked contrast with the US. The inability to make 'health' or 'functional' claims is an impediment to commercial investment in the significant research and development spend that is required to take a concept for a novel or functional food through to a patent and petition with all the attendant supporting data to confirm efficacy.

1.4 Conclusions

Through the slow evolution of EU treaties, public health nutrition at EU level has slowly come to life, but will receive a major boost with the forthcoming establishment of the EU Food Safety Agency with a remit in nutrition. However, such is the divergence of food consumption patterns in the EU the likelihood is that much of this activity will remain devolved within member states. Even when nutrient-based guidelines are to be considered, difficulties of centralised values will arise with some countries having, for example, saturated fatty acids falling from high values toward the standard dietary goal (e.g. Finland) and others moving in the opposite direction (e.g. Greece). A major shortcoming is the absence of a centralised food consumption database and the fact that in every possible aspect of survey design, food intake surveys differ across the Union. Finally, as nutrition comes to the forefront of public health issues in the EU, it is regrettable that the organisation of nutritional science in the EU is so poorly structured. Each country has its own nutrition society, some with several such societies. And while the Federation of European Nutrition Societies and the European Academy of Nutritional Sciences attempt to garner together the various nutrition groupings in Europe, the overall effectiveness is nowhere near the US with its monolithic American Society for Nutritional Sciences and its proximity to the Federation of American Societies of Experimental Biology, for which no counterpart exists in the EU.

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2 Dietary Advice in North America: the Good, the Bad and the Unheeded

Julie Miller Jones

2.1 Introduction

Dietary advice in North America comes from many sources – some credible, some not. While the focus of this chapter will be on advice from the government and from recognised health promotion organisations, it must be pointed out that advice from less credible sources is often heeded, despite frequent inaccuracies and half-truths. This chapter will cover the following: (1) credible dietary advice from North America; (2) an assessment of how well the population follows the advice; and (3) a brief overview of the consequences of these dietary indiscretions and how dietary fibre fits into the whole picture.

2.2 Specifics of dietary advice in North America

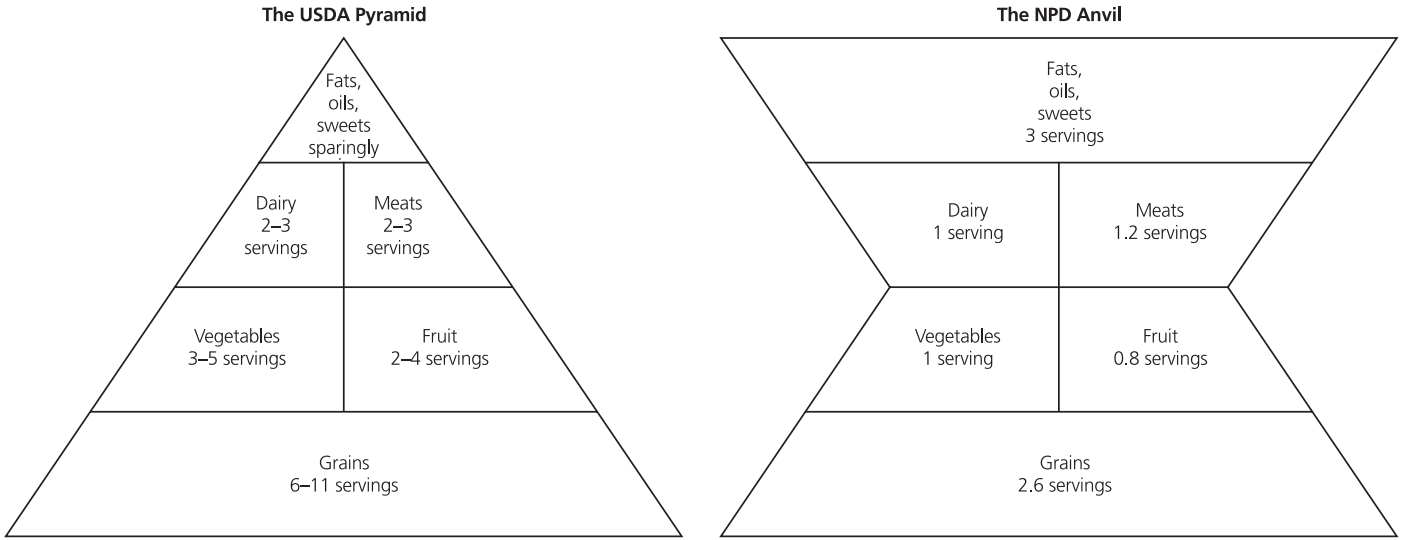
There are many sources of dietary advice from the US and Canadian governments. Dietary Reference Intakes (DRIs) from the Institute of Medicine (1997, 2000) outline what nutrients are required, and at what levels for a specific population group. DRIs have been developed as a joint effort of the USA and Canada, with committees of eminent scientists from both countries evaluating latest scientific information and condensing this into a single set of recommendations. The first DRIs were issued in 1997 and replace the Recommended Dietary Allowances (RDAs) in the USA and Recommended Nutrient Intakes for Canadians (RNIs). Recommendations for 13 vitamins and five minerals have been completed, and more are in process. The four key elements of the DRIs – the Recommended Dietary Allowances (RDAs), Adequate Intakes (AI), Tolerable Upper Intake Levels (UL), and Estimated Average Requirements (EAR) – are elaborated in Table 2.1.

While DRIs provide generous allowances for healthy people, they do not meet nutrient needs for all conditions, and do not provide guidance in food selection. Thus, the USDA Food Guide Pyramid (United States Department of Agriculture 1992) (Fig. 2.1) and the Canadian Food Guide to Healthy Eating (Department of Health and Welfare Canada 1992) are designed to translate nutrient requirements into food choices. If a variety of foods is chosen from the

Table 2.1 The four components of the dietary reference intakes.

RDAs	nutrient goals for individuals
Adequate intakes (AI)	nutrient goals for individuals for substances with inadequate scientific information
Tolerable upper intake levels (UL)	levels which should not be exceeded
Estimated average requirements (EAR)	establishes population average requirements for research and policy

Reality check
NPD Group Inc., a market-research firm that monitors Americans' eating habits, has created an anvil that it says reflects people's actual diets, heavy on the fats and sweets. The anvil contrasts sharply with the US Agriculture Department's pyramid, which recommends a daily diet rich in grains, fruits and vegetables.



Source: US Agriculture Department and NPD Group Inc.

Fig. 2.1 The USDA Pyramid and the Anvil.

various rungs of the Pyramid or parts of the Food Guide, the diet will contain all the nutrients. The dietary advice from these food group plans should be used in combination with the Dietary Guidelines for Americans (USDA 1995) to aid consumers in selection of foods that contain the right levels of fat, sugar, sodium, fibre and calories. In the Dietary Guidelines for 2000 (USDA 2000), the number of guidelines expands from seven in the 1995 version to ten (Table 2.2). Differences from the 1995 version are as follows:

- Goal number one directs the consumer to use the pyramid to direct food choice rather than the previous wording to select a variety of foods.
- The goal covering weight and physical activity was split into two goals to give importance to each part. One goal is to aim for a healthy weight. The other goal is to be physically active each day.
- The goal referring to fat, changes from recommending a diet low in total fat to one that is moderate in fat. Both the old and the new version recommend a diet low in both saturated fat and in cholesterol.
- The goal referring to grains, fruit and vegetables has also been divided into two goals – one recommends choosing a variety of fruits and vegetables daily. The second recommends choosing a variety of grain products on a daily basis, and advocates the selection of whole grains.
- The goal covering sugar, changed from admonition to choose a diet moderate in sugars to one that recommends choosing beverages and foods to limit intake of sugars.

The initiative *Healthy People 2010* has been published by the US Department of Health and Human Services, Office of Disease Prevention and Health Promotion (2000). It contains dietary strategies that are designed to reduce risk of heart disease, stroke, hypertension, obesity, diabetes and cancer and are part of a recommended battery of risk reduction strategies.

Table 2.2 1995 and 2000 dietary guidelines.

1995	2000
Eat a variety of foods	Let the pyramid guide your food choices Keep foods safe to eat
Balance the food you eat with physical activity – maintain or improve your weight	Aim for a healthy weight Be physically active daily
Choose a diet low in fat, saturated fat, and cholesterol	Choose a diet low in saturated fat, and cholesterol and moderate in total fat
Choose a diet with plenty of grain products, fruits and vegetables	Choose a variety of grain products daily, especially whole grains Choose a variety of fruits and vegetables daily
Choose a diet moderate in sugars	Choose beverages and foods to limit your intake of sugars
Choose a diet moderate in salt	Choose and prepare foods with less salt and sodium
If you drink alcoholic beverages, do so in moderation	If you drink alcoholic beverages, do so in moderation

(It is built on the 1979 *Surgeon General's Report*, *Healthy People*, and *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*.)

Many other health-promotion groups, including the American Cancer Society, American Heart Association, National Cancer Institute and National Cancer Institute in Canada in coalition with others, namely 5-A-Day for Better Health, the American Institute for Cancer Research, the National Heart and Stroke Foundation of Canada, have their own set of recommendations (Tables 2.3 and 2.4). All give roughly the same advice, although the emphasis may differ with the specific disease involved.

With all this advice and its seeming congruence, it would be logical to assume that the diets in North America are optimal. Unfortunately, the average North American is either confused with what he or she sees as constantly conflicting advice, or he or she chooses not to heed the advice. The pyramid is either lop-sided (Fig. 2.2) (National Cattlemen's Beef Association) or anvil-shaped (Fig. 2.1) (NDP Group). Instead of the recommended 'eat sparingly' from the tip of the pyramid, the average US consumer is eating 3.5 servings per day from the top rung of the pyramid (National Cattlemen's Beef Association 1994). These foods are high in sugar and sweeteners, fat, and salt and often offer little, except additional calories to a diet, which already has a caloric surfeit and an expenditure deficit.

The dietary guideline with respect to sugar are expressions of concern about the intake of added fat and sweeteners from the top of the pyramid. Sweetener intake has increased since 1909 from ~40 kg (86 lb.) per year per capita to ~70 kg (154 lb.) per year. Fats and oils have increased from 17 to 30 kg (38 to 66 lb.) per capita per year. According to United States Department of Agriculture (USDA) surveys, added sweeteners provide on average 16% of calories. For persons under 55 years of age, soft drinks comprise the number one source of sweeteners. According to a *Newsweek* survey in August 1999, 23% of males said they drank three or more cans of soda on a typical day (Cowley 1999). The mean adjusted energy intake in a sample of over 1500 school-aged children who consumed 250 ml (9 oz.) of soda or more per day was 300 calories more than for those who did not (Harnack *et al*. 1999). Candies, bread spreads, cookies, cakes and other sweetened bakery and cereal products, sweetened fruit-flavoured beverages and milk products such as ice cream make up the top contributors to

Table 2.3 American Cancer Society: guidelines on diet, nutrition and cancer prevention.

(1)	Choose most of the foods you eat from plant sources. Eat five or more servings of **fruits and vegetables** Eat other foods from plant sources, such as **breads**, **cereals**, **grain products**, **rice**, **pasta**, **or beans**
(2)	Limit your intake of high-fat foods, particularly from animal sources
(3)	Be physically active: achieve and maintain a healthy weight.
(4)	Limit consumption of alcoholic beverages, if you drink at all.

Table 2.4 American Heart Association dietary guidelines.

Total fat intake	<30% of calories.
Saturated fat	<10% of calories.
Polyunsaturated	8–10% of calories.
Monounsaturated	10–15% of calories.
Cholesterol intake	<300 mg/day
Sodium intake	<2400 mg (2.4 g)/day

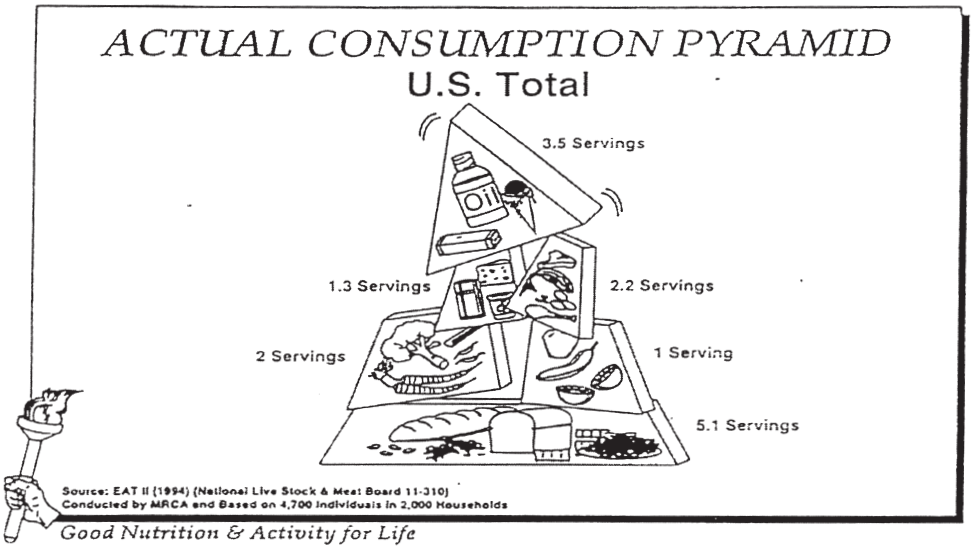


Fig. 2.2 Lop-sided pyramid.

sweetener intake (Anon. 1999). Interestingly, an article assessing the accuracy of food intake date, found these items to be among those most likely to be underreported, perhaps making some of the epidemiology data and conclusions inaccurate (Johnson 2000).

Underconsumption of recommended foods in the other rungs of the pyramid also contributes to the lop-sided pyramid. Despite active campaigns such as '5-A-Day for Better Health' (National Cancer Institute 2000) and news reports extolling the importance of antioxidants and phytochemicals from fruits and vegetables in preventing cancers, coronary disease, hypertension and stroke, fruit and vegetable consumption fails miserably. On a given day, 10–25% of Americans ate no vegetable, 47% did not consume even one serving of fruit or full-strength juice, 79% ate no vegetable or fruit high in vitamin A, 72% ate no vegetable or fruit high in vitamin C, and only 18% consumed a cruciferous vegetable (Patterson *et al.* 1990).

News that approximately 28 g (1 oz.) of nuts per day reduces the risk of coronary disease by 50% (Hu *et al.* 1998; Kris-Etherton *et al.* 1999), and many reports about benefits of whole grains (Slavin *et al.* 1999), soy (Potter 1998; Potter *et al.* 1998; Moyad 1999), and legumes (Kushi *et al.* 1999) have had little impact on most consumers' choices. On a given day, 79% of Americans do not consume one serving of legumes or nuts (Enns *et al.* 1999). Both men and women eat fewer servings of grain than recommended, and most of the grain consumed is not whole grain (Jacobs *et al.* 1999).

In terms of dairy and calcium intake, US intakes fall short of the mark. This is occurring even though recommendations abound about the importance of calcium and dairy in reducing hip fractures and preventing osteoporosis (Looker *et al.* 1993). However, the average calcium intake is only 400 –600 mg/day. Put another way, 11% of women over age 11 meet the calcium requirement (Pennington *et al.* 1996, 1997; Albertson *et al.* 1997). Excess soda intake may again exacerbate this problem. Adolescents who consumed 26 oz. of soft drinks

or more per day were about four times more likely to consume less than one cup (225 g) of milk per day than teens not consuming soft drinks (Anon. 1999).

While all of the above problems are significant, the number one nutrition problem in North America is overweight. According to a 1999 *Newsweek* survey (Cowley 1999), 60% of the men and 43% of the women were classified as overweight or obese using accepted medical standards. Interestingly, when asked to classify themselves, only 4% and 8% of men and women classified themselves as obese compared with 21% and 18% using a medical classification. Depending on the definition of obesity, somewhere between 33% and 40% of US adults (some 58 million people) are obese. Thus, there is higher risk for conditions associated with obesity such as heart disease, high blood pressure, high cholesterol and diabetes.

With the increase in fatness, the incidence of non-insulin-dependent diabetes mellitus (NIDDM) is rising dramatically, and the condition is now known to affect 10.3 million people. As many as 80% may be overweight at the time of diagnosis. Many more are believed to have the disorder and to be unaware of it. There is also another alarming trend, there being a sharp rise in the incidence of NIDDM in pre-adolescent and school-age children (American Diabetes Association 2000).

North Americans eat too many calories and expend too few. Moreover, Americans are exercising less than they were a few years ago (Kate 1995), and the trend is toward even less exercise. More than three-quarters of adults say they exercise, but exercisers do not sweat often enough: 7% of adults exercise less than once a month, 13% exercise just 1–3 days a month, and only 37% exercise frequently and strenuously enough for optimal cardiovascular activity (Fig. 2.3; Table 2.5). Furthermore, little energy expenditure occurs in daily life as conveniences such as television remote controls mean less movement. Walking is almost an un-American activity.

Physical activity among youth has declined over the past several decades, with less time being spent in all types of physical activity. Many popular activities such as watching television and computer games, involve minimal physical exertion (Luepker 1999).

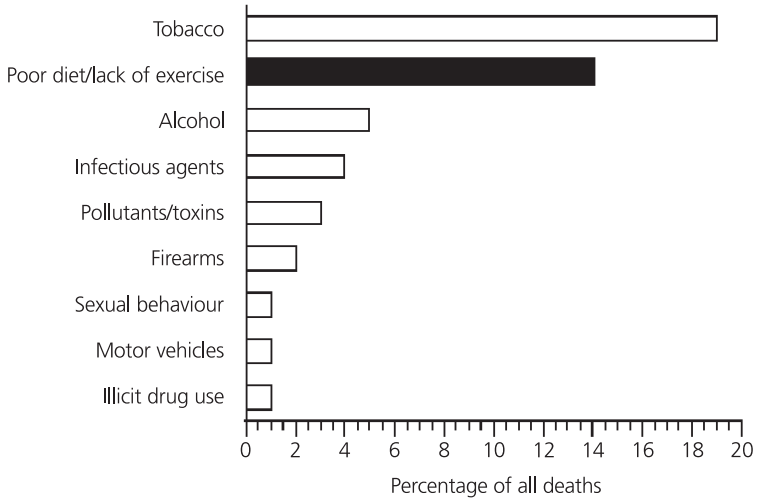


Fig. 2.3 Poor diet and exercise as causes of death in the US.

Table 2.5 Frequency of exercise.

Frequency	%
Less than once a month	7
One to three days per month	13
Three or more times a week: long enough and frequently enough to protect the heart	37

Newsweek survey (August 1999)

Another factor that affects calories and nutrition is the demise of family meals or the 'three squares' (Table 2.6). A recent survey (Gillman *et al.* 2000) showed that 17% of families ate together never or on 'some days'. Compared with the group that ate together, those not eating as a family ate fewer fruits and vegetables and more fried foods and sodas, and were very unlikely to get the recommended five servings of fruit and vegetables (Table 2.7). When the families ate together, diets contained more fibre, vitamins and minerals.

Overall, Americans are grazing (snacking more) (Baranowski *et al.* 1997; Siega-Riz *et al.* 1998), eating fewer meals, and purchasing more vending machine and other fast food. They are eating in locations other than at a traditional mealtime table. Astonishingly, the second most common place to eat is the car.

Another factor is that North Americans now spend nearly half their food dollar on food eaten away from home (National Restaurant Association 2000). In some venues, it is difficult to get a full serving of fruit or vegetable other than fried potatoes. For some, eating out has become an everyday occurrence, even an every meal occurrence. Still, restaurant meals are

Table 2.6 The vanishing family dinner.

Time to eat together	%
Never or some days	17
Every day	43
9-year-olds	50
14-year-olds	33

$n = 16\,202$
Source: Gillman *et al*. (2000) *Archives of Family Medicine,* **9**, 235–240.

Table 2.7 Effect of eating together versus not eating together.

Get five servings fruits and vegetables per day	RR = 1.45
Eat fried foods	RR = 0.67
Drinking any soda	RR = 0.73

$n = 16\,202$
Source: Gillman *et al*. (2000) *Archives of Family Medicine,* **9**, 235–240.

Table 2.8 Effect of eating in a restaurant on nutrition choices of pre-menopausal women.*

Nutrient	Out	Home
Calories	2057	1769
Fat (g)	79.5	60.6
Sodium (mg)	3299	2903

n = 129
Source: Clemens *et al.* (1999) *Journal of the American Dietetic Association*, **99**, 442–444.

often viewed as treats and thus not needing to count nutritionally. A recent study (Clemens *et al.* 1999) showed that restaurant and fast-food diners ate an average of 300 more calories and 19 g more fat than those dining at home. Despite more calories, there was no increase in some important nutrients or fibre (Table 2.8). Another problem with restaurant eating is gargantuan portions (McCrory *et al.* 2000). Some chain restaurants are super-sizing everything and offering food at a relatively low price. 'All-you-can-eat' buffets are popular, and so the calories eaten are often far greater than needed.

This book is focusing on one aspect of a healthy diet – fibre. While debate will occur as to whether fibre cures a variety of problems, the fact remains that adequate intake of fibre is associated in epidemiological studies with reduction in risk of most of the major dietary problems in the North America – cancer (Gold & Goldin 1998; Earnest *et al.* 1999; Fuchs *et al.* 1999; LaVecchia & Chatenoud 1999; Lupton & Turner 1999; Reddy 1999; Young & Lee 1999), coronary disease (Rimm *et al.* 1996; Brown *et al.* 1999; Ludwig *et al.* 1999; Wolk *et al.* 1999), diabetes (Salmerón *et al.* 1997) and obesity (Miller *et al.* 1994; Kimm 1995). It remains to be determined if fibre is the reason for the positive health effects or whether it is merely the marker of a good diet –one lower in fat and saturated fat and higher in fruits and vegetables, whole grains, vitamins, minerals, antioxidants and other phytochemicals. As chapters in this book delve into the many important health and nutritional issues associated with fibre, scientists are still attempting to determine the precise role of fibre in modifying the course of disease and maintaining health. What is crucial to keep in mind, is that people must eat fibre-containing foods before the various health benefits can be achieved. Therefore, we must develop foods that are likely to be selected. Secondly, we must frame clear, understandable, consistent nutrition messages that are meant not only to be heard, but also to be adopted.

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Part 2

Chemistry, Structure and Rheology

3 Light Microscopic Investigations on Dietary Fibre

Karin Autio

3.1 Introduction

Plant cell-wall material containing hemicellulose, cellulose, pectic substances and lignin are the major components of dietary fibre, with small amounts of other components, such as phenolic substituents and acetyl groups (Selvendran *et al*. 1987). The cell walls of the different microstructural components of the seed kernel differ in terms of chemical content and architecture. The main components of rye primary cell-walls are arabinoxylans; the β-glucan content is much lower. In barley and rye, ferulic acid is esterified to arabinoxylans (Harris & Hartley 1980; Ahluwalia & Fry 1986). Ferulic acid–arabinoxylan complexes are concentrated in the aleurone layer (Pussayanawin & Wetzel 1987; Pussayanawin *et al*. 1988). No ferulic acid is associated with β-glucan. Structurally, cereal arabinoxylans form a very heterogeneous group, in which the ratio of arabinose to xylose, the pattern of arabinose substitution, the feruloyl group content and the degree of polymerisation can vary significantly (Izydorczyk & Biliaderis 1993). Mixed linked β-glucan is the major cell-wall component in barley, but it is not evenly distributed in the grain. The aleurone cell walls consist of 67% arabinoxylans and 26% mixed linked β-glucans, whereas the starchy endosperm cell walls contain about 20% arabinoxylans and 70% mixed linked β-glucans (Bacic & Stone 1981). Mixed linked β-glucans also form a non-homogeneous group. Variations exist in the amounts of adjacent 1,3- and 1,4-linkages (Fincher & Stone 1986) and in the degree of polymerisation. Primary cell walls (walls of aleurone, starchy endosperm and scutellum) consist of an amorphous matrix in which cellulose microfibrils are embedded (Fincher & Stone 1986). Despite the enormous amount of published data on cereal grains, we have a poor understanding of the interaction of components in the matrix phase of the primary cell wall.

The structure of plants can be divided into different structural level: tissues, cells, cell walls and polymers (Waldron *et al*. 1997). Every level of the structural hierarchy will influence the functionality. Cross-links, arrangements and thickness of cell walls have a great effect during processing and digestion (Waldron *et al*. 1997). Cells constitute the next level of structure, differing in size, shape and content. Cells are joined to one another at the middle lamella, the extent and strength of adhesion being dependent on the degree of ripening. Cell-wall structures in grain serve the growing plant by maintaining the tissue integrity, as a conduit structure for the movement of water and low-molecular-weight solutes, thus helping to maintain osmotic pressure and acting as a barrier against microbe and insect penetration (Autio 1996). During processing, such as malting, milling and heating, great changes in cells and cell walls occur. In foods, the tissues, cells, cell walls and their components control texture, water-binding and sensory properties, and are an important source of nutrients and dietary fibre (Fincher & Stone 1986). Ferulic acid-containing cell-wall polysaccharides of fruits and vegetables play a key role in the thermal stability of cell-cell adhesion (Waldron *et al*. 1997). Cell separation is a very important factor in tissue softening.

Microscopy techniques are effective methods in studying the structure of tissues, cells and cell walls. Bright-field and fluorescence microscopy can reveal the distribution of different chemical components in different parts of cereal grains, since they allow selective staining of different chemical components. In this chapter, the microstructures of the main cereal grains and the effect of processing will be presented, as well as staining systems for the demonstration of different cell-wall components. The chemical components in the cell walls can also be mixed at a molecular or supra-molecular level, which does not allow visualisation of the components in the magnitude of light microscopy. Microscopy of thin sections of cereal grains incubated with purified enzymes can give additional information about the localisation of specific components. Examples of this method in studying cell wall structures of barley and rye cell walls will be shown.

3.2 Staining of the main chemical components of cereal cell walls for light microscopy

For microstructural characterisation, the samples must be prepared either by freezing with or without fixation or by plastic embedding, including fixation, dehydration and embedding in plastic. The most commonly used staining systems for cereal cell walls in bright field (Flint 1988) and fluorescence microscopy (Fulcher *et al*. 1989), are presented in Tables 3.1 and 3.2. Table 3.1 shows the most commonly used stains in bright-field microscopy. Some components exhibit autofluorescence. In cereal cell walls, the main sources of autofluorescence are polyphenolic compounds, such as ferulic acid and lignin. The use of fluorescently labelled antibodies and lectins that bind to specific protein or carbohydrate components of cell walls, increases the range of the specific components that can be studied (Miller *et al*. 1984). Toluidine blue is an example of a non-specific stain which is widely used for visualisation of cell walls (Flint 1988).

Table 3.1 The most commonly used stains for cell walls in bright-field microscopy.

Component	Stain	Colour
Cellulose	Thionin	Violet
Protein	Light green	Green
Lignin	Phloroglucinol	Red
Pectin	Ruthenium red	Rose

Table 3.2 The most commonly used stains for cell walls in fluorescence microscopy.

Component	Stain	Colour
Mixed-linked β-glucans	Calcofluor	White/blue
	Congo red	Red
	Immunostaining	Green
Arabinoxylans	Immunostaining	Green
Proteins	Acid fuchsin	Red

3.2.1 *Microscopic investigations of cereal cell walls*

The four morphologically different tissues common to all cereal grains are the layers of seed coat, embryo, aleurone and starchy endosperm (Evers & Bechtel 1988). The pericarp (fruit coat) is composed of an outer epidermis, hypodermis, remnants of thin-walled cells, intermediate cells, cross and tube cells. The outer layers are mainly composed of lignin and cellulose. Although the morphology of cereal grains share many similar features, differences exist especially in the chemical composition and distribution of components. Figs 3.1–3.4 show fluorescence micrographs of barley, oat, rye and wheat. The aleurone layer of barley (Plate 3.1) is two to three cells thick, whereas those of oat (Plate 3.2), rye (Plate 3.3), wheat (Plate 3.4) and maize grains (Plate 3.5) are only one cell thick. The cell walls of barley, rye and wheat (around the aleurone cells) are rich in polyphenolic compounds – mainly ferulic acid – since only aleurone cell walls and the layers of fruit coat (mainly lignin) exhibit autofluorescence (Plate 3.5). In the case of maize, rice and sorghum, the inner endosperm cell walls are also autofluorescent, suggesting that they too contain large amounts of polyphenolic compounds. The aleurone cells are cubic and their cell walls are thick. The aleurone cell walls of barley or wheat are reported to contain about 65–67% arabinoxylan and about 26–29% mixed linkage (1→3),(1→4)-β-D-glucan (Fincher & Stone 1986). The cells contain large nuclei and storage materials, including lipids, phytin, minerals, protein and phenolic compounds. The aleurone is important both botanically and industrially. It has a significant role, both in germination and in animal nutrition. The aleurone cells are an important source of the hydrolytic enzymes that degrade the starchy endosperm during germination. Nutritionally, the aleurone is a rich source of dietary fibre, minerals, vitamins and other health-promoting compounds.

In rye and wheat, the thickness of cell walls in different parts of the grain shows little variation between different varieties, whereas in the case of barley and oat, great differences exist between varieties. In barley, the thickness of cell walls is strongly related to malting quality. Plate 3.6 shows different barley varieties: Chariot Hja is an easily, and Minerva a poorly, modified variety. Easily modified barley samples usually have thin cell walls. Waxy barley (Prohashonupana), which has a β-glucan content of about 15%, has been developed. The endosperm cell walls of this sample have clearly thicker cell walls than barleys usually have (Andersson *et al*. 1999). According to Fulcher and Miller (1993), the distribution of β-glucan in oat kernel varies in low- and high-β-glucan varieties. The clear trend of their study was that β-glucan was evenly distributed in high-β-glucan varieties, whereas it was concentrated in the subaleurone layer in the low-β-glucan varieties. The distribution of β-glucan is of great practical importance in oat bran manufacture. In rye grains with a high falling number, the thickness of the cell walls is usually uniform in the different parts of the grain (Autio & Salmenkallio, in press). Pre-harvest sprouting may cause degradation of cell walls (Fabritius *et al*. 1997), and generally, cell walls in the subaleurone layer, and especially those of the ventral endosperm, are not stainable by Calcofluor. In wheat grain, the cell walls in the subaleurone layer are very thin, whereas the inner endosperm cell walls are thicker (see Plate 3.4).

3.3 The effect of purified cell wall degrading enzymes on cell walls

Microscopy of thin sections of cereal grains, incubated with purified enzymes, can be used as a means to localise specific components. In mature barley grains, three morphologically different cell types are apparent in the starchy endosperm: (1) cells of the dorsal endosperm,

which are elongated; (2) cells of the ventral endosperm, which are rounder; and (3) the small subaleurone cells. The major changes due to *endo*-β-glucanase treatment occurred in the cell walls of the outer starchy endosperm (Autio *et al*. 1996a). The inner starchy endosperm cell walls seem to be much more resistant to hydrolysis. The peripheral areas in the barley kernel have been shown to have a lower concentration of β-glucan than the central endosperm (Miller & Fulcher 1994). The consecutive incubation of thin sections of barley with purified *Trichoderma reesei endo*-xylanase and endo-β-glucanase (EG II) significantly weakens the aleurone cell walls (Autio *et al*. 1996a). This is consistent with the conclusion that the aleurone cell walls of barley have an outer coating of arabinoxylan, the removal of which by *endo*-xylanase allows access to a β-glucan layer, which can be subsequently degraded by *endo*-glucanase.

Incubation of rye kernel sections with purified *Trichoderma reesei endo*-xylanase and *Aspergillus niger* (Megazyme, Ireland) showed that only the cell walls of the very outer layers of the endosperm were degraded by xylanase, whereas both xylanase and arabinosidase were needed to hydrolyse all endosperm cell walls (Autio *et al*. 1997a).

3.4 The effect of processing on the microstructure of cell walls

Malting and baking have a great influence on the microstructure of cell walls. The malting of barley is one of the most studied plant processes and, in particular, the structure and physiology of the germinating grain has been well characterised (Palmer 1989). The structure of cell walls, their thickness and cell size have a significant effect on the malting performance of barley. Water uptake during steeping and diffusion of enzymes during germination determine the rate of modification. Thin cell walls, large cells and a loosely structured endosperm improve the water uptake during steeping and the diffusion of growth regulators and enzymes during germination.

Most of the cell walls present in wheat and rye dough are insoluble. In wheat dough and bread, the cell walls exist as cell wall fragments (Autio *et al*. 1997b; Laurikainen *et al*. 1998). The addition of cell wall-hydrolysing enzymes caused the area of insoluble cell walls to decrease (Laurikainen *et al*. 1998). In fibre-enriched baking, the added enzymes mainly affected wheat endosperm cell walls, but had very minor effects on aleurone layers, which are very stable during processing. Dough and bread from wholemeal rye contain seed coat, embryo, aleurone and starchy endosperm, with particles varying in size from under 10 μm up to 1 mm (Parkkonen *et al*. 1994, 1997; Autio *et al*. 1996b; Fabritius *et al*. 1997). The area of blue fluorescence due to intact cell walls is much higher in rye than in wheat bread. The addition of cell wall-hydrolysing enzymes, *endo*-xylanase or β-glucanase into the dough, caused fragmentation and fading of endosperm cell walls. The cell walls of the aluerone cells did not change to any degree.

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4 Assembly and Rheology of Non-starch Polysaccharides

Edwin R. Morris

4.1 Introduction

In addition to their role as constituents of dietary fibre, polysaccharides are also used widely, after extraction and purification, as thickeners and structuring agents for manufactured foods and related products. In the context of these industrial applications, the relationships between their structure, rheology and functional interactions have been studied extensively. Although there are, of course, substantial differences between extracted polysaccharides and intact plant tissue, the general principles that have emerged from such research provide a useful starting point for rationalising the physiological effects of dietary fibre.

4.2 Composition and shape of polysaccharide chains

The physical properties of polysaccharides are dominated by the shape (conformation) of the individual chains, and the way in which they interact with one another (Rees *et al*. 1982). Each polysaccharide molecule typically contains several thousand monomer (monosaccharide) units. These are often arranged in a linear sequence, like a (very long) string of beads, although more complex branched arrangements also occur. In contrast to globular proteins, polysaccharides normally have structures based on regular repeating sequences. The simplest arrangement is where all the monosaccharides are the same, and are linked together in the same way along the chain. Disaccharide repeating sequences (-A-B-A-B-) are also common, and larger repeating units (up to octasaccharide) can occur, particularly in polysaccharides produced by bacteria.

The constituent monosaccharides have a ring structure, which can be either five-membered or six-membered, and are linked together by 'glycosidic bonds' with a shared oxygen atom between adjacent sugars. The polysaccharides of greatest practical importance, both as commercial 'hydrocolloids' and as constituents of dietary fibre, are built up from six-membered (pyranose) rings consisting of one oxygen atom and five carbon atoms, which are numbered sequentially from the ring oxygen as C-1 to C-5, and with a sixth carbon atom, numbered as C-6, lying outside the ring. As a consequence of the tetrahedral bonding arrangement of carbon, and the requirement to avoid steric clashes between adjacent groups, the pyranose ring is locked in a fixed, chair-like geometry (Stoddart 1971), and the overall shape of the polysaccharide molecule is dictated by the torsional angles (Fig. 4.1) characterising the relative orientation of neighbouring sugars. These angles may be either fixed at the same values for equivalent linkages along the polymer chain, giving regular, ordered chain geometry, or constantly fluctuating, to give the disordered 'random coil' geometry typical of polysaccharide solutions.

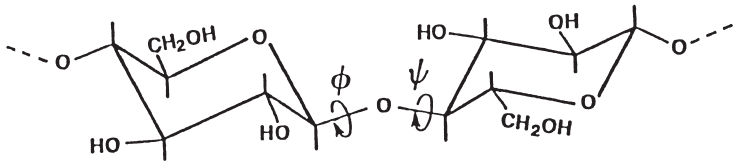

Fig. 4.1 Conformational variables in carbohydrate chains (illustrated for cellulose).

The nature of the ordered structures adopted by different polysaccharides depends strongly on the way in which the individual sugars are linked together. In the chair conformation shown in Fig. 4.1, the hydroxyl groups attached to C-2, C-3 and C-4 may be present either in equatorial locations around the periphery of the sugar ring, or in axial positions vertically above or below the plane of the ring. In glucose – the constituent monosaccharide of cellulose and starch – all three hydroxyl groups (i.e. on C-2, C-3 and C-4) are equatorial. Other sugars have one or more of these hydroxyl groups in axial positions. The group at C-6 is always positioned equatorially. In simple sugars (glucose, galactose and mannose), the C-6 substituent is a hydroxymethyl (-CH_2OH) group; in 6-deoxy sugars (such as rhamnose) this is replaced by a methyl (-CH_3) group, and in uronic acids (such as the galacturonic acid monomers of pectin) it is replaced by a carboxyl (-COOH) group, which at neutral pH exists in the charged carboxylate form (-COO^-). Each sugar can be synthesised in two different mirror-image forms, identified as D or L, although (with the notable exception of D and L galactose) only one of these normally occurs in nature.

Formation of glycosidic bonds can be regarded as a condensation reaction between OH groups on adjacent sugars, with formal elimination of water. Linkage invariably occurs between the OH group at C-1 of one of the participating monosaccharides and any of the other OH groups on the next sugar residue (including the hydroxyl group lying outside the ring on C-6). The OH at C-1 is chemically different from the other hydroxyls, since it forms part of a hemiacetal group which also includes the ring oxygen, and which converts to an acetal group on formation of the glycosidic bond. Like the other oxygens attached to the carbon atoms of the sugar ring, O-1 can be positioned either axially or equatorially; sugars in which O-1 is axial are termed α and those in which O-1 is equatorial are known as β. The position of linkage between adjacent sugars, and the axial or equatorial orientation of the bonds to the glycosidic oxygen, can have a profound effect on overall chain geometry, both in the disordered coil form and in the ordered structures found in the solid state (Rees *et al*. 1982).

Three patterns of linkage geometry (Fig. 4.2) are particularly relevant to dietary fibre. The first occurs in cellulose, which is a (1→4)-linked linear polymer of β-D-glucose. The bonds to and from each residue in the cellulose chain are diagonally opposite one another across the sugar ring and both are equatorial [since O-1 is equatorial in all β-linked sugars, and O-4 is equatorial in glucose]. Thus, as indicated in Fig. 4.2a, they are parallel, and only slightly offset from each other. As a direct consequence of this linkage arrangement, the individual chains in cellulose microfibrils exist as flat, extended ribbons, which pack together like planks of wood. The same (1→4)-diequatorial linkage pattern occurs in the mannan (i.e. poly-β-D-mannose) backbone of plant galactomannans such as guar gum and locust bean gum, and these materials also pack together as extended ribbons in the solid state.

The second characteristic arrangement (Fig. 4.2b) occurs in the (negatively-charged) poly-α-D-galacturonate sequences of pectin. Linkage is again at carbons 1 and 4, but through

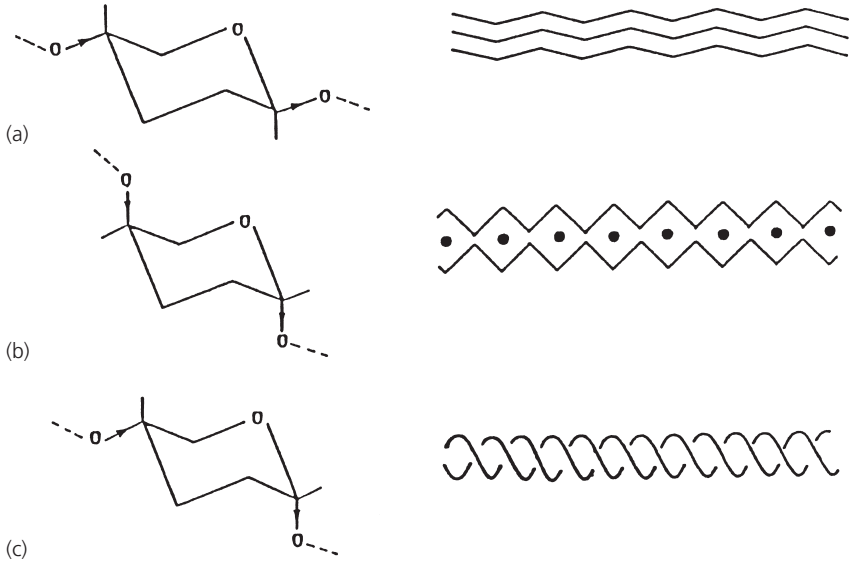

Fig. 4.2 Relationship between the relative orientation of bonds to and from component residues of polysaccharide chains and the nature of the ordered structures adopted in the solid-state: (a) extended ribbons; (b) buckled ribbons; (c) hollow helices.

axial bonds at each position. The bonds to and from each residue are again parallel, but are now offset from each other by the full width of the sugar ring, giving buckled, zig-zag chain geometry with large cavities which can accommodate metal cations (typically Ca^{2+}). These cations allow compact, ordered assemblies to form, by balancing the charge of the carboxylate groups on the galacturonate residues and thus suppressing electrostatic repulsion between the constituent polysaccharide chains. A similar arrangement of buckled polysaccharide chains with long arrays of site-bound cations sandwiched between them (known as an 'egg-box' structure) is adopted by the (1→4)-diaxially-linked poly-α-L-guluronate sequences of alginate, an algal (seaweed) polysaccharide which is used widely in the food industry as a gelling agent.

In the third class of linkage arrangement (Fig. 4.2c), the bonds to and from each residue are no longer parallel to one another. This introduces a systematic twist in the direction of the chain, and the resulting ordered structures are hollow helices which stabilise one another by packing together coaxially. Non-parallel bonding can occur in two ways. The first is when the sugars are again linked through carbons 1 and 4, as in the extended and buckled ribbon structures shown in Fig. 4.2a and b, but with one bond axial and the other equatorial. This arrangement occurs in the (1→4)-linked poly-α-D-glucose sequences of amylose and amylopectin – the constituent polysaccharides of starch – and leads to formation of coaxial double helices. Helical structures can also arise from linkage through carbons 1 and 3, with the bonds to and from each residue no longer directly opposite one another across the sugar ring. For example, the bacterial polysaccharide curdlan, which is used as food ingredient in Japan, is a (1→3)-linked linear polymer of β-D-glucose, and forms coaxial triple helices (i.e. with three chains wound together). In polysaccharides with more complex repeating sequences, where some of the linkages are of the parallel type (Fig. 4.2a and b) and others are non-parallel

(Fig. 4.2c), the twist introduced by the non-parallel linkages again promotes overall helical chain geometry. Examples of this include the coaxial double helices formed by algal polysaccharides in the carrageenan and agar families, and by gellan, a new bacterial polysaccharide recently approved for food use.

Linkage through carbons 1 and 2 is rare, although it does occur in 'rhamnogalacturonosyl' regions of the pectin backbone (which have an alternating sequence of galacturonate and rhamnose residues, linked at positions 4 and 2, respectively). When attachment is at O-6, the linkage includes an extra bond, between C-5 and C-6, and is therefore more flexible, and less amenable to ordered packing, than the normal glycoside linkage shown in Fig. 4.1.

4.3 Solids, solutions and hydrated networks

The physical state of polysaccharides may have profound effects on their physiological role as constituents of digest. At one extreme, the chains may adopt regular, ordered conformations of the types shown in Fig. 4.2, and pack together into insoluble 'crystalline' assemblies. Ordered packing confers resistance to enzymatic attack, and thus structures such as cellulose microfibrils may undergo only limited degradation during colonic fermentation, even when appropriate enzymes are secreted. Similarly, the effectiveness of human digestive enzymes in breaking down starch is critically dependent on chain conformation and packing. Starch granules contain densely packed assemblies of ordered (double helical) amylopectin, together with disordered amylose. On heating in water, the amylopectin helices melt, and the granules swell (gelatinise), with release of disordered amylose. In this freshly cooked, disordered form, starch is highly susceptible to enzymatic hydrolysis. On cooling and storage, however, 'retrogradation' occurs: the amylose component forms large bundles of aggregated double helices, and at longer times the amylopectin may also revert to the ordered form. These products of retrogradation, together with raw starch (e.g. from bananas), because of their packed, ordered structure, are far less susceptible to enzymatic attack, and are known as 'resistant' starch.

At the opposite extreme, polysaccharide chains may exist in solution as disordered coils and generate viscosity by restricting the flow of the solvent. Between these two extremes of packed, ordered assemblies and hydrated, disordered coils lie hydrated networks, such as food gels and the soft tissue of plants. Formation of a cohesive network requires enough ordered association (as in the solid state) to hold the network together, but there must also be sufficient residual disorder (as in solution) to maintain hydration and prevent the network collapsing to a close-packed solid.

The balance between association and hydration depends on a number of factors, the most important of which is charge. Neutral polysaccharides (such as cellulose or starch) have a strong tendency to self-association; charge promotes solubility by introducing electrostatic repulsion between the chains. Negatively charged polysaccharides may, however, associate in the presence of appropriate metal ions that can bind to the chains and balance their charge. One way in which this can occur is by formation of an 'egg-box' structure of the type shown schematically in Fig. 4.2c. Other types of ordered structure – and in particular coaxial helices involving negatively charged polysaccharides – however, can also form aggregated assemblies incorporating metal cations to balance the charge on the polymer chains.

Ordered packing can also be inhibited by structural irregularities or branching. The individual polysaccharide chains within ordered assemblies are held together by non-covalent

bonds. These are individually weak, and can promote stable association only when a sufficiently large number of them act together in a concerted array. Ordered junctions therefore have a minimum critical length below which they become unstable. The minimum length can vary substantially from one polysaccharide to another, but is typically in the range 8 to 20 residues. Thus any structural irregularities (i.e. deviations from the regular repeating sequence) that occur at a separation less than this critical length will terminate ordered association. Similarly, irregularly spaced side chains, or branch points separated by less than the minimum critical sequence length for stable association, can inhibit ordered packing and promote solubility. For example, guar gum is solubilised by sugar side chains attached irregularly to a backbone similar to cellulose.

Another important determinant of the stability of ordered structures is, of course, temperature. As in any chemical process, conversion between ordered and disordered conformational states will occur only if the accompanying change in free energy (ΔG) is negative (i.e. if there is an overall reduction in the free energy of the system). Melting of ordered junctions requires heat to break the non-covalent bonds between the chains, and thus the change in enthalpy (ΔH) is positive. Offset against this, however, the associated change in entropy (ΔS) is also positive, because of the increase in conformational freedom on conversion to the disordered coil form. The balance between the enthalpic drive to ordered association and the entropic drive to disorder and conformational mobility is related to absolute temperature (T) by the Second Law of Thermodynamics ($\Delta G = \Delta H - T\Delta S$). Thus entropy, favouring the disordered solution state, becomes progressively more important as the temperature is raised. This is why many gel networks melt, often quite sharply, on heating, and why most polysaccharides dissolve more readily in hot water than in cold water.

However, although the balance between entropy and enthalpy determines which state will be adopted at thermodynamic equilibrium, the kinetics of interconversion between ordered and disordered states may be very slow. Thus if soluble polysaccharides are consumed as part of a dry, or low-moisture, food product, their effect on digesta viscosity will be limited by their rate of dissolution and release, which in turn will depend on the overall structure of the food, and its susceptibility to mechanical and enzymatic degradation.

4.4 Rheological measurements

As a starting point for discussing the physical properties of complex systems such as digesta, it is useful first to consider two idealised extremes: perfect (Hookean) solids and perfect (Newtonian) liquids, which are approximated reasonably closely by, respectively, cross-linked rubber and sugar syrups. If a rubber band is stretched, the increase in length (at least initially) is directly proportional to the force applied: pull twice as hard and it stretches twice as much. The force required to stretch the band by a fixed amount will obviously depend on its thickness and on its initial length, as well as on the strength of the rubber. To eliminate these effects of size, the extension can be expressed as *strain*, defined as the increase in length divided by the initial length, and the resistance to deformation can be expressed as *stress*, which is given by the force divided by the cross-sectional area of the sample. The strength of the material can then be characterised by the ratio of stress/strain, which is known as the *modulus*.

When dealing with liquids or soft solids, there are obvious practical difficulties in making extensional measurements. Instead, the sample is normally subjected to a *shear* deformation.

If we imagine a block of material glued to a table, the sample can be sheared by applying a force to the upper surface, in a direction parallel to the surface of the table. The strain is then defined as the distance moved divided by the height of the sample, and the stress is again defined as the force per unit area. In practice, measurements under shear are normally made by sandwiching the sample between two circular plates (or in the gap between two cylinders), one of which rotates while the other remains stationary.

For Newtonian liquids, the resistance to rotation (i.e. the shear stress) is directly proportional to the rate of rotation (or, more strictly, to the shear rate, which is defined as the amount of strain applied per second). The ratio of shear stress/shear rate gives the *viscosity* of the liquid. Since stress is defined as force per unit area, it has units of pressure (i.e. Pa), and because strain is a dimensionless ratio, shear rate has units of reciprocal time (i.e. s^{-1}); viscosity (stress/shear rate) is therefore measured in units of Pas. As a useful point of reference, the viscosity of water at 20°C is 1 mPas, which in older units is also known as 1 cP (centipoise).

Most materials of relevance in discussing dietary fibre (polysaccharide solutions, hydrated networks, and slurries of dispersed particles) have a combination of liquid-like and solid-like properties. One way in which these can be resolved and quantified is by subjecting the sample to an oscillatory deformation. The principle of this approach, which is known as mechanical spectroscopy, is fairly simple. For perfect solids, the resistance to deformation (i.e. stress) is directly proportional to the extent of deformation (i.e. strain) and is therefore greatest at the extremes of the oscillation and drops to zero in the middle of the oscillatory cycle. For perfect liquids, the resistance is independent of the extent of deformation, but directly proportional to the rate of deformation, which is greatest in the centre of the oscillation and drops to zero at the extremes (at the point where the moving element switches from rotating in one direction to rotating back in the opposite direction, and is therefore stationary). Thus stress and strain are exactly in phase for a perfect solid and exactly out of phase for a perfect liquid.

For materials with intermediate rheological properties, the overall resistance to deformation (stress) can be resolved into an in-phase component and an out-of-phase component, both of which can be expressed as a modulus by dividing by the applied strain. Since the energy used in deforming a solid is stored, and released again when the deformation is removed (e.g. when a stretched rubber band springs back to its original length), the ratio of in-phase stress/applied strain is known as the *storage modulus* (G'). The energy used in deforming a liquid, by contrast, is lost (e.g. when a beaker of syrup is stirred with a spoon, the spoon does not spring back to its original position when the force applied to the handle is removed); the ratio of out-of-phase stress/applied strain, characterising the degree of liquid-like response, is therefore known as the *loss modulus* (G"). Since strain is a dimensionless ratio, both moduli have the same units as stress (Pa). The overall, unresolved stress divided by the applied strain gives the complex modulus (G*), which is related to G' and G" by: $G^* = (G'^2 + G''^2)^{½}$. Dividing the complex modulus by the frequency of oscillation (ω), expressed in units of radians per second (rad s^{-1}), gives the dynamic viscosity ($\eta^* = G^*/\omega = (G'^2 + G''^2)^{½}/\omega$), which can be regarded as the oscillatory analogue of conventional (steady-shear) viscosity (η) from rotational measurements.

4.5 Rheology of polysaccharide solutions, gels and dispersions

The mechanical properties of cross-linked, hydrated networks are predominantly solid-like.

Figure 4.3a shows the mechanical spectrum (frequency-dependence of G', G" and η*) for a typical polysaccharide gel. Solid-like resistance (characterised by G') exceeds solution-like response (G") by more than an order of magnitude, and the individual moduli show little variation with frequency. This insensitivity to frequency (i.e. rate of deformation) is again analogous to the behaviour of a rubber band, where the resistance to deformation is independent of the rate at which the stretching force is applied. As outlined above, η* is given by the root-mean-square value of G' and G", divided by frequency (ω), and since G' and G" are virtually constant, η* is almost inversely proportional to ω; thus the slope of log η* versus log ω in Fig. 4.3a is close to –1.

Although the nature of the intermolecular junctions (Fig. 4.2) depends critically on primary structure, the variation of rigidity (G') with polymer concentration (c) follows a common pattern for most gelling polysaccharides. Plots of log G' versus log c have a slope of 2 (i.e. c^2 dependence of gel strength) at high concentrations, with a progressive increase in

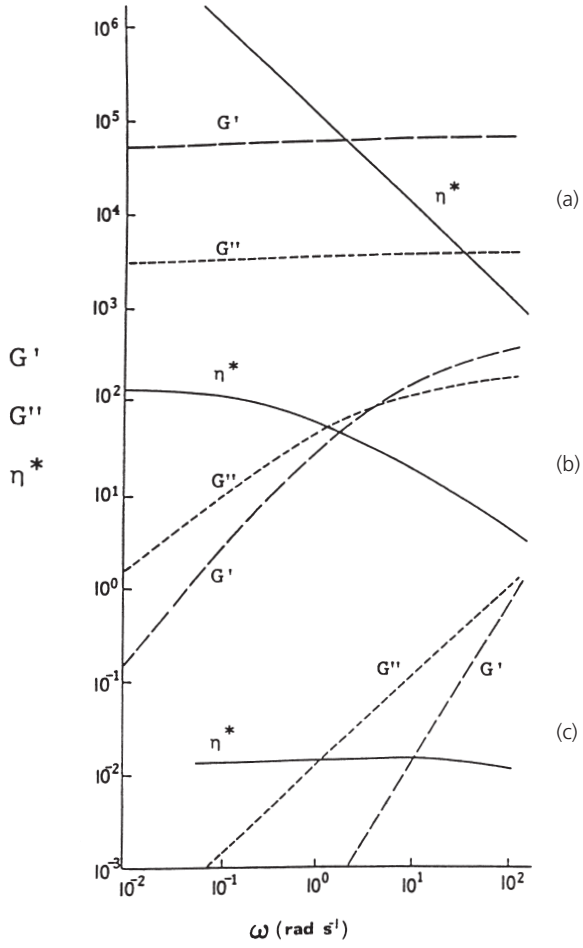

Fig. 4.3 Typical mechanical spectra for (a) a polysaccharide gel; (b) an entangled network; (c) a dilute solution of disordered coils free to move independently.

slope as the concentration is decreased towards the minimum critical gelling concentration (c_0), below which formation of a continuous network is no longer possible.

The rheology of solutions of disordered polysaccharide coils is also strongly dependent on concentration. In dilute solutions, the individual coils are free to move independently, and generate viscosity by tumbling around and interfering with the flow of the solvent. Dilute polysaccharide solutions are essentially Newtonian (i.e. the resistance to flow is almost directly proportional to the shear rate, so that the viscosity remains virtually constant). When subjected to oscillatory shear, the predominant response at low frequencies of oscillation is viscous flow, by movement of the polymer chains through the solvent. As the frequency is raised, it becomes progressively more difficult for the large polymer molecules to move backwards and forwards at the rate of the imposed deformation, and an increasing proportion of the energy is stored elastically, by contortion of individual chains. These effects can be seen in the mechanical spectrum shown in Fig. 4.3c for a typical dilute solution of disordered polysaccharide coils. As would be expected for a solution that is almost Newtonian, η^* is virtually independent of frequency (i.e. rate of deformation); viscous response (characterised by G") predominates at low frequency, but at higher frequencies G' rises towards G".

As the polymer concentration is raised, a point is reached at which the individual coils begin to touch, and at higher concentrations they are forced to overlap and interpenetrate one another. The transition from a solution of individual coils to an entangled network of overlapping chains is accompanied by a sharp increase in the concentration-dependence of viscosity, and by the onset of non-Newtonian behaviour. At low rates of deformation (i.e. low shear rates), where there is sufficient time for entanglements pulled apart by the imposed stress to be replaced by new entanglements with different chains, there is no change in the overall degree of overlap, and the viscosity remains constant at the maximum (Newtonian) value, η_0. However, at higher shear rates, where there is less time for new entanglements to form, the extent of coil overlap is decreased and the viscosity falls. This phenomenon is known as 'shear thinning', and at high concentrations of polymer it can reduce the viscosity by two or three orders of magnitude from the maximum 'zero shear' value (η_0).

The mechanical spectrum of a typical solution of entangled polysaccharide coils is shown in Fig. 4.4b. At low frequencies, where there is sufficient time for the entangled network to rearrange within the period of oscillation, the spectrum is similar in form to those observed for dilute solutions, where the chains are free to move independently (Fig. 4.3c). At higher frequencies, however, where the rate of deformation becomes much faster than the rate at which the chains can disentangle, the entanglements behave almost as permanent junctions, and the response becomes similar to that observed for cross-linked gel networks (Fig. 4.3a), with G' > G" and little frequency-dependence of either modulus. The shear thinning behaviour observed in rotational measurements (reduction in viscosity, η, with increasing shear rate, $\dot{\gamma}$) is also evident in the frequency-dependence of η^*. Indeed, the variation of log η^* with log ω is virtually identical to the dependence of log η on log $\dot{\gamma}$, both for entangled networks and for dilute solutions. This generality is known as the 'Cox–Merz rule'.

Susceptibility to shear thinning can be characterised (Morris 1990) by the shear rate needed to reduce viscosity to some fixed fraction of η_0 (e.g. $\dot{\gamma}_{½}$, the shear rate at which $\eta = \eta_0/2$). Plots of log η versus log $\dot{\gamma}$ for solutions of different polysaccharides, or different concentrations of the same polysaccharide, differ in maximum viscosity (η_0) and in the shear rate at which the reduction in viscosity begins (i.e. different values of $\dot{\gamma}_{½}$), but are identical in shape. Thus the shear-thinning behaviour of all entangled solutions of all disordered polysaccharides can be reduced to a single 'master curve' (Fig. 4.4) by expressing measured viscosi-

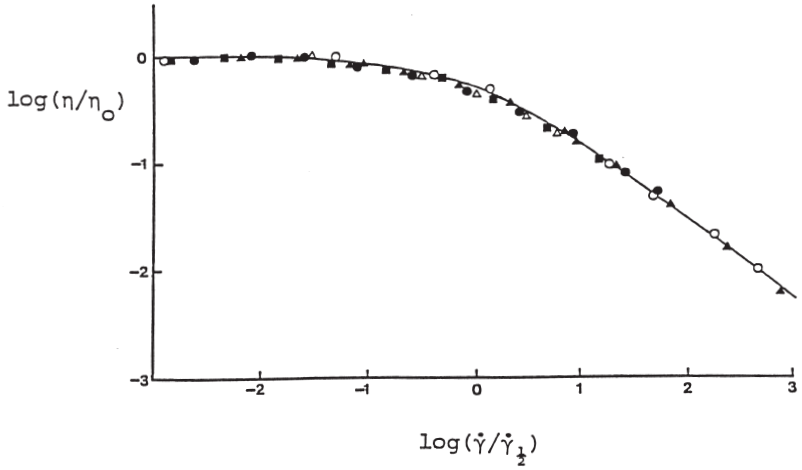

Fig. 4.4 'Master curve' for shear thinning of entangled polysaccharide coils. Adapted from Morris *et al*. (1981), with permission.

ties as a fraction of η_0 and similarly scaling applied shear rates to $\dot{\gamma}_{½}$ (i.e. by plotting log $\{\eta/\eta_0\}$ *versus* log $\{\dot{\gamma}/\dot{\gamma}_{½}\}$).

The concentration-dependence of η_0 for most disordered polysaccharides (Morris *et al*. 1981) can similarly be reduced to a single master plot (Fig. 4.5). To remove the direct contribution of solvent viscosity (η_s) to the overall viscosity of very dilute solutions, the values of η_0 are expressed as specific viscosity (η_{sp}), which is defined as $(\eta - \eta_s)/\eta_s$ (i.e. the fractional increase in viscosity due to the polymer). At higher concentrations, the correction becomes negligible, and specific viscosity (which is a dimensionless ratio) has virtually the same numerical values as absolute viscosity (η) expressed in mPas (water having a viscosity of 1 mPas).

Measurements of the specific viscosity of very dilute solutions of disordered polysaccharides (within the range $0.2 < \eta_{sp} < 1.0$) can also be used to characterise the extent of coil overlap in more concentrated solutions. To a first approximation, the fractional increase in viscosity per unit concentration of polymer (i.e. η_{sp}/c) is directly proportional to the volume occupied by the individual polymer coils. To eliminate the effect of interactions between different chains, however, the experimental values must be extrapolated to zero concentration. The resulting parameter is known as the intrinsic viscosity, $[\eta]$, and provides a direct index of coil volume. Since η_{sp} is dimensionless, intrinsic viscosity has units of reciprocal concentration (i.e. dl g^{-1} if concentration is expressed as % w/v). Thus the product of intrinsic viscosity (proportional to the volume occupied by each of the polymer coils) and concentration (proportional to the number of coils present) is dimensionless, and characterises total degree of space-occupancy by the polymer. The abrupt change in slope in Fig. 4.5 corresponds to the transition from a dilute solution to an entangled network, and occurs at $c[\eta] \approx 4$ and $\eta_{sp} \approx 10$.

Plots of log η_{sp} versus log c for individual polysaccharide samples are identical in form to the 'master curve' in Fig. 4.5, but are displaced from one another along the concentration axis, with the onset of coil overlap and entanglement occurring at progressively lower concentrations as the size of the individual polymer coils (characterised by $[\eta]$) increases. Intrinsic

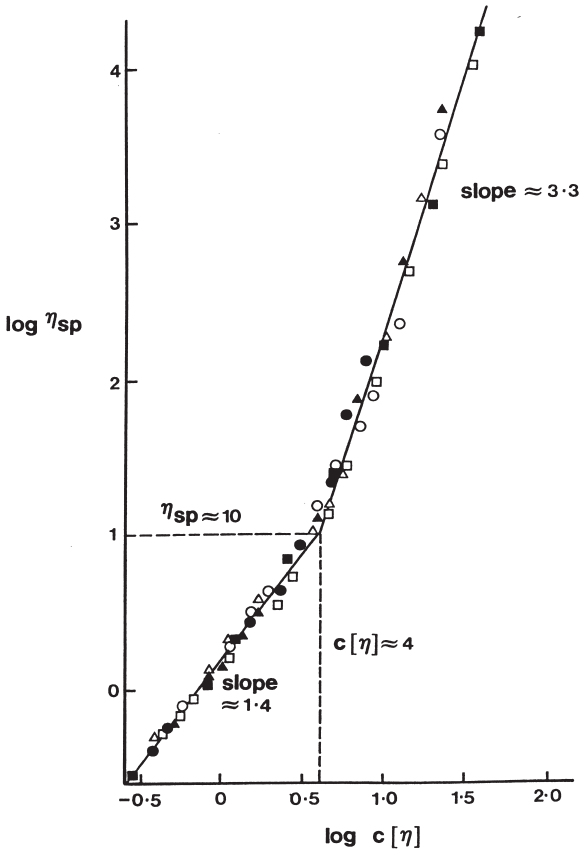

Fig. 4.5 Variation of maximum 'zero shear' viscosity with degree of space-occupancy for various disordered polysaccharides (shown by different symbols). From Morris *et al*. (1981), with permission.

viscosity is related to molecular weight (M) by the Mark–Houwink equation: $[\eta] = kM^{\alpha}$. For most polysaccharides, α is close to 1, so $[\eta]$ is roughly proportional to molecular weight. The scaling factor, k, depends on the geometry of the polysaccharide chain. Bonding arrangements that give ribbon-like ordered structures (Fig. 4.2a and b) also give extended coil dimensions in solution (i.e. high values of k). The systematic twist in chain direction that promotes formation of ordered helices (Fig. 4.3c) also promotes compact coil dimensions in the disordered form (i.e. low values of k).

In the dilute solution regime, where the chains are free to move independently through the solvent, log η_{sp} versus log c has a slope of ~1.4, so that doubling concentration increases viscosity by a factor of about 2.5. After the onset of coil overlap, where individual coils can move only by the much more difficult process of 'wriggling' (reptating) through the entangled network of neighbouring chains, the slope increases to ~3.3, which means that doubling concentration now increases viscosity by about a factor of 10. The maximum viscosity prior to entanglement is about 10 mPas. To place this value in context, it would be hard to distinguish such solutions from water by pouring or stirring them. Thus virtually all disordered

polysaccharide solutions of practical significance involve entangled coils, and are therefore extremely sensitive to small changes in concentration.

The viscosity generated by dispersions of solid particles is largely independent of their size, but is directly related to their phase volume, ϕ (i.e. the volume occupied by the particles, expressed as a fraction of the total volume). For dilute dispersions, viscosity is related to phase volume by: $\eta_{sp} \approx k\,\phi$ (Everett 1988). Since the density of food materials is usually close to that of water (1 g ml^{-1}), ϕ is approximately equal to c /100 , where c is the concentration of dispersed particles expressed as % w/v. Thus $[\eta] \approx \eta_{sp}/c \approx k/100$. The constant of proportionality, k, has a value of 2.5 for spherical particles, and increases to ~4 for extended rods. Dispersed particles therefore have intrinsic viscosities in the approximate range 0.025 to 0.04 dl g^{-1}. By comparison, the intrinsic viscosities of polysaccharides normally fall within the range 5–15 dl g^{-1}, and in some cases can be as high as 70–80 dl g^{-1} (Morris & Ross-Murphy 1981). It is evident, therefore, that in fluid digesta with any significant content of dissolved polysaccharides, these will dominate the overall viscosity.

The same is not, however, true at higher solids content (as in faeces). With decreasing water content, and thus increasing phase volume of particulate material, the viscosity generated by the particles increases very steeply as they approach close-packing. This is analogous to the sharp increase in the concentration-dependence of viscosity for disordered polysaccharides when the individual coils are forced into contact with each other (Fig. 4.5), but the rheological changes are much greater since, unlike polymer coils, solid particles cannot interpenetrate one another. At phase volumes near the close-packing limit, a small (1-2%) increase in the concentration of dispersed particles can lead to a massive change in mechanical spectra (D'Haene & Mewis 1995) from a form similar to that observed for entangled polysaccharide coils (Fig. 4.3b) to the predominantly solid-like response observed for cross-linked gels (Fig. 4.3a).

The behaviour of swollen, hydrated particles is broadly similar, but with the important distinction that they can go above the close-packing limit, by reduction in the size of the individual particles. There is again a massive increase in modulus (G') as the particles come into contact with one another (Evans & Lips 1990), but this is then followed by a more gradual increase at higher concentrations, as the particles are compressed to progressively smaller volume.

The general trend to be expected from the extreme sensitivity of entangled networks and particulate dispersions to comparatively small changes in concentration is a progressive increase in the viscosity and, ultimately, solid-like character of digesta as they pass along the gastrointestinal tract, due to absorption of water from the lumen. However, studies using a pig model (Roberts *et al*. 1990; Ellis *et al*. 1996) have shown a substantial increase in the total volume of digesta passing through the small intestine with increasing concentration and molecular weight of soluble polysaccharide in the test meals. The mechanism of this 'feedback' process is not yet fully understood, but it is clearly an important consideration in rationalizing the rheology and physiological effects of dietary fibre in the gut.

4.6 Overview

The dominant role of dietary fibre in the stomach and small intestine is to limit the rate of release of nutrients. One way in which this may occur is by physical trapping of sugars, lipids and proteins within solid particles or hydrated networks. Another mechanism is by dissolved

polysaccharides increasing the viscosity of the digesta and hence reducing the rate of transport to the epithelium. Both mechanisms centre on the physical properties of the fibre, rather than on its chemical composition.

As outlined in Section 4.2, association of polysaccharides into solid particles or the junction zones of hydrated networks depends on the way in which the constituent sugars are linked together, rather than on the types of sugar present. Different sugars linked in the same way can give materials with similar physical properties (e.g. pectin and alginate); conversely, the same sugar linked in different ways can give polysaccharides with entirely different properties (e.g. cellulose, curdlan, amylose and amylopectin, all of which are polymers of glucose). Similarly, the rheology of polysaccharide solutions and dispersions (Section 4.5) is critically dependent on the extent of space-occupancy (which is determined by the volume occupied by the individual coils or particles and their concentration), but has no direct relationship to chemical composition.

Susceptibility to fermentation in the colon is, of course, dependent on chemical structure, but even here the physical state of the fibre is an important consideration, since ordered packing can inhibit or abolish enzymatic degradation.

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5 The Structures and Architectures of Plant Cell Walls Define Dietary Fibre Composition and the Textures of Foods

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5.1 Introduction

As research advances in nutrition and health sciences, we become ever more aware of the unique role of plant cell walls as dietary fibres. Most of our dietary fibre comes from the cell walls of fruits, vegetables, cereal products and seeds. Food scientists today find new ways to improve the nutritional benefits without compromising the enjoyment factors that reside in the variety of tastes, flavours, aromas and textures of fruits and vegetables. The diverse cell walls of these plants are the main contributors to texture.

The primary cell wall is a complex network of cellulose and cross-linking glycans embedded in a matrix of pectic substances and reinforced with structural proteins and aromatic substances (McCann & Roberts 1991; Carpita & Gibeaut 1993). Almost all of the different plant cell types can be identified by unique chemical and structural features of their cell walls. On the other hand, the walls of many fruits and vegetables have similar polymer compositions, but the polymers are assembled into such different architectures that they result in completely different textures.

For many years, traditional methods of chemical analysis of cell walls have provided information on bulk cell wall composition and architecture. Unfortunately, such methods do not yield information on individual cells of plant tissues that may impact the properties of dietary fibres (McCann *et al.* 1995). Such complexity of polysaccharide structure can be resolved using highly specific probes. This has been achieved using the natural specificity of enzymes for their polymeric substrates or antibodies against particular carbohydrate epitopes. The advent of microspectroscopies, in which a microscope with modified optics is attached to a spectrometer, has made chemical imaging possible. Hence, the distribution of functional groups of molecules can be mapped at the single-cell level (McCann *et al.* 1997). This chapter addresses the diversity in structural and chemical composition of the plant cell wall, and how these different architectures might influence the taste and texture of dietary fibres. In this era of genetically modified crops we have the capacity to identify the genes responsible for cell wall diversity and to use these genes as targets for genetic manipulations resulting in future 'designer dietary fibres'.

5.2 Cell-wall carbohydrates

Cellulose is the most abundant plant polysaccharide on earth, accounting for 15–30% of the dry mass of all primary cell walls. In secondary walls, cellulose may even account for over one-half of the mass (McNeil *et al.* 1984). Cellulose is a linear polymer composed of

(1→4)β-D-glucan (for carbohydrate nomenclature, see Appendix). About three dozen linear chains associate via hydrogen bond or steric interactions to form para-crystalline microfibrils (Fig. 5.1). The β-glucan chains are arranged parallel to one another. Although each chain may be only several thousand units long, they begin and end at different places so that microfibrils are very long and able to spool around each cell (Delmer 1999).

As seen in the micrograph of an onion wall, fine thread-like glycans tether the cellulose microfibrils into a very strong network (McCann *et al.* 1990). These threads are fucogalacto-**xyloglucans** (**XyGs**), which, like cellulose, have linear backbones of (1→4)-β-linked D-glucose. Numerous xylosyl units are attached along these backbones at regular sites (Fig. 5.2a). Using sequence-dependent hydrolases it is possible to obtain diagnostic oligosaccharides from the XyGs, which can be fully characterised and whose structures may vary among species. In most plants the fundamental structure of XyG is composed of a heptasaccharide repeating unit, a cellotetraose with three subtending xylose residues, which may bear additional galactosyl and fucosyl units on some of the xyloses. Many galactoses on the side chains, and even some of the glucosyl units on the backbone, are acetylated (York *et al.* 1988).

A few plants, instead of fucogalacto-XyGs have arabino-XyGs, with α-L-arabinosyl units attached to the xylose residues (Fig. 5.2b). Only two of every four glucosyl units contain a xylose residue, and an acetyl group replaces the third xylose (Sims *et al.* 1996). Cell walls from some cereal species have a small amount of galacto-XyG, with two xyloses for every four glucosyl units and a few galactosyl units attached to xylose residues (Bacic *et al.* 1988). In several species, the cotyledon and endosperm cell walls have galacto-XyGs with more side-chain substitutions (Gidley *et al.* 1991).

Only one surface of the XyG backbone is able to bind to a microfibril. One hypothesis postulates that the trisaccharide side chain attached to alternate heptasaccharide units in the XyG is necessary for the binding to cellulose. Alternating side chains straighten the backbone (Levy *et al.* 1991), and this conformation may facilitate the close packing of XyG to cellulose. In some cases, α-L-arabinose is attached at a position that interferes with cellulose binding (Levy *et al.* 1997; Bacic *et al*. 1988). Therefore, XyGs occur in at least two domains in the wall. In one, they align with and bind tightly to cellulose microfibrils, and in the other, they span the distance between the microfibrils to tether microfibril neighbours (Fig. 5.1). There is evidence developing that the fine structure of XyGs in these domains is quite different (Pauly *et al.* 1999).

Glucuronoarabinoxylans (**GAXs**) are linear chains of (1→4)-β-D-xylan with single units of α-L-arabinose attached along the xylose backbone and, less frequently, single glucuronic acid residues (Fig. 5.3a, b). The amount of arabinose and glucuronic acid substitutions varies markedly, ranging from GAXs with substitutions at almost all xyloses to GAXs with only 10% or less of the xyloses bearing side groups (Carpita & Gibeaut 1993).

Other non-cellulosic polysaccharides, such as **mannans** (Fig. 5.4a), **gluco-** and **galactoglucomannans** (Fig. 5.4b), and **galactomannans** (Fig. 5.4c), also interlock the microfibrils in cell walls, but are found in much lower amounts (Bacic *et al.* 1988). However, cell walls of seeds are specially enriched in these polysaccharides. Mannans have a backbone composed of linear (1→4)-β-D-mannose chains. Glucomannans are also linear polymers of both (1→4)-β-D-glucose and (1→4)-β-D-mannose runs with a ratio that varies somewhat between species. Acetyl esters are usually present on both the glucose and mannose residues (Meier & Reid 1982). In some species single galactosyl units are present as side chains of the mannosyl units, and they are called galactoglucomannans. Galactomannans have the same

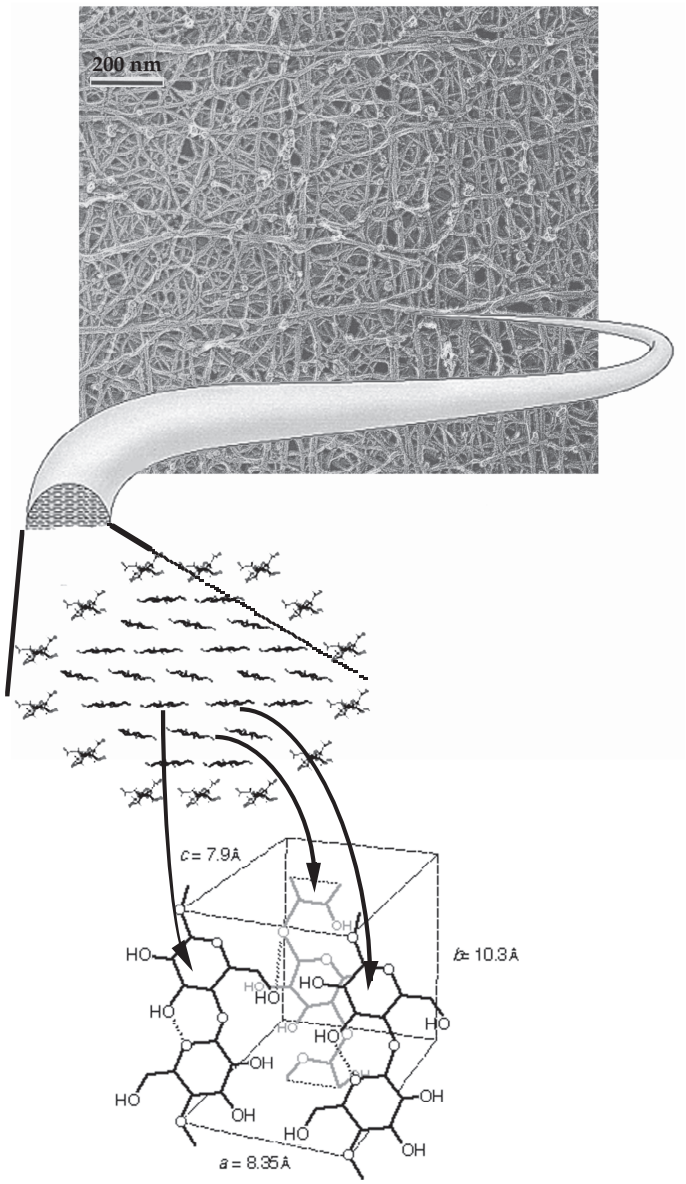


Fig. 5.1 A fast-freeze, deep-etch, rotary-shadowed replica technique is used to image cell-wall architecture as close as possible to that expected *in vivo*. Pectins have been extracted from this onion parenchyma cell wall to expose the cellulose-XyG network. An individual cellulose microfibril is a para-crystalline array of several dozen (1→4)β-D-glucan chains that are tightly associated to each other, both side-to-side and top-to-bottom. The arrangement of the glucan chains in a cross-section of a single microfibril, and the arrangement of atoms in the unit structure of the microfibril core, are shown. The glucan chains in the core of the microfibril have a precise spacing, as determined by X-ray diffraction. From studies involving solid-state NMR spectroscopy, glucan chains at the surface of the microfibril are thought to adopt a slightly different alignment from 180° to accommodate binding to the XyGs. (Micrograph courtesy of M. McCann, John Innes Centre; diagram of microfibril cross-sectional structure courtesy of M. Jarvis; diagram of X-ray crystal structure from R. J. Preston (1974) *Physical Biology of the Plant Cell Wall*, Chapman & Hall, London.)

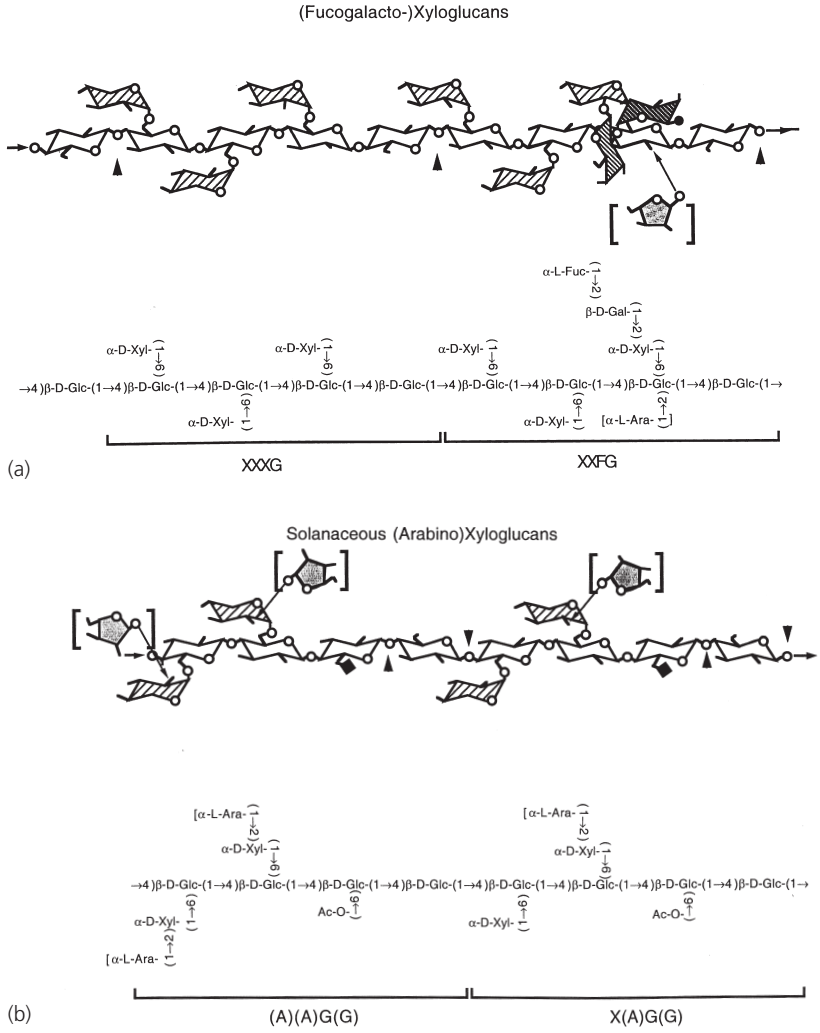


Fig. 5.2 (a) Fucogalacto-xyloglucans. In most XyGs, the α-D-xylosyl units are added to three contiguous glucosyl units of the backbone to produce a heptasaccharide unit structure (for a review of nomenclature, see Appendix). On about one-half of these unit structures, an α-L-fucosyl-(1→2)β-D-galactose- is added to the O-2 of the xylosyl side group nearest to the reducing end, forming a nonasaccharide unit. Attachment of an α-L-arabinosyl unit to the O-2 of backbone glucose units bearing the trisaccharide side groups blocks hydrogen bonding of the XyG to cellulose at these regions. The arrowheads denote the only linkages able to be cleaved by the *Trichoderma* endo-β-D-glucanase to yield the diagnostic oligomers. A single letter designator convention has been adopted, which is based on the last sugar in the side-group (Fry *et al.* 1993). G = a glucose remaining unbranched in the backbone, L = galactose, F = fucose, A = arabinose. The two oligomers depicted here are XXXG and XXFG, where F implies that the entire side chain is α-L-fucosyl-(1→2)β-D-galactosyl-(1→2)α-D-xylosyl-(1→6). (b) Arabino-xyloglucans. In the Solanales and Lamiales, the major repeating unit is a hexamer, rather than a heptamer, with one or two α-L-arabinosyl units added directly to the O-2 position of the xylose residues. The Solanaceae XyG units are separated by two unbranched glucosyl units rather than one, and the penultimate glucose contains an acetyl group at the O-6 position. The arrowheads denote the linkages able to be cleaved by the *Trichoderma* endo-β-D-glucanase. Using the single letter designator convention, these two oligomers are AAG(G) or XAG(G), if the arabinosyl units are attached, or both XXG(G) if they are not attached.

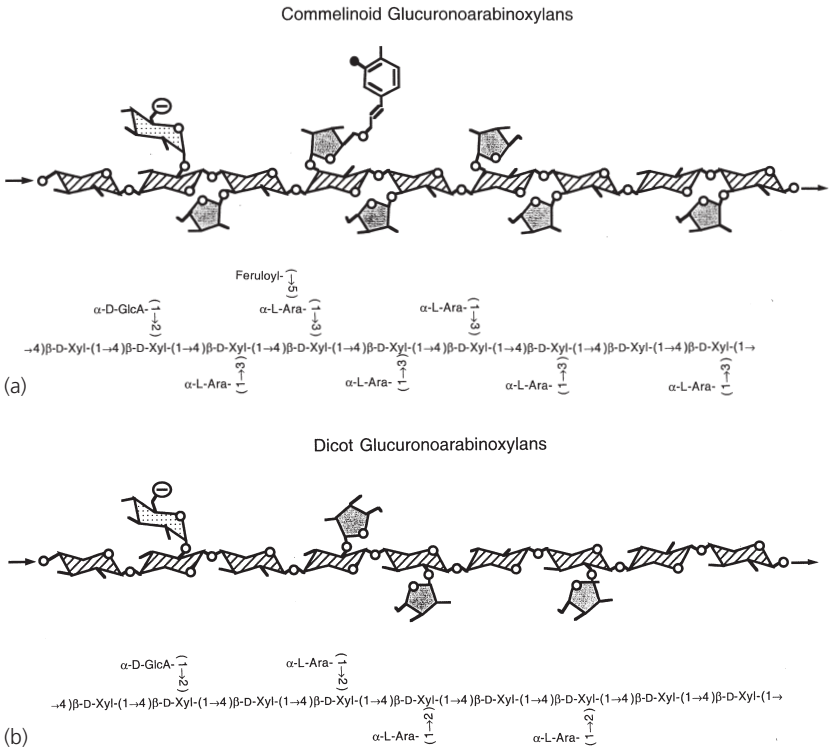


Fig. 5.3 (a) Commelinoid glucuronoarabinoxylan (GAX). In GAXs from the walls of the commelinoid line of monocots, which includes the cereals, the α-L-arabinosyl units are added strictly to the O-3 position of the xylosyl units of the backbone polymer. Feruloyl groups and other hydroxycinnamic acids are esterified to the O-5 position of the α-L-arabinose units and are spaced about 50 xylose residues apart in the backbone. The α-D-glucuronic acids are added to the O-2 position of the xylosyl units. (b) Non-commelinoid Glucuronoarabinoxylans. Non-commelinoid monocots and all dicots also contain GAX in addition to the more abundant XyG. However, the α-L-arabinosyl units of these GAXs are attached primarily to the O-2 position of the xylose residues, although they may be attached to some of the O-3 positions as well. Like the commelinoid GAX, the α-D-glucuronic acid units are attached only at the O-2 position.

linear mannose backbone with galactose side chains in different amounts. The variable degree of substitution of these mannan backbones gives a family of polymers with different solution properties (Dea *et al*. 1986; Reid & Edwards 1995).

Callose is a polymer of (1→3)-β-D-glucan, which is found at specific stages of wall development, such as growing pollen tubes and in cell plates formed during cytokinesis. Callose may also accumulate in response to wounding or pathogen attack.

Mixed-linkage (1→3),(1→4)-β-glucans are present only in the grass species and cereals (Fig. 5.5). The mixed linkage glucan is an unbranched polymer containing predominantly (1→4)-β-D-glucosyl linkages, and these cellodextrins are connected by single (1→3)-β-D-glucosyl linkages, which introduce 'kinks' in the unbranched polymer (Wood *et al.* 1994). In the sequence structure from oat or barley endosperm, over 90% of the polysaccharide is composed of cellotriosyl and cellotetraosyl residues in a 2–3 : 1 ratio. The distribution of these residues in the molecule is not known. There are also longer blocks of up to 10 contigu-

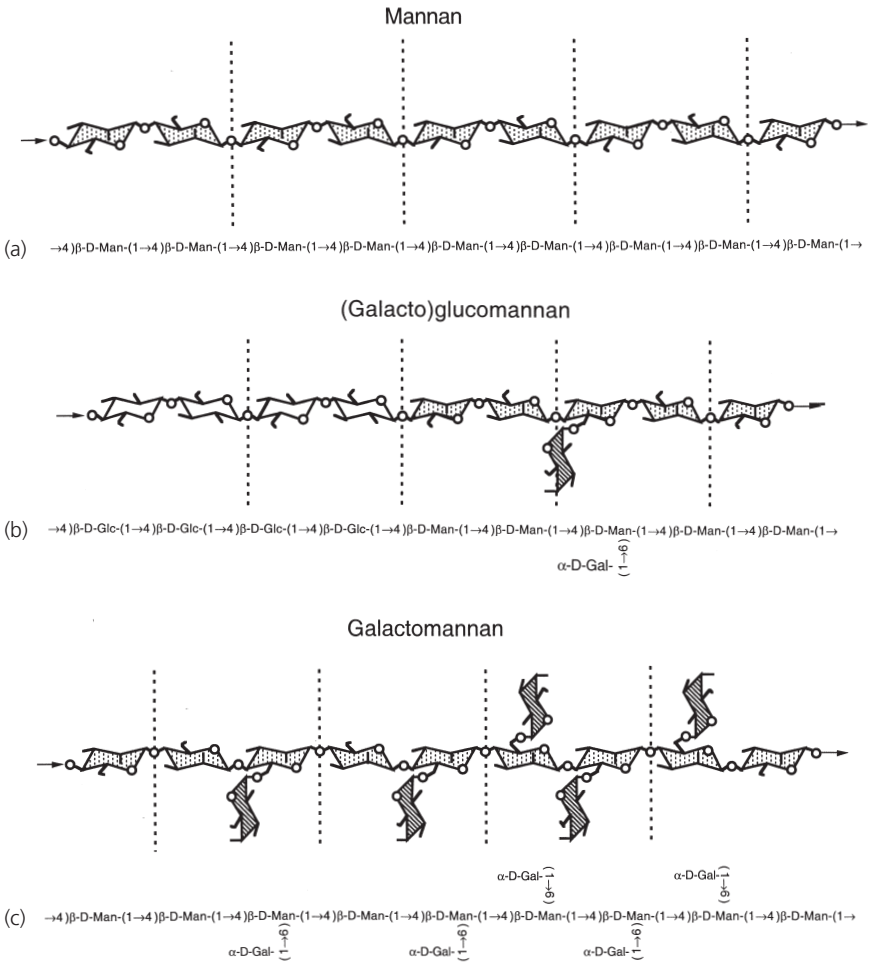


Fig. 5.4 (a) Unbranched mannans are able to hydrogen bond into para-crystalline arrays similar in structure to cellulose. (b) Gluco- and (galacto)glucomannans are roughly 1:1 mixtures of (1→4)β-D-mannosyl and (1→4)β-D-glucosyl units, with small amounts of terminal α-D-galactosyl units added to the O-6 position of the mannose residues. (c) Galactomannans have backbones composed exclusively of (1→4)β-D-mannan, and generally contain substantially more α-D-galactosyl units subtending the O-6 positions.

ous (1→4)-β-linkages in a small proportion distributed throughout the length of the polymer (Staudte *et al.* 1983).

Pectic polymers are major components of most higher plant cell walls. The major constituents of pectins are **homogalacturonan** (**HGA**) and **rhamnogalacturonan I** (**RG I**). The HGAs are helical homopolymers of (1→4)-α-D-galacturonic acid (Fig. 5.6a). The carboxylic acid group may be methyl esterified, and the degree of esterification is an important architectural factor. **Xylogalacturonan** and **rhamnogalacturonan II** (**RG II**) are two other pectin polymers with HGA-like backbones. The xylogalacturonan has xylosyl units attached to about one-half of the backbone residues (Fig. 5.6b). This polymer is enriched in floral and reproductive tissues, particularly fruits (Schols *et al.* 1995). The RG II molecules have back-

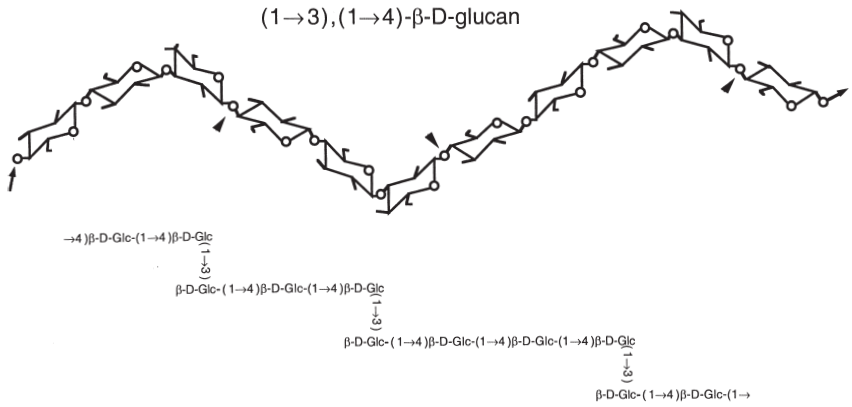


Fig. 5.5 The mixed-linkage (1→3),(1→4)β-D-glucan unique to the Poales. The long arrows along the polymer indicate cleavage sites by the *Bacillus subtilis* endoglucanase. This enzyme has the unique property to cleave a (1→4)β-D-glucosyl linkage, but only if it is preceded by a (1→3)β-D-glucose residue (Wood *et al.* 1994). Like the *Trichoderma* endoglucanase-released XyG oligomers, these cellodextrin-(1→3)β-D-glucose oligomers of β-glucan are diagnostic of the polymer and used for absolute quantitation. The linear cellodextrin oligomers are connected by single (1→3)β-linkages that introduce bends in the chain, and iteration of this pattern produces a corkscrew-shaped polymer.

bones with extremely complex side chains, not only in sugar content but in linkage structure (Fig. 5.6c). This complex structure is highly conserved among flowering plants. Most of the element boron – an essential nutrient in plants – is tied up in di-diester linkages between apiosyl units in the complex side chains to form dimers of RG II (Fig. 5.6d) (Ishii & Matsunaga 1996; O'Neill *et al.* 1996).

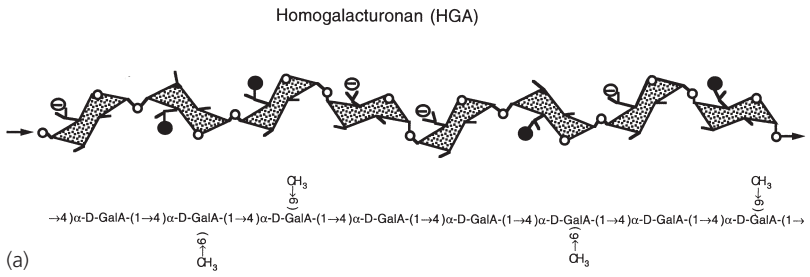
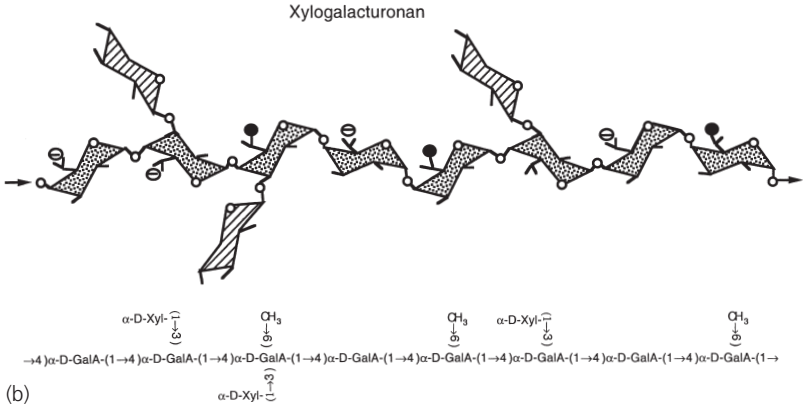


Fig. 5.6 The pectic polysaccharides of plants. (a) Highly methyl esterified chains of (1→4)α-D-galacturonic acid (HGA) are secreted by the plant cell and are de-esterified to varying degrees in certain wall domains by pectin methyl esterases. The methyl groups are denoted by filled circles, whereas the free acids are encircled negative charges. (b) Xylogalacturonans are a separate class of substituted HGAs, with appendant α-D-xylosyl units at the O-3 position of about one-half of the galacturonic acid units. (c) Rhamnogalacturonan (RG) II contains an HGA backbone with four distinct side groups containing several different kinds of sugars and sugar linkages found only in this polymer. They include apiose, aceric acid (3-C′-carboxy-5-deoxy-L-xylose), 2-O-methyl fucose, 2-O-methyl xylose, Kdo (3-deoxy-D-*manno*-2-octulosonic acid), and Dha (3-deoxy-D-*lyxo*-2-heptulosaric acid). (d) RG II monomers of about 4200 Da can dimerise as boron di-diesters of the apiose residues. The central boron residue is shown circled. Filled circles are methyl groups and the filled squares denote acetyl groups. (*Continued.*)



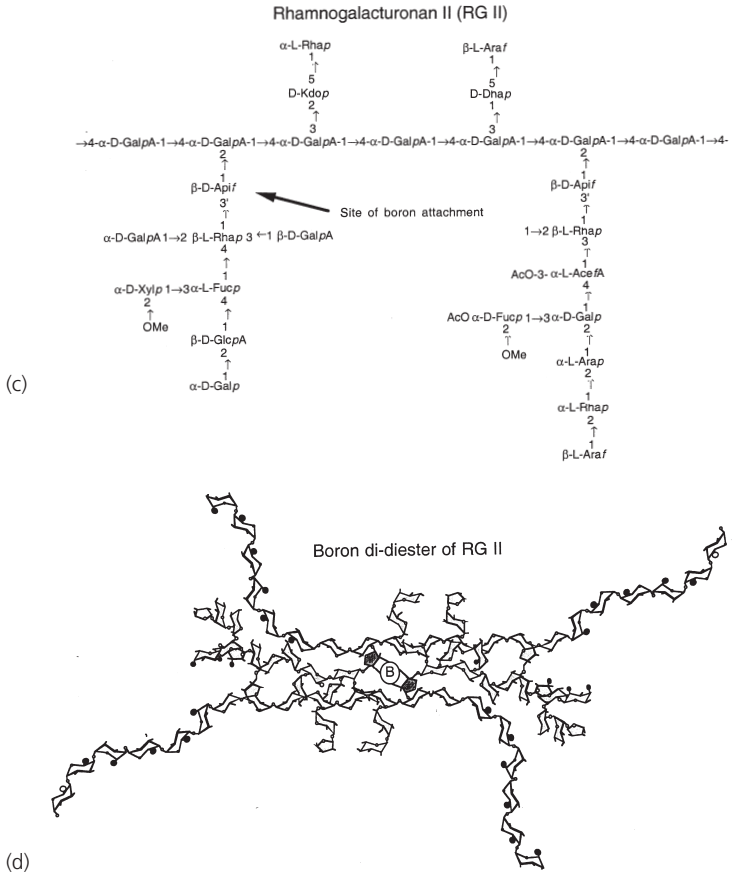


Fig. 5.6 (*Continued.*)

RG I is a contorted rod-like heteropolymer of repeating (1→2)-α-L-rhamnosyl-(1→4)-α-D-galacturonic acid disaccharides (Lau *et al.* 1985). About one-half of the rhamnose residues are substituted with side groups. These substitutions comprise **arabinans**, **galactans** and

highly branched **type I arabinogalactans** (**AGs**) (Fig. 5.7). The arabinose residues can attach to one another in several ways to form a diverse group of branched polymers. Type I AGs are composed of (1→4)-β-D-galactan chains with mostly arabinose residues, and are found only associated to pectins. RG I is a block polymer composed of *smooth* regions, with few side-group substitutions, and *hairy* regions, with substantial substitutions (Mutter *et al.* 1998). The length of RG I is unknown because it can be associated with HGA at the ends (Jarvis 1984).

5.3 Structural proteins

Cell walls are mainly composed of carbohydrates, but some structural proteins are present. They may form a scaffold around the carbohydrate matrix and contribute to the variety of textures. There are four major classes of structural proteins, with three of them named for their uniquely enriched amino acids: the **hydroxyproline-rich proteins** (HRGPs), the **proline-rich proteins** (PRPs), the **glycine-rich proteins** (GRPs), and the fourth class, a proteoglycan, **arabinogalactan-proteins** (AGPs).

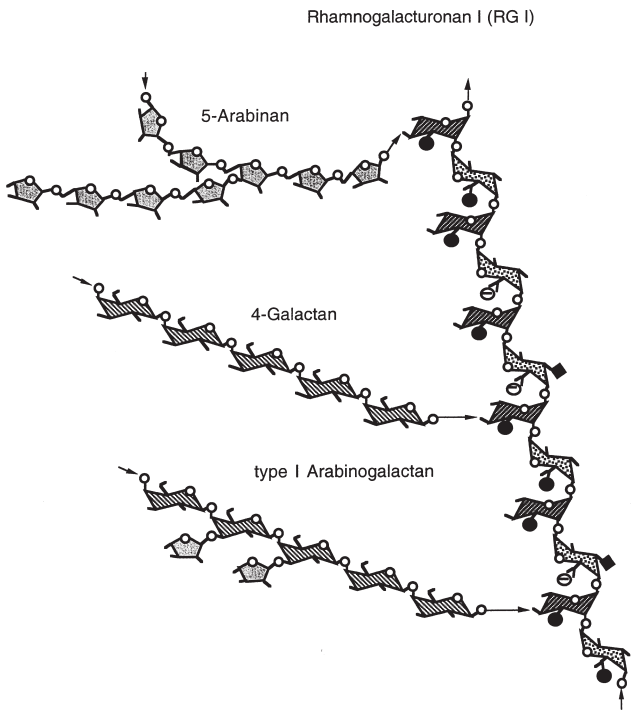


Fig. 5.7 Contorted, rod-like RG I is composed of a repeating →2)α-D-rhamnosyl-(1→4)α-D-galacturonic acid-(1→ disaccharide. About one-third of the galacturonic acid units are acetylated at secondary alcohol groups (denoted by filled squares). Rhamnose is a deoxysugar, with the methyl group at the O-6 position denoted by a filled circle. Three types of side groups attach to about one-half of the rhamnose units of RG I: the (1→5)α-L-arabinans, the unbranched (1→4)β-D-galactans, and the type I arabinogalactans, which contain α-L-arabinosyl units at the O-3 position along the 4-linked galactan backbone.

The genes encoding these proteins are tightly regulated, and their expression patterns show cell-type specificity and developmental regulation (Cassab 1998). Extensin is one of the best-studied HRGPs of plants. It is a rod-shaped protein consisting of repeating hydroxylated amino acid sequences to which arabinosides, each one to four sugars long, are added (Kieliszewski & Lamport 1994). The structure of PRPs is unknown, but their similarity to extensin suggests they are also rod-shaped. GRPs, some of which contain more than 70% glycine, are predicted to be β-pleated sheets rather than rod-shaped molecules. The cell-wall face of the pleated sheet contains a linear arrangement of aromatic amino acids that may function in cross-linking this protein into the wall.

The AGPs are more aptly named proteoglycans because they can consist of more than 95% carbohydrate. The carbohydrate portion, called type II arabinogalactans, constitute a group of short (1→3)- and (1→6)-β-D-galactan chains connected to each other by (1→3,1→6) branch-point residues, with arabinose added to most of the remaining available O-3 and O-6 positions of the galactans (Schultz *et al.* 1998). AGPs constitute a broad class of molecules that are concentrated in the pectic fraction of the cell wall. Some of these proteoglycans may have architectural roles, e.g. for wall assembly, or by directly binding polymers together, or more subtle biological roles as signalling molecules (Schultz *et al.* 1998).

5.4 Aromatic and other substances

Lignins, present in some secondary cell walls, are complex networks of aromatic compounds. They are composed mainly of the monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols, and linked together by ester, ether or carbon-carbon bonds. The diversity of monolignols and their possible inter-molecular linkages give a remarkably complex structure. The aromatic network also tightly associates with cellulose and other polysaccharides in the cell wall (Campbell & Sederoff 1996).

In some unlignified cell walls, other aromatic compounds may be present, such as ferulic and *p*-coumaric acid. They are attached via ester linkages to the arabinose or galactose residues of some wall polysaccharides. Ferulic acids may form several kinds of dimers, which could form cross-links between polysaccharides within the wall and between cells (Ralph *et al.* 1994).

Suberin is a material found in specific tissues and cell types, like the cork cells of the cork-oak. The core of suberin is lignin-like, with ester-linked fatty acids attached to it (Bernards *et al.* 1995). **Cutin** is the main substance present in the cuticle, which is a layer that consists of cutin embedded in wax. It is localised to the outside surface of the epidermis in some species. Cutin is a complex mixture of fatty acids and fatty esters. The fatty acids are mostly hydroxylated C_{16} and C_{18} straight-chain saturated molecules, and they are often extensively esterified to each other to form a cross-linked network (Kolattakudy *et al*. 1981).

Silica is particularly abundant in grass species, mostly in inclusion bodies in epidermal and other specialised cells (Carpita 1996). Other species form **calcium oxalate crystals** (Webb *et al*. 1995).

5.5 Dynamics in cell-wall architecture

Elongation and differentiation of individual plant cells is achieved by alteration of the struc-

ture of the wall. These alterations are a direct response to developmental cues within each cell. Hence, cell-wall architecture has to be considered as distinct for dividing cells, for elongating cells, and for differentiating cells. A view of the major cell-wall polysaccharides found in higher plants reveals at least two distinct types of walls. Within each type there may be subclasses unique to a particular order (Fig. 5.8).

The **type I** cell wall, which typifies most flowering plants, is a network of cellulose microfibrils interlocked with fucogalacto-XyGs (Plate 5.1). This strong framework is embedded in a dense matrix of pectic polysaccharides and reinforced by a separate network of structural proteins. The cellulose/XyG framework accounts for about 50% of the wall mass. With the development of new techniques used for electron microscopy, it is now possible to make *in-situ* observations of this cellulose/XyG network (see Fig. 5.1)**.**

The cellulose/XyG network is embedded in a pectin matrix, which comprises some of the most complex polymers known in plants. The pectin network participates in cell–cell adhesion in the middle lamella. It determines wall porosity and provides charged surfaces that modulate wall pH and ion balance (Jarvis 1984). The helical chains of HGAs can condense by cross-linking with Ca^{2+} to form 'junction zones,' linking two anti-parallel chains. Maximally strong junctions occur between two chains of at least seven unesterified galacturonic acid units each. The methyl group eliminates the negative charge associated with the galacturonic

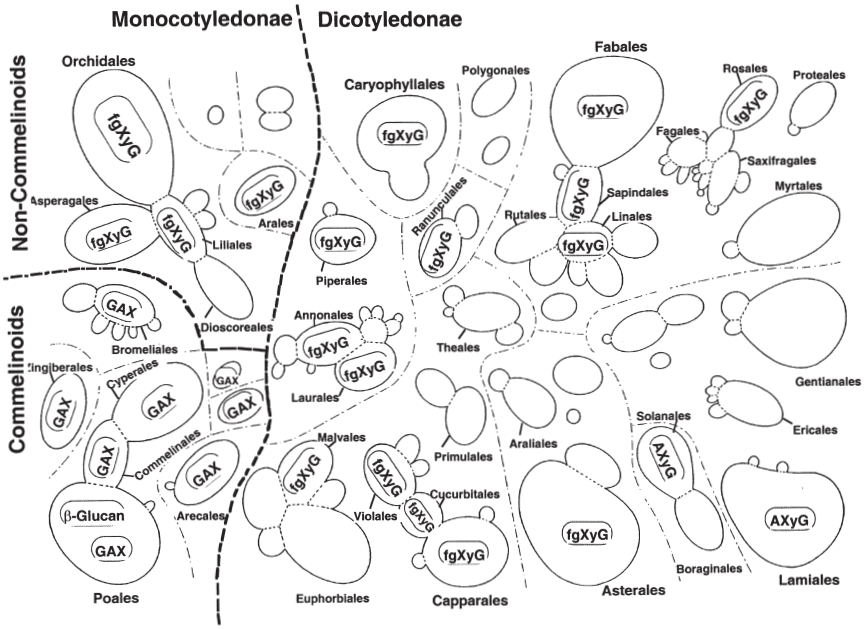


Fig. 5.8 Orders of the flowering plants with symbolic descriptions of the major cross-linking glycans and major distinctions illustrated between grasses, commelinoid and non-commelinoid monocots, and between Solanales and Lamiales and the other dicot orders. fgXyG = fucogalacto-xyloglucan and AXyG = arabinoxyloglucan (see Fig. 5.2a,b), GAX = glucuronoarabinoxylan (see Fig. 5.3a), and β-glucan = (1→3),(1→4)β-D-glucan (see Fig. 5.5). In addition, the fgXyG- and AXyG- containing walls are enriched in pectic substances, whereas the GAX-containing walls are not. Cell-wall structure in the vast majority of angiosperm species has never been determined. [The relationship of the orders is adapted from G. Dahlgren (1989) *Botanical Journal of the Linnaen Society*, **100**:197.]

acid, and thus prevents Ca^{2+} bridging (Jarvis 1984). Provided that sufficient Ca^{2+} is present, some methyl esters can be tolerated in the junction, and the HGAs can bind in both parallel and anti-parallel orientations (Selvendran 1984). The spacing of the junctions is postulated to create a cell-specific pore-size. The rhamnosyl units of RG I and their side chains interrupt the Ca^{2+} junctions and contribute to the pore definition. Another contributing determinant of wall porosity is the extent of boron cross-linking of RG II monomers (Fleischer *et al.* 1998).

HGAs are thought to be secreted to the primary cell wall as highly esterified polymers. The deposition of highly esterified HGA into the wall is followed by a de-esterification to remove methyl groups and initiate the binding with Ca^{2+}. The cell walls of meristematic and elongation zones are characteristically low in Ca^{2+}, and Ca^{2+}-HGA junction zones are observed more frequently after cell elongation has stopped. The middle lamella is formed by unesterified pectins with Ca^{2+} junction zones associated with the cell–cell adhesion. Some HGA and RG I are cross-linked by ester linkages to pectin or other polymers held more tightly in the wall matrix (McCann *et al.* 1994). Some type I walls contain large amounts of proteins, which may bind to one another by intermolecular bridges and form a network, with or without association with other networks in the wall.

The cell-wall architecture changes markedly during cell expansion. Two integrated processes are needed: cell-wall loosening to permit microfibril separation, and insertion of new material to maintain constant wall thickness. Two kinds of activities are being evaluated as possible wall-loosening enzymes, and both affect the cellulose/XyG network. One is **xyloglucan endotransglycosylase** (XET) (Nishitani 1997), and the other is **expansin** (Cosgrove 1997). The latter protein probably catalyses breakage of hydrogen bonds between cellulose and XyGs. XETs and expansins may not be the only wall-loosening agents, and work continues to determine the roles played by additional hydrolases. While plant biologists are concerned about the role of these proteins in growth physiology, as will be discussed later, these proteins are also investigated heavily for their involvement in fruit ripening.

The **type II** cell wall of grasses and cereals provide a different kind of dietary fibre. The chemical structure of these walls differs from those of all other flowering plants, and are found exclusively in the commelinoid line of monocotyledonous plants (Fig. 5.8). The type II cell wall is composed of cellulose microfibrils cross-linked by glucuronoarabinoxylans (GAXs) instead of fucogalacto-xyloglucans (XyGs) (Plate 5.2). The degree of branching with arabinose residues is the major determinant of the ability of GAXs to bind to one another or to cellulose.

Because the arabinosyl units increase xylan's solubility in water and interfere with hydrogen bonding, the GAXs, like XyG, may form two distinct domains within the wall. One domain is the HS-GAX (highly substituted-GAX)-rich domain that is structurally continuous with the pectic matrix, and the other is the domain formed by relatively unbranched GAXs that bind tightly to cellulose (Carpita 1984). In dividing and elongating cells, HS-GAXs are abundant, but after elongation and differentiation, more unbranched GAX accumulates. The HS-GAX, with six of seven xylosyl units bearing appendant groups, is most likely the form that is synthesised and secreted by the cells. Type II walls contain small amounts of XyG bound to cellulose (Sims *et al.* 2000), but the unit structure of this polymer is different from that in type I walls. The pectin content in these type of walls is low. Chemically, the pectins of the type II walls are composed of HGA and RG, but HS-GAX is also associated with these pectins (Carpita 1989).

The GAXs are cross-linked by aromatic substances, such as esterified and etherified hydroxycinnamate residues attached to the GAX units, and form an extended polyphenolic

network at maturity (Scalbert *et al.* 1985). The accumulation of phenolic esters and ethers is important for locking the cells into their final shape and in providing strength. These aromatic compounds give the commelinoids a property that sets them apart from the other plants. They fluoresce in UV light because of the accumulation of ferulic acid and *p*-coumaric acid.

Another remarkable characteristic of the type II cell wall of Poales is that it changes its constituents during cell expansion. Some arabinans found during cell division are no longer found during cell growth (Carpita 1984). β-Glucans that contribute to interlocking of the cellulose microfibrils are synthesised along with the GAXs. The β-glucan is also enriched in the walls of grain endosperm. Its abundance is correlated with growth, as it accumulates during cell elongation, and disappears when growth is completed (Carpita 1996).

The β-glucan is substrate for two glucanohydrolases located in the expanding wall. In addition to XETs and expansins, other enzymes implicated in wall extension and growth are those involved in pectin assembly, turnover of AGPs, assembly of GAX, and the breaking of hydrogen bonds between cross-linking glycans (Carpita 1996).

5.6 Texture of fruits and vegetables

There is no better everyday illustration for how cell-wall polymer structure and dynamic architecture define the character of dietary fibre than that observed in the ripening textures of fruits and vegetables. Most fruits that soften during ripening develop swollen primary walls that are greatly enriched in pectic substances. There are fruit-specific choices for arabinan, galactan, arabinogalactan side chains of the RG I polymers (Redgwell *et al.* 1997a).

The texture of the ripe fruit pulp is governed by the extent of wall degradation and the loss of cell–cell adhesion. For example, the walls of the apple cortex undergo little change in rigidity, and exhibit little separation, whereas the walls of the peach and tomato soften considerably through wall swelling and loss of cell adhesion. Wall softening is correlated with wall swelling. Redgwell *et al.* (1997b) showed that certain fruit walls swell *in vivo* and *in vitro* 3- to 10-fold greater than do walls from unripe fruit. This enormous volume change occurs without significant pectin depolymerisation and without correlation with the galactose or arabinose loss that occurs during the ripening. Swelling of cell walls may be associated with covalent bonds, because only when these linkages are broken does the swelling increase, and this has been observed in *in-vitro* experiments (Redgwell *et al.* 1997b).

During cooking, canning and other food processing treatments, fruits and vegetables soften markedly. This is due in part to the degradation of pectic polysaccharides involved in cell adhesion. Excessive cell separation results in over-softening in cooked vegetables (Waldron *et al.* 1997a). Inappropriate fruit storage can lead to development of mealiness, extensive loss of cell–cell adhesion, and over-softening.

The softening process in tomato is associated with the enzymatic loss of methyl esters of HGA. The de-esterified HGA backbone is then susceptible to the activity of pectin-degrading enzymes. However, despite extensive de-esterification and depolymerisation of the pectin polymers during ripening, fruit softening does not appear to result directly from these modifications to the pectic network. Transgenic tomatoes engineered to eliminate pectic depolymerisation exhibited no reduction in softening (Smith *et al.* 1988). In addition to glycan hydrolases, attention has been directed to the role that expansins and XETs play in maturation and ripening. Rose *et al.* (1997) discovered members of the expansin family of genes whose expression is restricted to maturation stages, when cell expansion occurs, and others whose

expression is restricted to ripening stages, when the walls begin to swell and soften. However, a pectate lyase is implicated in the softening of bananas (Dominguez-Puigjaner *et al.* 1997), and expression of a cellulase in tomatoes is correlated with softening (Gonzalez-Bosch *et al.* 1996).

Softening of the pericarp tissue may occur in a cell- and wall-domain-specific manner. Recently Steele *et al.* (1997) found that de-esterification occurs in distinct block-like domains that are spatially restricted. Of course, the impact of all of these developmental and structural modifications on the beneficial properties of pectins as dietary fibres has not been investigated systematically.

The special properties of dietary fibres not only reside in the polymer structure but in the way they are cross-linked into a special architecture. For example, Chinese water chestnuts (*Eleocharis dulcis*, Cyperaceae) and the mature storage organs from sugarbeet (*Beta vulgaris*, Chenopodiaceae) remain crisp after cooking. The water chestnut is a commelinoid with type II cell walls (Parr *et al.* 1996), whereas sugarbeet is a dicot with a type I wall (Waldron *et al.* 1997b). Despite their varied constituents, cross-linking of polysaccharides by phenolic dimers, such as diferulic acid, are thought to be responsible for the thermal stability of their texture (Waldron *et al.* 1997a, b). This is a good example of how the interactions between polymers represent yet another level of complexity affecting the properties of dietary fibres.

5.7 The special secondary walls of seeds

Another site of diversity among plant species is in the special storage secondary walls of the cotyledon and endosperm of developing seeds. These walls contain little or no cellulose, but rather consist of a single non-cellulosic polysaccharide typically found in the primary wall. These secondary walls serve two functions. First, they provide a thick wall to protect the embryo or to impose mechanical dormancy. Second, they contain specialised storage carbohydrates that are digested during germination and converted to sucrose for transport to the growing seedling.

The cotyledon walls of *Tamarindus* and similar legumes, as well as families that include primrose, flax and buttercup, are rich in galacto-XyGs, whereas glucomannans predominate in the cotyledon walls of some lilies and irises. Dates, coconuts and other palm seeds, coffee beans, ivory nuts and seeds of some Umbellifereae, all contain thick walls of almost pure mannan. The crystalline nature of mannans makes the seeds extremely hard. The endosperm wall of lettuce seeds, which constitutes the mechanical determinant of dormancy, is over 70% mannan. All endospermic legumes store galactomannans, but the mannose:galactose ratio can vary markedly, yielding a variety of polymers with quite different physical properties. For example, the galactomannans of fenugreek are almost fully branched, whereas those of guar, carob and honey locust (*Gleditsia triacanthos*) are much less branched (Meier & Reid 1982; McCleary *et al.* 1985). The degrees of substitution of the mannans greatly influence their viscosity and other solution properties. Seeds of yet other species accumulate neutral polysaccharides typically associated with pectins. For example, lupins contain large amounts of galactans and some arabinans. All of the grasses accumulate β-glucans in the walls of the endosperm and maternal tissues surrounding them. Oat and barley brans are notably enriched in β-glucans, comprising up to 70% of the aleurone layer cell walls at maturity (Fincher & Stone 1986).

Many of these seed polysaccharides are used in the food industry, and some – like β-glucans – are recognised for their special roles as dietary fibres. Surprisingly, the vast majority of plant seeds have never been examined as potential sources of unique dietary fibres (see Fig. 5.8).

5.8 The biotechnology of dietary fibres

These are truly challenging and exciting times for the study of plants and their cell walls. We are finally bridging the gap between our understanding of the structural elements of the cell wall and the genes that encode the machinery for cell-wall biogenesis.

The structural differences between the type I and the type II walls give rise to different end products, which have a direct impact on the taste and texture of dietary fibres. We are beginning to identify unique genes responsible for the structural differences. These genes are the ultimate targets for genetic manipulation to improve fibre content and textural properties.

One route to gene discovery is the identification of mutations where a constituent is absent or the structure of a polymer is altered because of the lack of a particular sugar. In the model organism *Arabidopsis*, such mutants have already been recognised by deficiencies in certain cell-wall sugars, such as fucose, arabinose and rhamnose (Reiter *et al.* 1997).

A major breakthrough in the area of polysaccharide synthesis was the identification of a gene that encodes the catalytic subunit of cellulose synthase (*Ces*A) (Pear *et al.* 1996). This discovery was instrumental in the identification of many other related genes that are involved in the synthesis of cellulose as well as many other non-cellulosic polysaccharides (Delmer 1999). An *Arabidopsis* mutant, called *rsw1*, a temperature-sensitive root tip cell-swelling mutant that is defective in cellulose synthesis, provided the first genetic evidence for function of *Ces*A. The root-swelling phenotype was traced to a defective *Ces*A gene, and when a healthy *Ces*A gene was introduced, normal cellulose synthesis resumed (Arioli *et al.* 1998). Thus, this gene complementation provided the genetic proof that *Ces*A indeed encodes a cellulose synthase.

Identification of mutants affecting enzymes and proteins that function in wall rearrangement during growth, such as XET and expansins, will not only aid in our understanding of the wall assembly mechanisms, but also in understanding the function of wall dynamics during fruit maturation and ripening. Defects at this 'disassembly' stage will probably result in the reduction of sugars in the wall, which will directly affect the fibre content of fruits and vegetables.

Given the impact of the special cell wall of grasses and cereals in dietary fibre production, considerable efforts are aimed towards research in this field. The alteration of the structure and biogenesis of the cell wall of grasses will enhance the nutritional value of cereals as the content and composition of important wall constituents like β-glucans can be modulated.

The cell walls of fruits and vegetables are also of central importance in human nutrition, and investigations on these will yield knowledge that will impact on features such as taste and texture. Undoubtedly there is a need to substantially augment the mutant collections in order to be able systematically to study cell-wall biogenesis and modification. Even then, the challenge will be to characterise these mutants in order to understand the role that these genes play in the developmental and functional scenario of cell-wall biogenesis – a critical process in plant growth and development.

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Appendix: sugar nomenclature

The conformational models of the monosaccharides are illustrated to distinguish between the relative axial and equatorial positions of the hydroxyl groups around their ring structures (Fig. 5.A1). **Pyranose** sugars, such as the pentose, xylose, and the hexoses, mannose, galactose and glucose, are six-membered rings that adopt a 'chair-form', whereas **furanoses**, such as arabinose, are five-membered rings with a slightly 'puckered' conformation. The α or β designations denote the position of the hydroxyl on the **anomeric** carbon, which forms the glycosidic linkage, relative to the last asymmetric carbon [C-5 for hexoses, C-4 for pentoses]. α-D-Glucose is the most stable of the hexoses because every hydroxyl group of the ring and the C-6 primary alcohol group are in the equatorial position, which is energetically more favourable. In mannose, the hydroxyl group at the O-2 position is in the axial position instead of the equatorial position, and in galactose it is the O-4 hydroxyl that is axial. Hence, iterative linkage at these different positions results in different polysaccharide shapes. A glycosidic linkage between two sugars is always through the anomeric carbon of one sugar [C-1 in all aldoses] to the hydroxyl position of the next sugar. The **reducing end** of a mono- or polysaccharide, a classical term reserved for the ability of the free aldehyde to reduce copper, refers to the single, unbound anomeric carbon. Because the linkage of all sugars always involves the anomeric carbon, there is always only one reducing end regardless of polymer size.

The linkage designation for cellobiose, β-D-glucose-(1→4)-D-glucose, describes the linkage in the β configuration of one glucose via its anomeric carbon to the O-4 position of the next sugar, which in this instance is a reducing sugar. Because the D- or L- designation deter-

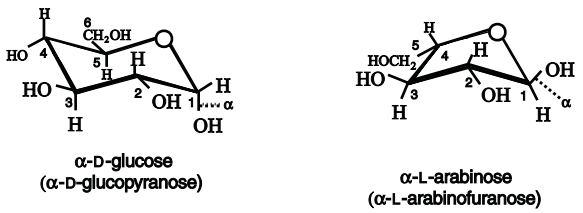

Fig. 5.A1 α-D-glucose and α-L-arabinose models.

mines whether the α or β configurations of hydroxyl groups of the anomeric carbon are axial or equatorial, both elements are requirements of this nomenclature. For example, note that the equatorial position is β for D-glucose but α for L-arabinose. The hydroxyl group of the anomeric carbon of the reducing sugar is able to mutarotate between the α and β configurations, no designation is assigned. The (1→4)β-D-glucosyl, mannosyl, and xylosyl linkages share the feature that each sugar is inverted almost 180° with respect to each neighbour to produce a nearly linear polymer. When unbranched, all of them will share the propensity to hydrogen bond tightly to each other to form para-crystalline arrays. Iterative linkage to any position other than (1→4)β- for these sugars creates helical polymers of widely varying periodicities (Rees 1977). The (1→4)β-D-galactosyl linkages are not linear because the change in angle by conversion of the O-4 hydroxyl group from the equatorial to axial position results in polymer orientation.

Part 3

Measurement of Dietary Fibre and Dietary Fibre Components

6 What is Dietary Fibre? A New Look at the Definition

Leon Prosky

6.1 Introduction

The generally accepted definition of dietary fibre is that of Trowell *et al.* (1976), that dietary fibre consists of the remnants of edible plant cell polysaccharides, lignin, and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans. In Japan, the food tables list the dietary fibre content of animal as well as plant tissues. According to the Food and Agricultural Organisation (FAO) of the United Nation World Health Organization (WHO), dietary fibres are constituents of substances of both animal and plant origins that are resistant to hydrolysis by human alimentary enzymes. Animal, as well as plant dietary fibres do assay in the Association of Official Analytical Chemist International (AOAC) method for dietary fibre. While many countries accept saccharides of degree of polmerisation (DP) <10 as dietary fibre (inulin, oligofructose, Fibersol-2, polydextrose, fructo-oligosaccharides, galacto-oligosaccharides, etc.), these shorter-chain oligosaccharides do not precipitate as dietary fibre in the standard AOAC method, which is accepted by the US Food & Drug Administration (FDA), the US Department of Agriculture (USDA) and the FAO of the WHO for nutrition labelling purposes. In the United Kingdom, the term dietary fibre has been replaced in nutrition labelling by non-starch polysaccharides. The Executive Board of the American Association of Cereal Chemists (AACC) convened an *ad hoc* committee of scientists to evaluate continuing validity of the currently used definition, and if appropriate, to modify and update that definition. Obtaining scientific input from the community of analysts, health professionals and dietary fibre researchers was considered a high priority. To this end, three meetings were held during a one-year period to ensure input from all persons cognisant with the field, and the committee's response is expected during 2000.

6.2 What is dietary fibre? A new look at the definition

A great deal of debate has existed over the definition of dietary fibre, as well as methods for measuring the amount of dietary fibre. This results principally because some people want to describe dietary fibre by its physiological attributes, and some by its chemical composition. Dietary fibre was first defined by Hipsley (1953) to includes lignin, cellulose and hemicellulose, and broadened to include soluble substances (non-cell-wall derived materials) such as pectins, gums and mucilages (Trowell *et al.* 1976). This broad definition acknowledges the significance of fibre as a chemical and physiological component of the diet as compared with the static definition of crude fibre – 'the residue of plant food left after extraction with solvent, dilute acid, and dilute alkali' (Browne 1940). After the acceptance of the crude fibre method came the AOAC acceptance of the acid detergent fibre method (ADF), which measured cellulose and lignin (Van Soest 1963), and the AACC acceptance of the neutral detergent fibre

method (NDF) which gave higher values for the fibre because of the improved recoveries of cellulose, hemicellulose and lignins (Van Soest & Wine 1967). Complete removal of the starch by conventional means was difficult for some food samples, so the method was modified to include an α-amylase digestion treatment to remove residual starch (Schaller 1976; AACC Method 32–20 1978).

Since neither the ADF nor the NDF comprise all the components that have been encompassed by the term dietary fibre, Prosky and Harland (1979), announced their intention of seeking a definition and method for the determination of total dietary fibre (TDF) which could then be subjected to a collaborative study under the auspices of the AOAC.

Following the survey, an AOAC Spring Workshop was held in Ottawa where Asp, Southgate, Baker, Van Soest and Heckman presented their fibre methodology research (Prosky 1981). The scientists present at the workshop concluded that two methods for the determination of TDF in foods should be developed: (1) a rapid, enzymatic–gravimetric method based on the procedure developed by Asp *et al.* (1983), Furda *et al.* (1979) and Schweizer and Wursch (1979); and (2) a more comprehensive method, such as a modification of the methods of Southgate (1969) or Theander and Aman (1982) to determine the individual dietary fibre components. In the rapid enzymatic–gravimetric method, the sum of soluble and insoluble polysaccharides and lignin would be defined and measured as a unit; in the second method, each of the specific components of TDF would be identified and measured separately (Prosky & Harland 1981). Even at this time there was recognition that the needs of cereal scientists, who were mainly interested in the fibre content, were different from those of physiologists, who were primarily interested in identifying the fibre fractions that most consistently elicited physiological responses. Little thought was given to the dietician and the consumer – who were to be the ultimate users of the information.

At the time, the results of the survey showed that the preferred methods for determining dietary fibre were modifications of Southgate's procedures (Southgate 1969). Because of complexities and time involved with this procedure (acknowledging that the method gave the most complete results), an intermediate method was desired. Such a method would include the determination of the soluble and insoluble fractions, and yet would be much simpler than the Southgate procedure, so that it could be carried out in most general chemical laboratories.

The basic definition of dietary fibre accepted by scientists throughout the world was proposed by Trowell *et al.* (1976). It stated that dietary fibre is composed of the remnants of plant cells resistant to hydrolysis by human alimentary enzymes, and that it included all indigestible polysaccharides (celluloses, hemicelluloses, oligosaccharides, pectins, gums) plus waxes and lignin. We endeavoured to turn this definition into a method which could pass a collaborative study and be certified by the AOAC International. This definition and method served the scientific community well until the early 1990s, when it became clear that there were several classes of compounds which did not precipitate in the 78% ethanol (the classical AOAC method precipitates dietary fibre from a 78% ethanol solution), and which possessed many of the characteristics commonly associated with dietary fibre, e.g. effects on serum cholesterol, blood glucose and laxation, distribution of fatty acids produced in the large intestine, absorption of minerals, promotion of favourable intestinal bacteria, etc. and yet were resistant to hydrolysis by the enzymes in the small intestine of humans. At that time, a Committee of scientists, appointed by the Executive Committee of the AACC were holding meetings, and with invitations extended to the scientific community at large to participate in this effort, set out to determine if the definition of dietary fibre should remain as originally

proposed or – due to the accumulation of scientific data – be changed or altered in any way to accommodate these new compounds. There was also discussion as to whether or not animal-origin polysaccharides (e.g. chitin) fit into the definition of dietary fibre. The Committee set up to define dietary fibre took on this very challenging task in its attempt to redefine a very diverse group of food components in the diet, for an equally diverse group of users, including consumers, educators, research scientists, nutritionists, medical doctors, food processors, government regulators and others. This was a difficult but essential task, more difficult than could be envisioned by the Committee. The nutritionist thinks of dietary fibre as a new dietary component, especially when it is compared to lipids, carbohydrates, proteins and vitamins. From the consumers' point of view, there is a desire by some to seek out foods that are believed to promote health and/or prevent diseases. Dietary fibre, as components of fruits, vegetables and whole cereals grains, has gained such identification. From the analytical chemists' point of view, there will never be a universal method for determining dietary fibre because of the diverse components that comprise dietary fibre, the matrices that contain dietary fibre, and the complexity of the mixture of substances. The marketing strategy of food processors is to promote foods through the use of health claims. Regulatory agencies, which monitor the accuracy of health claims, play an important role in this area, deciding on a case-by-case basis the validity of the dietary fibre claims. The clinicians will continuously question the role and mechanism of action of dietary fibre in maintaining heath and preventing disease. Much more research will have to be done to satisfy their needs. The educators need a simple and practical definition that they can pass on to their students and the lay public. We can see from the foregoing discussion that the definition will have to encompass some physiological effects of dietary fibre to be meaningful to a majority of the people concerned.

Dietary fibre is described as a class of compounds, mainly polysaccharide in nature (but also including lignin) which, when ingested in the form of plant material, escapes hydrolysis, digestion and absorption in the small intestine of the human. This definition – which is basically physiological in nature – has been accepted by the majority of scientists working in the field, and has formed the basis for much of the scientific research on dietary fibre and data assemblage that has taken place during the past 25 years (Lee & Prosky 1995). From time to time, questions have arisen concerning compounds which may be dietary fibre but which do not precipitate in 78% ethanol as dietary fibre – as called for in the AOAC method for dietary fibre analysis.

6.3 The available methods

Many analytical methods have been developed during the past 25 years, including those of Williams and Olmstead (1935), Hellendorn *et al.* (1975), Asp and Johannson (1981,1984), Asp *et al.* (1983), Schweizer and Wursch (1979), Theander and Aman (1978, 1979, 1982), Southgate (1969, 1982), Englyst *et al.* (1983), Englyst and Cummings (1984, 1988), Englyst and Hudson (1987), Furda (1981), Furda *et al.* (1979), Baker (1981); Baker *et al.* (1979), Van Soest (1978), Van Soest and McQueen (1973) and Heckman and Lane (1981). The main aim of these methods was to remove the digestible portion of the food using enzymes and leave the indigestible portion, i.e. mimicking the dietary fibre digestive process in the small intestine. Enzymatic methods were first employed by Williams and Olmstead (1935). Pancreatin was used to remove starch and protein, followed by acid hydrolysis and subsequent identification and measurement of the sugar fractions. Helledoorn *et al.* (1975) used pepsin for the hydroly-

sis of protein and pancreatin for subsequent starch hydrolysis. Furda (1977) examined existing analytical methods and suggested new methodology that would be based on the use of appropriate enzymes and inclusion of the soluble dietary fibre fraction. Asp *et al.* (1977, 1983) evaluated some of the more widely used methods for determination of dietary fibre, and proposed enzymatic modifications using pepsin, pancreatin and Termamyl – a heat-stable α-amylase. Theander and Aman (1979) introduced the use of the Termamyl enzyme for combined gelatinisation and starch removal.

Subsequently, Heckman and Lane (1981) analysed several foods by various dietary fibre methods (Southgate,1969; Goering & Van Soest 1970; Hellendoorn *et al.* 1975; Schaller 1976; Robertson & Van Soest 1977; Furda *et al.* 1979). The results showed that the enzyme-modified NDF was lower than the non-enzyme-modified NDF, and depended principally on the starch content of the food examined. Starch removal from the food became an important determinant of the success of the measurement of dietary fibre. The success of this approach was limited by the availability of pure-enough enzymes that were not contaminated by dietary fibre digesting enzymes (glucanase, cellulase, hemicellulase, pectinase, etc.) and capable of the removal of starch.

The methodological approach used by Asp, Furda, Schweizer and their co-workers was deemed the best, and with the combined effort of Prosky, DeVries and Harland, a single method suitable for a collaborative study was developed. The collaborative study conducted by 43 laboratories in 29 countries was limited by the amount of sample available. An initial disappointment (Prosky *et al.* 1984) with the enzymatic–gravimetric method was corrected by making minor modifications to the method which resulted in a successful collaborative study (Prosky *et al.* 1985). The method was adopted by the Official Method of Analysis (AOAC Official Method 985.29, 1995). The method was also adopted as AACC Approved Method 32–05 (AACC Approved Method 1995).

6.3.1 *The enzymatic-gravimetric method*

The key to success with the enzymatic–gravimetric method were the specifications on enzyme purity, particularly with regard to avoiding contaminating enzymes which digest the dietary fibre, and precise handling of the digestion steps.

The method became routine world-wide, being used for analytical as well as nutrition research purposes and always emphasising the positive effects of ingesting dietary fibre. Because its measurement met the goals of the Ottawa workshop, and because of its world-wide acceptance, the analytical method for dietary fibre became the *de facto* operating definition of dietary fibre. The AOAC method was adopted as the official method of analysis for dietary fibre for nutrition labelling of foods by the US Food and Drug Administration (US Food and Drug Administration 1993) and US Department of Agriculture (US Department of Agriculture 1993), and was accepted as official by the Codex Committee of WHO/FAO (18th Session of Codex Committee on Method of Analysis and Sampling 1992). Further, as important distinctions between the physiological consequences of ingesting soluble and insoluble dietary fibre became apparent, methods were developed to determine these two components. Method 985.29 was modified to allow quantitation of soluble and insoluble dietary fibre (Prosky *et al.* 1992, 1994). The distinction between soluble and insoluble dietary fibre is somewhat arbitrary, and is based on the solubility of the dietary fibre in a pH controlled system.

The de facto method

In the *de facto* method, the total dietary fibre is precipitated in a mixture of 1 volume of aqueous enzyme solution with 4 volumes of 95% ethanol – a system long used by chemists to separate complex from simple carbohydrates. While this may be the case, the dietary fibre definition does not imply insolubility in aqueous ethanol as a requirement for calling the substance dietary fibre. Further, it was thought that this method of precipitation precipitated oligosaccharides/polysaccharides of DP ≥10. It has been demonstrated recently that with some materials (e.g. starch dextrins), none of DP 10 and only some of DP 11 and 12 are precipitated in the alcohol step (Ohkuma *et al.* 2000). Lee and Prosky (1994) had recommended that a new dietary fibre definition include resistant oligosaccharides of DP 3 and higher. The modified methodology for soluble and insoluble dietary fibre was adopted as AOAC Official Method 991.42, 1995 and AOAC Official Method 993.19, 1995) following successful collaborative studies.

With a 'gold standard' method in place, nutritional research and analytical chemists began to add improvements or alternative approaches, but all having the same objective and results. Lee *et al.* (1992), Mongeau and Brassard (1993), Li and Cardozo (1994) and Theander *et al.* (1994) all developed, validated and carried out successful collaborative studies which were adopted as official by the AOAC. Noteworthy is the method of Lee *et al.* (1992), who substituted MES-TRIS buffer for the phosphate buffer in the original AOAC dietary fibre determination. The results showed that using different methods and alternative approaches to determining dietary fibre led to the same result. In an article which appeared in *Cereal Foods World*, Lee and Prosky (1992) explained why the method for nutrition labelling of dietary fibre was chosen, and what the future considerations for labelling should be.

The situation in the United Kingdom

The story is different in the United Kingdom, where the Ministry of Agriculture, Fisheries and Food (MAFF) adopted the Englyst and Cummings (1988), non-starch polysaccharide (NSP) procedure as official designation for food labelling purposes for 'dietary fibre' (MAFF validated methods of analysis of foodstuffs 1995). In fact, The British Nutrition Foundation recommended that the term 'dietary fibre' should become obsolete in the scientific literature (The British Nutrition Foundation 1990), and instead the term NSP should be used. It was suggested that the consumer did not understand the term dietary fibre, and was quite confused as to the advantages and disadvantages of ingesting it in the diet. This type of reasoning was not borne out in our surveys, which showed the public to understand what dietary fibre was; moreover, the large amount of data already collected on the dietary fibre composition of food was certainly too valuable to relinquish. Leeds (1993) stated in an editorial comparing the use of term dietary fibre versus NSP, 'A number of readers, particularly in the United Kingdom and other European countries will take issue with our discontinuing the use of the term "dietary fibre". There are two reason for this: First, the term "dietary fibre" is now reasonably well understood world-wide, whereas "non-starch polysaccharide" is not. Secondly, "non-starch polysaccharide" is not a term which easily trips off the tongue, nor is it easily incorporated into other than a rather clumsy book or newsletter title.'

The situation in the European Union

The European Union is still working on a suitable definition and method for dietary fibre labelling of foods. To this end, a Scientific Committee on Foods (SCF) and a Management Committee of the European Concerted Action for Co-operation on Science and Technology (COST 92) 'Metabolic and Physiological Aspects of Dietary Fibre in Foods (CMC)' were established. James (1993) prepared the recommendation of the SCF, which stated that dietary fibre be re-defined as 'plant cell wall non-starch polysaccharides', and that the method of Englyst was to be used for the determination of dietary fibre for food labelling purposes. However, when this proposal was presented to the CMC, representatives of 12 of the 13 countries voted to adopt a definition of dietary fibre based on physiological properties, including edibility, resistance to digestibility in the small intestine, and faecal bulking that influences bowel habits. Only the United Kingdom voted for the NSP definition and method for dietary fibre (Cummings 1994). The CMC was of the opinion that the definition should not be limited to carbohydrates, but that lignin, polyphenolic compounds, inositol phosphates, etc. should be included in the definition. It was also suggested that digestion-resistant oligosaccharides should also be included in the dietary fibre complex. The CMC pointed out that most European countries were using AOAC Official Method to quantitate the dietary fibre according to the definition. In Copenhagen, Denmark (October 1994), at a follow-up COST 92 Workshop on methods of dietary fibre analysis, participants of the workshop affirmed the recommendation of the CMC (European Scientific Workshop 1994).

Finally, the MAFF Joint Food Safety and Standard Group (JFFSG) released a proposal (August 9, 1999) which states, 'This letter issues new proposals: again these are intended to ensure consistent labelling of dietary fibre, which will allow consumers to make meaningful comparisons of the fibre contents of different products. JFSSG now proposes adopting AOAC International methodology as the UK's preferred method for analysis for fibre for nutrition labelling purposes. This move would facilitate harmonisation of fibre declarations across the EU and more widely and improve the usefulness of information available to consumers. Revised claims criteria have also been developed to reflect the increased values produced by this method of analysis' (Hignett 1999).

6.4 Dietary fibre: the definition

Now that food labelling for dietary fibre is uniform around the world, we still have to arrive at a definition which will include those plant oligo- and polysaccharides which do not precipitate in 78% ethanol, and which are not hydrolysed by the enzymes of the small intestine. The AOAC, in the person of the General Referee for Dietary Fibre and Complex Carbohydrates, has taken the approach that a specific method could be developed for measurement of specific food saccharides and that value could be added to the dietary fibre value obtained by using the standard AOAC method for dietary fibre, to give the TDF value for the food product (Prosky 1999; Prosky & Hoebregs 1999). The first food saccharides (that did not precipitate in 78% ethanol) which passed an AOAC collaborative study were inulin and oligofructose. The method for inulin and oligofructose was approved by the AOAC after a successful study in 1997 (Hoebregs 1997). A second, simpler, method for carrying out the determination of oligofructose and fructose polysaccharides, was developed by Megazyme International Ireland, Ltd. This method utilises an enzymatic/spectrophotometric approach, and has also

received AOAC approval (McCleary *et al.* 2000). The method is described in detail in a paper published in *Cereal Foods World* (McCleary & Blakeney 1999). Methods for the measurement of three other saccharide materials which are not quantitatively measured by the AOAC TDF method, are in various stages of the approval process. The first of these is for the product Fibersol-2®, an indigestible dextrin product which is made from corn starch. A collaborative study has been completed, and is in the possession of the evaluating committee of the AOAC, the Official Methods Board (OMB). A preliminary report was given at the AOAC annual meeting (Ohkuma & Gordon 1999). The Fibersol® material is 'generally recognised as safe' (GRAS) and is not hydrolysed in the small intestine; in addition, it has many of the physiological properties that we have come to expect from a dietary fibre. Another two compounds currently under review are polydextrose – for which the collaborative study has been completed – and galacto-oligosaccharides, for which a collaborative study is now underway. The US Food and Drug Administration does not have a definition of dietary fibre at the present time. It currently accepts the AOAC method as the determinant for what is dietary fibre, but this could change if the scientific community arrives at a modified definition of dietary fibre, which may include the lower DP oligosaccharides.

After much discussion and input from industry, academia, and government personnel from United States and abroad and a committee of the AACC, the definition arrived at for dietary fibre was:

> 'Dietary fibre is the remnants of the edible part of the plant and analogous carbohydrate that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibre exhibits one or more of either laxation (faecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation.'

This definition was sent to the Executive Board of Directors of the AACC (for possible forwarding to the FDA) but came back for further rewording. The definition was reworded and submitted as:

> 'Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibre promotes beneficial physiological effects, such as, laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.'

'Analogous carbohydrates' is defined as those carbohydrates-based food ingredients that are non-digestible and non-absorbable, and which are similar to plant dietary fibre for which most of the dietary fibre research has encompassed. Associated substances include the wax compounds, suberin and cutin. The definition of dietary fibre has also included some of the health benefits that we have ascertained are the benefits of ingesting dietary fibre. It is realised that the definition will be further modified and revised as new data become available.

6.5 The benefits of increased dietary fibre intake

The beneficial effects of increasing dietary fibre intake by the use of four dietary fibre products are outlined below.

6.5.1 *Partially hydrolysed guar gum*

The first example product is partially hydrolysed guar gum (PHGG). Guar gum is of particular interest to the food industry because of its viscogenic properties. The PHGG is considerably less viscous than guar gum, and therefore provides several technical effects such as extension of bowel life for ready-to-eat cereals, emulsification and foam stabilisation, moisture retention and texture improvement. The product, called Sunfiber® in Japan (Taiyo Kagaku Co. Ltd) and Benefiber® (Novartis Nutrition) in the USA, has the following functional food properties:

(1) It reduces postprandial blood glucose and insulin, especially in diabetics (Golay *et al.* 1995).
(2) It prevents ileum shrinkage due to low-residue diet (Takahashi *et al.* 1995).
(3) It has a modest effect on stool weight and increased frequency, and also softens stools (Takahashi *et al.* 1994a).
(4) It increases the bioavailability of calcium and magnesium and dietary iron in deficiency (Hara *et al.* 1996; Takahashi *et al.* 1994b).
(5) It increases *Bifidobacterium* (Okubo *et al.* 1994).
(6) It lowers serum levels of cholesterol and triglycerides (Takahashi *et al.* 1993).
(7) It is metabolised to short-chain fatty acids in the large intestine.

6.5.2 *Fibersol®*

The second example product is an indigestible dextrin made from corn starch and called Fibersol-2® (Matsutani Chemical Industry Ltd, Japan). It has the following physiological properties:

(1) It reduces the postprandial rise in blood glucose levels (Wakabayashi 1992; Wakabayashi *et al.* 1993, 1995).
(2) It increases faecal frequency and volume (Satouchi *et al.* 1993; Flickenger *et al.* 1998).
(3) It prevents intestinal mucosal atrophy due to long-term administration of enteral nutrition (Satouchi *et al.* 1993; Wakabayashi 1998).
(4) It increases *Bifidobacterium* (Ohkuma *et al.* 1990; Wakabayashi 1998).
(5) It lowers serum levels of cholesterol and triglyceride, but does not reduce HDL-cholesterol levels (Matsuoka *et al.* 1992; Nomura *et al.* 1992; Fujiwara & Matsuoka 1993; Wakabayashi 1998; Tokunaga & Matsuoka 1999).
(6) It prevents fat accumulation in internal organs (Watanabe *et al.* 1993; Wakabayashi *et al.* 1995).
(7) It is metabolised to short-chain fatty acids in the large intestine (Kishimoto *et al.* 1995; Flickenger *et al.* 1998; Wakabayashi 1998).

6.5.3 *Inulin and oligofructose*

The third example products are inulin and oligofructose (Orafti Active Food Ingredients, Belgium). These products have the following metabolic properties (Nutritional and Health Benefits of Inulin and Oligofructose 1999):

(1) They cause no increase in serum glucose, no stimulation of insulin secretion, and have no influence on glucagon secretion.
(2) They cause increased stool frequency and stool weight.
(3) They decrease serum levels of triglycerides and cholesterol in hypercholesterolaemic patients.
(4) They stimulate *Bifidobacterium*.
(5) They suppress pathogenic bacteria (e.g. *E. coli* and *Clostridium* spp.).
(6) They improve calcium absorption.
(7) They may play a role in the prevention and inhibition of colon and breast cancer.
(8) They provides immune stimulation properties.

6.5.4 *Galacto-oligosaccharide*

The fourth example product is a transgalactosylated oligosaccharide (galacto-oligosaccharide) (Friesland Coberco Dairy Products, The Netherlands) with the following physiological properties:

(1) Non-cariogenic.
(2) Associated with protection against development of colon cancer (Rowland & Tanaka 1993; Wijnands *et al.* 1999).
(3) Enhancement of the absorption of minerals at the end of the ileum (Chonan & Watanuki 1994, 1996; Chonan *et al.* 1995).
(4) Stimulation of *Bifidobacterium* (Tanaka *et al.* 1983; Ito *et al.* 1990, 1993a, b; Bouhnik *et al.* 1997).
(5) Increased stool frequency and stool weight and ease of defecation (Deguchi *et al.* 1997; Teuri & Korpela 1998).
(6) Improved calcium absorption (Chonan *et al.* 1995).

6.6 Restrictions on beneficial claims for dietary fibre

The first product discussed above – Sunfiber (Benefiber®) – is the only one that is currently recognised as dietary fibre by the US Food and Drug Administration and US Department of Agriculture. However, the AACC committee, which is working on a definition of dietary fibre, has added to the definition a number of physiological consequences of ingesting dietary fibre, which may permit the inclusion of the other products, including Fibersol-2®, inulin and oligofructose and galacto-oligosaccharides.

There are restrictions on dietary fibre claims because the data for the presumed effects are not conclusive! Are the effects due to high dietary fibre, low fat or a combination of the two dietary levels? Or is it the kind of dietary fibre (soluble or insoluble), or kind of soluble or insoluble dietary fibre, or fat component (cholesterol, saturated fat etc.) that has the specific effect. The allowed claims are:

(1) A grain product, fruit, or vegetable that contains dietary fibre; low fat and good source of dietary fibre (**without fortification**) may be beneficial in preventing some types of cancer.
(2) A fruit, vegetable, or grain product that contains dietary fibre: low saturated fat, low cholesterol, and low fat, particularly soluble fibre (0.6 g per Reference Amount [RA] **without fortification**), may reduce the risk of coronary heart disease.
(3) A fruit or vegetable, low in fat with good source of vitamin A, vitamin C or dietary fibre (**without fortification**) may reduce the risks of some types of cancer. Soluble fibre must be labelled.
(4) Soluble fibre from: (1) β-glucan soluble fibre from oat bran, rolled oats (oatmeal) and whole oat flour; and (2) psyllium husks may reduce the risk of heart disease if they are low in fat, saturated fat, cholesterol and include 0.75 g of whole oat soluble fibre or 1.7 g of psyllium husk soluble fibre per RA. Soluble fibre must be labelled.
(5) Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers. The food must contain 51% or more whole grain ingredients by weight per serving, and a dietary fibre content of at least 3.0 per RA of 55 g, 2.8 g per RA of 50 g, 2.5 g per RA of 45 g, 1.7 g per RA of 35 g, and be low in fat.

We can see that the attributes of the food are not necessarily due only to the ingestion of dietary fibre, but are due to a variety of factors in the diet – namely low fat, low saturated fat and low cholesterol. It is likely that, in the not-too-distant future, we will be able to better pinpoint the causes of the various diseases and their relationship to dietary fibre intake.

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7 Development of Dietary Fibre Methodology

Nils-Georg Asp

7.1 Introduction

When considering developments in dietary fibre methodology, two basic points are evident: (1) that the progress in understanding of dietary fibre physiology has been parallel with and dependent upon progress in analytical methodology; and (2) that dietary fibre is a fraction of total food carbohydrates – which means that any method or definition of dietary fibre should consider and be consistent with the corresponding methods and definitions of the other carbohydrates.

7.2 Early developments

The proximate analysis system for feed was developed in the nineteenth century. In the absence of specific analytical techniques, it was possible and reasonable to measure water, nitrogen (converted to protein by a standard factor), lipids extractable in an organic solvent and mineral matter (i.e. ash), and to consider the remaining material as carbohydrate (Henneberg & Stohmann, 1860). When the proximate analysis system was applied in studies of ruminant physiology, especially at the Weende Experimental Station in Germany, it became apparent that the feed value of carbohydrates measured by difference was not constant, an insoluble fibrous fraction being identified that was not digested. This led to the development of the crude fibre method using successive acid and alkaline digestion to isolate this undigestible fraction (AOAC 1980). The detergent methods introduced by Van Soest (1963a, b) and Van Soest and Wine (1967) meant considerably improved prediction of the feed value, compared with the crude fibre method.

The crude fibre method was brought into human nutrition at the turn of the century, when especially Atwater (1900) studied in detail the caloric value of different foods and diets. These studies suggested that crude fibre did not contribute to the metabolisable energy.

The differentiation made between 'available' and 'unavailable' carbohydrates by McCance and Lawrence (1929) may be regarded as a milestone in our understanding of the nutritional importance of different food carbohydrates. The main objective was to differentiate those carbohydrates that affected the blood glucose levels, i.e. those 'available' for digestion and absorption in the small intestine, in order to improve nutritional counselling to diabetic patients. In line with this differentiation, the recent FAO/WHO report 'Carbohydrates in human nutrition' (Anon. 1998) recommended the adoption of the concept of 'glycaemic carbohydrate', meaning 'providing carbohydrate for metabolism'.

In 1935, Widdowson and McCance developed methods for analysing reducing sugars, sucrose and starch in foods as a measure of the available carbohydrates. Unavailable carbohydrates were determined as the insoluble residue, corrected for protein and ash. Such car-

bohydrate figures were introduced into British food tables in 1940, which should be borne in mind considering the fact that most food tables still today rely upon carbohydrate-by-difference data.

The more recent development of dietary fibre methodology has occurred along two routes: (1) *enzymatic gravimetric* methods; and (2) *enzymatic, chemical* methods. The origin of enzymatic gravimetric methods can be traced to the work of Stutzer and Isbert (1888) and Remy (1931) in Germany. At the same time, Williams and Olmstedt (1935) in the USA sought a more physiological way to estimate undigestible material than the crude fibre method. They developed a method that simulated digestion by incubating the food sample with enzymes. Their work was the basis for an enzymatic gravimetric method developed later by Hellendoorn *et al*. (1975) in The Netherlands, using pepsin and pancreatin to isolate an 'indigestible residue'. In Berlin, Thomas (1972) and his group also used enzymatic methods to isolate what they called 'Ballaststoffe' in cereal flours and bran.

7.3 Definitions of dietary fibre

The definition and analysis of dietary fibre are intimately related. Analysis methods have to be developed in accordance with the conceptual definitions, but in practice compromises must be accepted due to constraints of costs and time. All types of dietary carbohydrates can be separated at different levels of complexity and determined separately for research purposes, though short-hand methods are needed for labelling and control purposes.

The term 'dietary fibre' was first used by Hipsley (1953) to describe plant cell walls in the diet that he thought were protective against toxaemia of pregnancy. Trowell (1972a, 1974) revived the term and defined it originally as 'the skeletal remains of plant cells that were indigestible' or 'that portion of food which is derived from cellular walls of plants which is digested very poorly by human beings' (Trowell 1972b). In 1976, dietary fibre was re-defined by a group of scientists to include polysaccharides and lignin that are not digested in the small intestine (Trowell *et al*. 1976). A main reason for this re-definition was that isolated polysaccharides had become increasingly used in animal and human studies, and found to have effects on carbohydrate and lipid metabolism.

Thus, undigestibility in the small intestine is a core property of dietary fibre in all definitions, whereas plant cell-wall origin is emphasised in some – but not all – definitions. One main argument for restricting dietary fibre to plant cell-wall components, or plant cell-wall polysaccharides (Englyst & Cummings 1990) has been the early studies of Heaton's group (e.g. Haber *et al.* 1977) showing that both disruption and depletion of dietary fibre in foods such as fruits and nuts had effects on glycaemic response and nutrient absorption, which could be regarded as unfavourable in relation to 'Western' diseases. Recent research, however, has identified a number of food-related factors affecting the glycaemic response, and none of the current methods of analysis is specific for plant cell-wall material, the isolation of plant cell-walls being a very complex procedure (if possible at all) in processed foods. Therefore, a definition of dietary fibre including all polysaccharides that are not digested and absorbed in the small intestine is to be preferred, both physiologically and analytically (Asp 1995, 1996)

7.4 Classification of food carbohydrates

Being a fraction of the total food carbohydrates, dietary fibre has to be considered in the context of food carbohydrates. These are usually classified according to their degree of polymerisation, initially into three main classes: sugars; oligosaccharides; and polysaccharides (Anon. 1998). Sugars can be further subdivided into monosaccharides, disaccharides and polyols, oligosaccharides into malto-oligosaccharides and other oligosaccharides, and polysaccharides into starch and non-starch polysaccharides (NSP) (Table 7.1). Figure 7.1 illustrates which fractions are included in various definitions and methods of dietary fibre analysis.

By convention, polysaccharides are defined as having 10 or more monomeric residues. In a nutritional context, the term 'sugars' is often used to mean mono- and disaccharides. Glucose and fructose, and the disaccharides sucrose and lactose are the quantitatively most important 'sugars' in most diets. The main forms of oligosaccharides are the raffinose series of α-galactosides, and inulin and lower fructo-oligosaccharides. Malto-oligosaccharides are derived mainly from starch hydrolysates.

The polysaccharides are usually divided into starches, which are linear (amylose) or branched (amylopectin) homopolymers of glucose with α-glucosidic linkages (α-glucans)

Table 7.1 Classification of main food carbohydrates. (Adapted from Anon. 1998; Asp 1996.)

Class (DP)	Subgroup	Components	Typical monomers	Digestibility*
Sugars (1–2)	Mono-saccharides	Glucose		+
		Galactose		+
		Fructose		+
	Disaccharides	Sucrose	Glu, Fru	+
		Lactose	Glu, Gal	+ (–)
		Trehalose	Glu	+
Oligosaccharides	Malto-oligo-saccharides	Maltodextrins	Glu	+
	Other oligo-saccharides	α-Galactosides	Gal, Glu	–
		Fructo-oligo-saccharides	Fru, Glu	–
Polysaccharides	Starch	Amylose	Glu Glu	+ (–)
		Amylopectin	Glu	+ (–)
		Modified starch	Glu	+ –
	Non-starch polysaccharides	Cellulose	Glu	-
		Hemicelluloses	Variable	-
		Pectins	Uronic acids	-
		Hydrocolloids	Variable	-
'New' carbohydrate food ingredients	Polydextrose		Glucose	-
	Pyrodextrins		Glucose	-

DP, degree of polymerisation.
*Digestion/absorption in the small intestine.

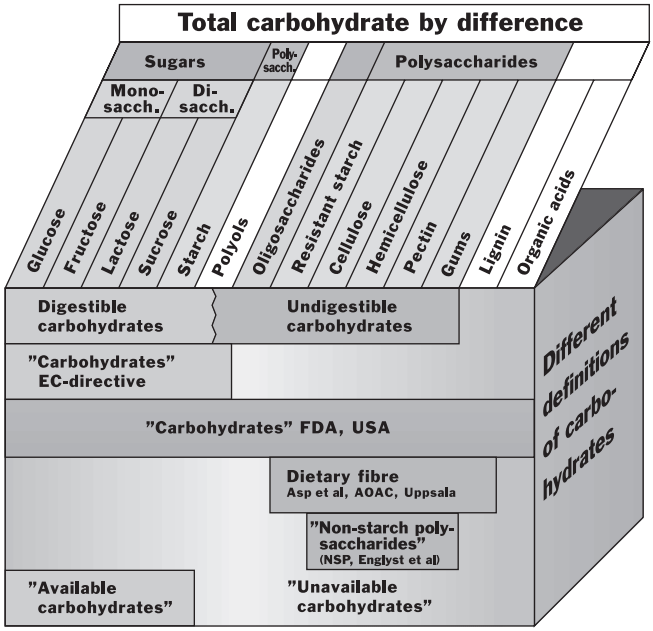

Fig. 7.1 Food carbohydrate fractions and their inclusion in various definitions. (Reprinted from *Food Chemistry*, **57**, Asp N.-G., Dietary Carbohydrates: classification by chamistry and physiology, pp. 9–14, copyright (1996), with permission from Elsevier Science.)

and NSP. Cellulose, which is a linear β-glucan, and hemicelluloses, pectins and hydrocolloids include a range of hetero-polysaccharides with variable degree of polymerisation, branching and monomeric composition. These main monomeric residues are another basis for classification of NSP, for example, arabinoxylan, galactan, galactomannan and arabinan.

7.5 Enzymatic-gravimetric methods

The main steps in the current gravimetric dietary fibre methods include enzymatic treatments for starch and protein removal, precipitation of soluble dietary fibre components by aqueous ethanol, isolation and weighing of the dietary fibre residue, and correction for protein and ash in the residue. A number of reviews on dietary fibre analysis, comparing different approaches, have been published (e.g. Asp & Johansson 1984; Asp *et al.* 1992; Cho *et al.* 1997).

7.5.1 *Modified detergent methods*

The neutral detergent fibre (NDF) method of Van Soest and Wine (1967) showed limitations when applied to starchy foods or ingredients in that starch was incompletely solubilised. Amylase treatment to solve this problem was introduced by Schaller (1977) and developed to a method approved by the American Association of Cereal Chemists (AACC Method 32-20 for insoluble dietary fibre).

Mongeau and Brassard (1986, 1990) added determination of a soluble fibre fraction to a modified NDF procedure. This rather rapid gravimetric method has shown excellent preci-

sion, but has limitations in incomplete removal of starch and/or protein in some kinds of samples and the risk of losses of some dietary fibre components that are soluble in the detergent system, but not under the conditions used to recover the soluble fraction (Asp *et al.* 1992; Cho *et al.* 1997, p. 54) (Table 7.2).

7.5.2 *Enzymatic methods using both amylases and proteases*

The first gravimetric procedures accounting for both insoluble and soluble parts of dietary fibre were developed at about the same time and independently by Furda (1977, 1981), Schweizer and Würsch (1979, 1981) and Asp and Johansson (1981); the procedure was finally published by Asp *et al.* (1983). As a result of a session at the annual AOAC meeting in Ottawa 1981, DeVries (and later also Prosky and Harland) joined these authors and designed the first version of the enzymatic gravimetric AOAC method. This was adopted for final action regarding total dietary fibre in 1986 (Prosky *et al.* 1984, 1985a, b), and later also for insoluble and soluble fibre. Lee *et al.* (1992) developed a further simplified method using MES-TRIS

Table 7.2 Main steps and comparison of enzymatic-gravimetric methods. (From Asp *et al.* 1992).

	Asp *et al.* 1983	AOAC 1988 Prosky *et al.* Schweizer *et al.* 1988	AOAC Modified Lee *et al.* 1992
Sample	1 g	1 g*	1 g*
Buffer Enzyme step 1	Na-phosphate pH 6 Termamyl† 100°C, 15 min	Na-phosphate pH 6 Termamyl 100°C, 15–30 min	MES/TRIS pH 8.2 Termamyl 95–100°C, 35 min
pH adjustment Enzyme step 2	To pH 1.5 Pepsin 40°C, 60 min	To pH 7.5 Protease 60°C, 30 min	Protease 60°C, 30 min
pH adjustment Enzyme step 3	To pH 6.8 Pancreatin 40°C, 60 min	To pH 4.0–4.6 Amyloglucosidase 60°C, 30 min	To pH 4.1–4.7 Amyloglucosidase 60°C, 30 min
pH adjustment	To pH 4.5		
Alcohol precipitation with 4 vol. 95% ethanol‡ Volume required	400 ml	280 ml	225 ml
Filtering aid	Celite 545	Celite 545	Celite
Protein correction Ash correction	N×6.25 (optional) Incineration 525°C	N×6.25 Incineration 525°C	N×6.25 Incineration 525°C

* Smaller samples can be analysed if samples are difficult to filter.
† Termamyl is thermostable α-amylase from Novo Nordisk, Denmark.
‡ The enzyme digest can be filtered before the alcohol precipitation to recover insoluble fibre separately. The soluble fibre is then precipitated from the filtrate and recovered in a separate fraction. Alternatively, the soluble fibre can be obtained as the difference between total and insoluble fibre as approved by the AOAC.

buffer that was approved by the AOAC in 1994. For details regarding AOAC approved methods, see Table 7.4 and Cho *et al.* 1997).

7.5.3 *Enzymatic chemical methods*

The enzymatic removal of starch is an essential first step also in the enzymatic chemical methods. Extraction or precipitation with 80% (v/v) ethanol is used to separate the soluble dietary fibre polysaccharides from low-molecular weight sugars and starch hydrolysis products.

The Southgate method

As described by Southgate (1992), the development of an enzymatic chemical method for measuring unavailable carbohydrates directly was driven by problems in calculating the energy values of food supplies after World War II. Carbohydrate by difference was used in the USA, and available carbohydrate in the UK, which resulted in the projected energy values of cereal supplies changing as they crossed the Atlantic! The Southgate procedure was developed (Southgate 1969a, b, 1981) following the principles of McCance and Widdowson, so that a complete carbohydrate analysis of sugars, starches, non-cellulosic polysaccharides, cellulose and lignin could be carried out sequentially on the same sample.

The carbohydrate analyses in the Southgate method were carried out by semi-specific colorimetric reactions for hexoses, pentoses and uronic acids. As in all other methods, an efficient starch digestion is essential to remove digestible starch (or all starch if resistant starch is not to be included). One reason for problems occurring with the Southgate method in this respect was probably the change from takadiastase, which contained some proteolytic activity, to more purified amylase preparations (Southgate 1981).

7.5.4 *Methods using gas-liquid chromatography (GLC)*

A meeting in 1977 organised by the EEC and the International Agency for Research on Cancer in Lyons was a starting point for efforts to introduce more modern and informative methods for dietary fibre analysis (Theander & James 1979). Schweizer and Würsch (1979) published a GLC method for characterisation of gravimetrically determined soluble and insoluble dietary fibre residues, which combined the advantages of the methods of Hellendoorn *et al.* (1975) and Southgate (1969b). In the same year, Theander and Åman (1979) published the first version of the Uppsala methodology using GLC for determination and characterisation of soluble and insoluble fractions of dietary fibre. A Klason lignin determination was included.

The Englyst methods

The methods of Englyst (Southgate *et al.* 1978; Englyst 1981; Englyst & Cummings 1984) was originally based on that of Southgate (1969b), with GLC replacing the colorimetric determination of neutral sugar components. The method has undergone a number of developments, not least regarding the choice of enzymes and conditions for removal of starch. The decision to exclude resistant starch from dietary fibre, i.e. to determine NSP only, led to the introduction of dimethyl sulphoxide to disperse the starch before amylolysis (Englyst

et al. 1982). A colorimetric variant was published in 1987 (Englyst & Hudson 1987). More recently, alternative procedures for measuring both neutral sugars and uronic acids by high-performance liquid chromatography (HPLC) have been developed (Quigley & Englyst 1992 1994).

The Uppsala methods

The initial method of Theander and Åman (1979) was modified to a more rapid analysis of total dietary fibre (Theander *et al.* 1990). After starch gelatinisation and hydrolysis by incubation with Temamyl (thermostable α-amylase; Novo Nordisk, Denmark) in a boiling water bath, soluble fibre polysaccharides are precipitated in 80% ethanol and the dietary fibre residue containing soluble (as well as insoluble fibre) is obtained by centrifugation. The content of neutral monomeric residues of dietary fibre polysaccharides is determined by GLC, after acid hydrolysis and preparation of alditol acetates. Colorimetric determination of uronic acids was introduced as a simpler alternative to the originally used decarboxylation method.

7.6 Collaborative studies

Methods for dietary fibre and NSP analysis have been subject to numerous collaborative trials during the 1980s and early 1990s (for a review, see Asp *et al.* 1992; European Commission 1995). The performance of both gravimetric and component analysis methods has improved gradually. In gravimetric methods, the coefficient of variation tends to become high at low dietary fibre content, because the corrections for protein and ash become proportionately larger, whereas component analysis methods have a comparable coefficient of variation over the range of fibre contents normally found in foods. On the other hand, the confidence interval is acceptable for practical purposes, e.g. labelling, over the range of fibre contents found in foods and raw materials (Asp *et al.* 1992). The AOAC International has approved several versions of the enzymatic gravimetric methods of Prosky *et al.* and Lee *et al.*, as well as the enzymatic chemical method of Theander *et al.* and the modified detergent method of Mongeau and Brassard, as shown in Tables 7.3 and 7.4. The Englyst methods has been approved mainly in the UK, subject to a corresponding number of collaborative studies conducted.

Few studies, however, have directly compared the different approaches in a conclusive way. Therefore, the collaborative studies supported by the EC Bureau of Community References (BCR) are particularly useful. After the first study, three reference materials could be certified regarding dietary fibre content measured with the enzymatic gravimetric AOAC Method 985.29, whereas results obtained with the Englyst method were still not regarded as reliable enough for certification.

A more extensive study of five different materials was then started. The most recent versions of the Englyst methods, the enzymatic gravimetric AOAC methods 985.29 and 991.43, and the enzymatic chemical method of Theander *et al.* (AOAC 994.13, the Uppsala method) underwent a detailed comparison. The gravimetric methods and the Uppsala method showed very similar mean values for the different test materials. The lower values obtained with the Englyst methods could be explained by the fact that resistant starch and lignin are not included in these methods (Pendlington & Brookes 1995).

Table 7.3 Main steps in the Uppsala method and the Englyst methods have approached each other, as shown for the 1990 versions of the two methods (Theander *et al.* 1990; Englyst & Cummings 1990). Only a few minor modifications have been made in the later versions. The main persisting differences are the dimethyl sulphoxide (DMSO) solubilisation of starch in the Englyst method, and the Klason lignin determination in the Uppsala method. (Adapted from Asp *et al.* 1992.)

Procedure	Uppsala method	Englyst method
Sample size (dry)	250–500 mg	50–300 mg
Starch removal	Termamyl (0.5 h, 96°C) Amyloglucosidase (16 h, 96°C)	DMSO (0.5 h, boiling water bath) Termamyl (10 min, boiling water bath) Pancreatin and pullulanase (0.5 h, and 50°C + 10 min boiling)
Analysis of neutral sugars	12 M H_2SO_4 (1 h, 30°C) Addition of internal standard myo-inositol 0.4 M H_2SO_4 (1 h, 125°C) Alditol acetate preparation Individual correction factors regularly determined for each sugar	12 M H_2SO_4 (1 h, 35°C) 2 M H_2SO_4 (1 h, 100°C) Addition of internal standard (allose) Alditol acetate preparation Individual standard correction factors for hydrolysis losses (0.89–0.96, 0.52 for rhamnose)
Analysis of uronic acids	Stoichiometric decarboxylation or colorimetry with 3,5-dimethylphenol (Scott 1979) Calibration with galacturonic acid	Scott procedure with 3,5-dimethylphenol Calibration with glucuronic acid
Lignin	Gravimetrically as Klason (sulphuric acid) lignin	Not determined

Table 7.4 AOAC INTERNATIONAL approved dietary fibre methods.

AOAC No.	Type of method	Total fibre (T) Soluble (S) Insoluble (I)	Action Year First/Final	Authors
	Dietary fibre			
985.29	Enzymatic gravimetric	T	1986 Final	Prosky *et al.*
991.42	Enzymatic gravimetric	I	1994 Final	Prosky *et al*
993.19	Enzymatic gravimetric	S	1995 Final	Prosky *et al*
991.43	Enzymatic gravimetric	T, S, I	1994 Final	Lee *et al.*
992.16	Enzymatic gravimetric, using neutral detergent	T	1992 First	Mongeau, Brassard
993.21	Non-enzymatic gravimetric	T	1993 First	Lee, Cardozo <2% starch, >10% fibre
994.13	Enzymatic chemical	T	1994 First	Theander *et al.*
	β-D-Glucan			
992.28	Enzymatic		1992 First	Zygmunt, Praisley
995.16	Enzymatic		1995 First	McCleary, Codd

7.7 Delimitation problems

Regardless of the exact definition of dietary fibre, a number of delimitation problems have to be considered in the analysis. These include the starch/NSP cut-off, as well as the separation of sugars from polysaccharides by alcohol precipitation.

7.7.1 *Starch*

The removal of starch is dependent upon the solubilisation and enzyme systems used, any remaining starch appearing as dietary fibre glucans. With the discovery of physiologically resistant starch, i.e. starch that is not digested or absorbed in the small intestine of humans with physiological effects regarded as typical of dietary fibre, there is much in favour of including such starch in the dietary fibre. However, this has been vigorously opposed by Englyst *et al.* (1982, 1990). The AOAC methods include *in-vitro*-resistant starch after rather vigorous amylase treatment at high temperature. This starch represents mainly retrograded amylase (RS_3) and is lower than the *in-vivo*-resistant starch (Asp *et al.* 1996; Champ, Chapter 9, this volume).

7.7.2 *Low-molecular weight carbohydrates*

Precipitation in 78–80% ethanol, which is employed in all the current dietary fibre and NSP methods, is not an exact delimitation of polysaccharides [degree of polymerisation (DP) 10 or more]. For instance, arabinans in sugarbeet fibre were soluble in spite of a considerably higher DP, and thus were lost in enzymatic gravimetric dietary fibre determination. The fact that a higher recovery of these components was obtained with the Englyst method indicates that fibre components were solubilised by the proteolytic treatments included in the gravimetric assay (Asp *et al.* 1990).

Non-digestible oligosaccharides (NDO) are soluble in 80% ethanol, and are therefore not included in dietary fibre or NSP estimates with any of the current methods. The present interest in the physiological effects of NDO has stimulated the development of methods for their determination (e.g. Quigley & Englyst 1992), and authorities in an increasing number of countries accept the inclusion of NDO as dietary fibre for labelling purposes.

7.8 Future perspectives

There is an increasing consensus that carbohydrate measurement and classification should be based on specific determination of the different chemical classes of carbohydrates, i.e. mono-, di-, oligo- and polysaccharides, the latter being divided into starch and NSP (Koivistoinen *et al.* 1996; Anon. 1998). It is also evident, however, that such a chemical classification is not always helpful for the understanding of the physiological and nutritional role of various carbohydrates.

Nutritional groupings such as dietary fibre can then be made on the basis of physiological properties. The term dietary fibre should always be qualified by a statement itemising those carbohydrates that are intended for inclusion (Anon. 1998). As small-intestinal digestibility is a key feature differentiating carbohydrates that provide carbohydrate substrate for body cells from those used as fermentation substrates by intestinal bacteria, a first classification

Table 7.5 Suggested physiological classification of food carbohydrates.

Primary classification	Secondary characteristics	Fermentation properties
Glycaemic carbohydrates (digested and absorbed in the small intestine)	- Rate of digestion/absorption (glycaemic index) - Proportions of absorbed monomers (fructose/glucose)	
Dietary fibre (not digested/absorbed in the small intestine)	- Viscosity - Binding of water - Binding of ions, organic molecules, microorganisms - Fermentation	- Rate and site of fermentation - Fermentation products

should be glycaemic carbohydrates, and dietary fibre defined as including all undigestible carbohydrates. Methods are at hand for the determination of NDO, whereas methods of resistant starch determination require further fine-tuning. Needless to say, glycaemic carbohydrates and dietary fibre should have the same cut-off in order to avoid any starch fraction to be accounted for twice, or not at all.

As recommended in the recent FAO/WHO report (Anon. 1998) the terms extrinsic and intrinsic sugars, complex carbohydrates, and available and unavailable carbohydrates should be discontinued. Furthermore, this report recommended that the use of soluble and insoluble dietary fibre be gradually phased out since this division was not considered useful either analytically or physiologically.

The present emphasis on food carbohydrates from the nutrition point of view calls for nutritional grouping to describe carbohydrates for the purpose of epidemiological and clinical studies, as well as for more differentiated recommendations regarding carbohydrate intake. Such a classification, based upon the nutritional properties that appear most important for the time being, is shown in Table 7.5.

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8 Measurement of Dietary Fibre Components: the Importance of Enzyme Purity, Activity and Specificity

Barry V. McCleary

8.1 Total dietary fibre: introduction

Interest in dietary fibre is undergoing a dramatic revival, thanks in part to the introduction of new carbohydrates as dietary fibre components. Much emphasis is being placed on determining how much fibre is present in a food. Linking a particular amount of fibre to a specific health benefit is now an important area of research.

The term 'dietary fibre' first appeared in 1953, and referred to hemicelluloses, celluloses and lignin (Theander *et al*. 1995). Trowell (1974) recommended this term as a replacement for the no longer acceptable term 'crude fibre'. Burkitt (1995) has likened the interest in dietary fibre to the growth of a river from its first trickle to a mighty torrent. He observes that dietary fibre 'was first viewed as merely the less digestible constituent of food which exerts a laxative action by irritating the gut', thus acquiring the designation 'roughage' – a term later replaced by 'crude fibre' and ultimately by 'dietary fibre'. Various definitions of dietary fibre have appeared over the years, partly due to the various concepts used in deriving the term (i.e. origin of material, resistance to digestion, fermentation in the colon, etc.), and partly to the difficulties associated with its measurement and labelling (Mongeau *et al*. 1999). The principal components of dietary fibre, as traditionally understood, are non-starch polysaccharides (which in plant fibre are principally hemicelluloses and celluloses), and the non-carbohydrate phenolic components, cutin, suberin and waxes, with which they are associated in nature. In 1976, the definition of dietary fibre was modified to include gums and some pectic substances, based on the resistance to digestion of these components in the upper intestinal tract. For the purposes of labelling, Englyst *et al*. (1987) proposed that dietary fibre be defined as 'non-starch polysaccharides (NSP) in the diet that are not digested by the endogenous secretions of the human digestive tract'. Methods were concurrently developed to specifically measure NSP (Englyst *et al*. 1994).

8.1.1 *Measurement of NSP*

In the Englyst procedure (Englyst *et al. 19*94; Quigley and Englyst 1994) for the measurement of NSP, the sample is first defatted (if necessary) and then starch is completely removed. Starch removal is achieved by cooking the sample in dimethyl sulphoxide (which completely solubilises the starch), treatment with bacterial α-amylase and pullulanase, and finally precipitation of the dissolved fibre components with acidic alcohol. The starch fragments remain in solution and are removed. Pancreatin is added with the pullulanase to effect protein degradation. The precipitated material is recovered by centrifugation, washed with ethanol and acetone, and dried. This residue is acid hydrolysed and the sugar and uronic acid components are quantified by GLC, HPLC or by a colorimetric procedure. This procedure is used in the United Kingdom, but in other countries the AOAC dietary fibre procedures are the methods of

choice. The Englyst procedure has not received widespread adoption because, from a physiological point of view, NSP is less relevant than total dietary fibre. It is now generally accepted that resistant starch should be considered as part of dietary fibre.

8.1.2 *Measurement of total, soluble and insoluble dietary fibre*

An alternative – and now generally accepted – method for the measurement of dietary fibre is AOAC Method 985.29 (Prosky 1985), which has been modified to allow measurement of soluble and insoluble components (Prosky *et al*. 1988), and to allow the use of alternative buffers (AOAC method 991.43) (Lee *et al*. 1992). In principle, samples are treated with petroleum ether (if necessary) to remove fat, and then with enzymes to depolymerise starch and protein, which are subsequently removed in an ethanol precipitation step. The residue is dried and weighed and samples are taken for protein and ash determination. An extension of this method involves acid hydrolysis of the residue and analysis for sugars and uronic acids (the Uppsala method; AACC Method 32-25) (AACC 1997).

These methods are the culmination of the work of several research groups over many years. A major difference between these methods and the NSP procedure is that values obtained using the AOAC procedures include resistant starch and lignin. Resistant starch should be included under the 'umbrella' of dietary fibre, because it behaves in a manner similar to other dietary fibre components, in that it resists digestion in the small intestine and it is largely fermented in the colon, producing short-chain fatty acids (Champ *et al*. 1999).

8.1.3 *Enzyme activity*

Measurement of total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) by AOAC methods (985.29, 991.42, 993.19) requires the use of three enzymes, thermostable α-amylase (*Bacillus licheniformis*), amyloglucosidase (*Aspergillus niger*) and protease (*Bacillus licheniformis*). The effectiveness and purity of these enzymes for dietary fibre analyses is usually determined by analysing specific control samples and monitoring recovery, e.g. starch, casein, β-glucan, pectin and larch galactan. With the first two samples, the recovery should be close to zero, whereas with the latter three, the recovery should be approximately 100%. The actual activity of the individual enzymes and of contaminants of importance is usually not provided or measured, and this is due (in part) to the absence of generally accepted assay methods. To fill this need, traditional methods for the measurement of each of these activities were evaluated, and new rapid and quantitative methods for the measurement of the major activities and important contaminants were developed (McCleary 1999).

Two methods were evaluated for the measurement of α-amylase in the commercially available thermostable α-amylase preparations used for dietary fibre analyses (McCleary 1999). One of these methods was based on the Nelson/Somogyi reducing sugar procedure (Somogyi 1952) with soluble starch as substrate, and the second used a defined *p*-nitrophenyl-maltosaccharide as substrate, in the presence of a thermostable α-glucosidase (Ceralpha method using Amylase HR reagent) (McCleary & Sheehan 1987) (Fig. 8.1). It was found that the activity of α-amylase preparations currently available from major suppliers is similar, i.e. ~10 000 U/ml (soluble starch) or ~3000 U/ml (Ceralpha method). However, the activity of preparations of this enzyme supplied for use in AOAC dietary fibre procedures over the past five years have ranged from 2000 to 10 000 U/ml (activity on soluble starch). From the results

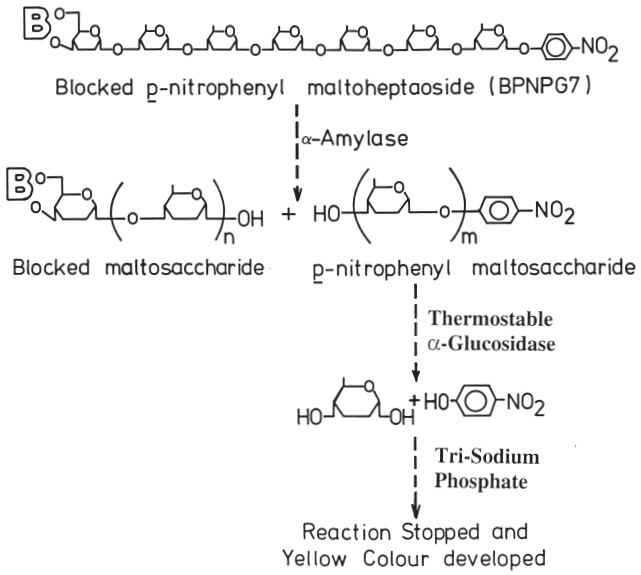

Fig. 8.1 Schematic representation of the measurement of α-amylase with Ceralpha reagent.

shown in Table 8.1, it is evident that the level of activity of this enzyme significantly affects the measured dietary fibre levels of certain samples, particularly resistant starch. Consequently, it is essential that the level of activity of this preparation be more clearly defined than has previously been the case. Since the preparations that are currently available commercially have an activity of approximately 10 000 ± 1000 U/ml on soluble starch (or 3000 ± 300 Ceralpha Units/ml), it would seem wise to set the required activity at this value.

Standardisation of the concentration of this enzyme is necessary to reduce the interlaboratory variations in reported values for resistant starch samples. From Table 8.1, it can be seen that for four commercial resistant starch materials (resistant starch A, B and C, and high-amylose maize starch), the level of α-amylase used in the assay has a very significant effect on the measured dietary fibre level. In AOAC Method 985.29, the recommended addition of α-amylase is 50 µl of preparations which are ~3000 Ceralpha Units/ml (i.e. 150 U/assay). It is evident, that higher or lower levels of enzyme will alter the determined dietary fibre levels for resistant starch samples. These variations have in the past caused conflict between analytical laboratories, but hopefully can now be avoided by using properly standardised enzyme preparations. Both described assays for the measurement of α-amylase give reliable and consistent results in the hands of a competent biochemist, but the Ceralpha method has the advantage of being extremely easy to use, even by analysts less experienced in biochemical assays.

Two methods have been compared for the assay of amyloglucosidase (McCleary 1999). One of these methods is more conventional; it employs soluble starch as substrate and the release of glucose is measured using glucose oxidase/peroxidase reagent. A second method employs *p*-nitrophenyl β-maltoside (PNPBM) as substrate in the presence of saturating levels of β-glucosidase (McCleary *et al*. 1991). In this method, as amyloglucosidase hydrolyses the terminal α-linked D-glucosyl residue, the excess levels of β-glucosidase give immediate

Table 8.1 Effect of the concentration of thermostable α-amylase and amyloglucosidase on determined dietary fibre contents of starch and resistant starch samples.

Polysaccharide	Enzyme preparation	Quantity (U/assay)	Total dietary fibre (%)
High-amylose*	Thermostable α-amylase‡	600 U	15.2
maize starch		300 U	23.4
		150 U	29.3
		75 U	34.2
		38 U	34.6
		19 U	38.3
Resistant starch A*	Thermostable α-amylase	600 U	29.5
		300 U	32.6
		150 U	34.8
		75 U	35.5
		38 U	35.8
		19 U	38.7
Resistant starch B*	Thermostable α-amylase	150 U	53.8
		75 U	55.5
		38 U	59.5
		19 U	61.0
Resistant starch C*	Thermostable α-amylase	600 U	28.1
		300 U	38.4
		150 U	43.3
Regular maize starch†	Amyloglucosidase§	40 U	0.08 ± 0.07
	Amyloglucosidase	20 U	1.3 ± 0.07
High-amylose	Amyloglucosidase	120 U	28.8
maize starch*	Amyloglucosidase	40 U	29.3
Casein†	Standard enzymes		1.38 ± 0.04
Pectin (ex. Sigma)†	Standard enzymes		89.0 ± 0.9
Pectin (ex. Megazyme)†	Standard enzymes		86.5 ± 1.0

* These samples were analysed once.
† Duplicate analyses were performed on these enzymes.
‡ α-Amylase activity is expressed as Ceralpha units. For all samples, an aliquot (50 µl) of enzyme is used, and the activities of the preparations are 380 to 12 000 U/ml. In the standard AOAC procedure, the concentration of the α-amylase is 3000 U/ml (or 150 U/assay).
§ Amyloglucosidase activity is expressed as PNP-Units bases on the assay using *p*-NP-β-maltoside.

hydrolysis of the β-linked D-glucosyl residue, releasing free *p*-nitrophenol that is detected by adding an alkaline solution. Both methods give quantitative measurement of amyloglucosidase. The level of activity of the amyloglucosidase preparation supplied by Megazyme is 200 U/ml on *p*-nitrophenyl β-maltoside (or 3300 U/ml on soluble starch), and 0.2 ml is used in the procedure (i.e. 40 U/test sample). Amyloglucosidase preparations supplied for use in TDF assay procedures have traditionally had an activity of about 130 U/ml on PNPBM (or ~2000 U/ml on soluble starch), and 0.3 ml of enzyme was used in standard AOAC methods (i.e. 40 U/test sample). This level of activity is adequate to ensure that all α-limit dextrins that are released on hydrolysis of starch by α-amylase, are hydrolysed to glucose in the standard assay format. The effect of using lower or higher levels of amyloglucosidase on the measured dietary fibre levels of regular maize starch and high amylose maize starch is shown in Table 8.1. With half of the recommended level of amyloglucosidase, the total dietary fibre values for regular maize starch increases only marginally (from <0.1% to 1.3%). Increasing the

level of amyloglucosidase to three times the recommended level has an insignificant effect on measured total dietary fibre in resistant starch samples, i.e. values for high-amylose maize starch decreased by less than 1%.

There are no reported problems associated with variations in the concentration of the protease used in dietary fibre determinations. This is most likely due to the fact that the level of enzyme used is more than adequate to give hydrolysis of all susceptible bonds in proteins in the samples being analysed. Also, any protein that is resistant to hydrolysis by the protease, and thus remains in the recovered fibre sample, is chemically measured and then subtracted in the calculations. However, since accurate and reliable assay procedures for standardising protease activity are desirable, two assay formats were evaluated (McCleary 1999). One method is a modification of traditional methods employing casein with TCA precipitation. This method was easy to use and give a linear standard curve over one absorbance unit. The second method employed Azo-Casein and a TCA precipitation step (Megazyme 1999). This assay has the advantage that it is more sensitive, the reaction products absorb in the visible range (440 nm) and the assay is specific for *endo*-protease. A standard curve for Subtilisin A on Azo-Casein is shown in Fig. 8.2. Activity Units for both assays were expressed as micromoles of tyrosine equivalents per min (Tyrosine Units). In evaluating enzyme preparations currently used in dietary fibre analyses (Subtilisin A), an activity of 6–8 tyrosine units/mg was obtained. In the standard AOAC dietary fibre methods, a protease concentration of 50 mg/ml is recommended (i.e. ~300–400 tyrosine Units/ml). Consequently, a concentration of 350 ± 50 U/ml was recommended for general use in dietary fibre determinations. A stabilised solution of this enzyme (350 U/ml) is commercially available (Megazyme cat. no. E-BSPRT).

8.1.4 *Enzyme purity*

Of the three enzymes used in dietary fibre determinations, only one – namely amyloglucosidase – was significantly contaminated with interfering activities. The thermostable

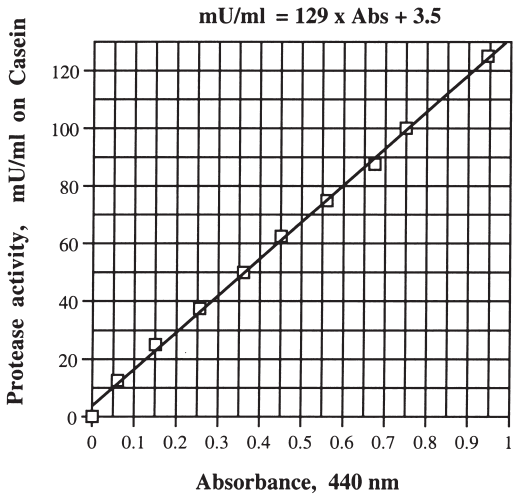

Fig. 8.2 Standard curve relating the activity of Subtilisin A on casein to action on Azo-casein (Megazyme Lot. 81001).

α-amylase and protease enzymes that are commercially available for use in dietary fibre measurement are generally free of interfering enzymes. Low levels of β-glucanase (*endo*-1,3:1,4-β–D glucanase; lichenase) have been detected in protease preparations, but these were well below the level which would interfere with the dietary fibre assay (i.e. that would result in a loss of β-glucan). The major contaminant in amyloglucosidase preparations (ex. *Aspergillus niger*) was shown to be *endo*-cellulase, and this resulted in *endo*-depolymerisation of mixed-linkage β-glucan from barley and oat, with a resultant underestimation of this component. The effect of cellulase contamination in amyloglucosidase preparations on the viscosity of barley β-glucan solutions is shown in Fig. 8.3, and the effect on analysed dietary fibre levels of pure barley β-glucan is shown in Table 8.2. It is evident that cellulase concentrations greater than 1.0 U/ml in the amyloglucosidase preparations (i.e. 0.2 U/assay) result in significant depolymerisation of barley β-glucan (Fig. 8.3) and loss of this component in dietary fibre analyses. Levels of cellulase equivalent to or higher than this concentration have been measured in some preparations of amyloglucosidase offered commercially for use in dietary fibre analyses. This contaminant can be readily detected and quantified using an assay procedure based on a substrate containing dyed, cross-linked β-glucan (Beta-Glucazyme tablets) (McCleary 1999). This simple test can assure that the enzyme is of adequate purity to ensure quantitative recovery of β-glucan, a major cereal based soluble dietary fibre component.

Another enzyme activity, which could cause significant underestimation of dietary fibre, particularly dietary fibre of fruit origin, is polygalacturonanase. This enzyme is most readily assayed using a viscometric method employing high-viscosity citrus pectin as substrate. It has been shown that the level of this enzyme in amyloglucosidase preparations used in

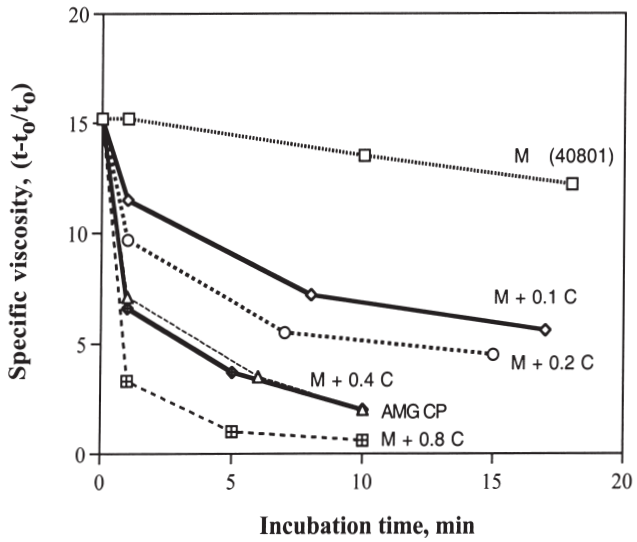

Fig. 8.3 Effect of *Aspergillus niger* cellulase on the viscosity of solutions of barley β-glucan. Assays were performed exactly as described in the text. To highly purified *A. niger* amyloglucosidase (lot 40801) cellulase was added at a concentration of 0.1, 0.2, 0.4 and 0.8 Units per 0.2 ml of amyloglucosidase, and 0.2 ml of these amyloglucosidase preparations were added to 10 ml of β-glucan solution (10 mg/ml, pH 4.5) in the viscometer.

Table 8.2 Effect of the concentration of cellulase contamination in amyloglucosidase on determined dietary fibre contents of pure barley β-glucan.

Polysaccharide	Added cellulase (Units/assay i.e./0.2 ml AMG)	Total dietary fibre (%)
Barley β-glucan (100 mg)*	0.0	99.9
	0.05	99.2
	0.10	99.1
	0.20	98.4
	0.40	90.0
	0.80	69.2

*All values are based on single determinations.

dietary fibre determinations were extremely low (McCleary 1999). On incubation of 0.2 ml of amyloglucosidase preparation (as used in dietary fibre analyses) with a solution of citrus pectin (10 ml, 10 mg/ml), there was only minor viscosity reduction over an incubation period of 20 min. This viscosity reduction was equivalent to a polygalacturonanase concentration of less than 0.01 U/assay (i.e. per 0.2 ml of amyloglucosidase), which is below the level which would result in pectin loss in AOAC dietary fibre assays (about 0.20 U/test). However, other enzyme preparations, that may be incorporated into AOAC total dietary fibre procedures, may contain this contaminating activity (e.g. Fructozyme® which is used to remove fructans) (Quemener *et al*. 1993, 1997).

The TDF values obtained for casein and pectin (ex. Megazyme TDF Controls Kit) and pectin from Sigma Chemical Co. (dietary fibre control) are also shown in Table 8.1.

The dietary fibre value obtained for casein was as expected, but the value for the control pectin samples was much lower than expected. This was not due to a pectinase contamination in the amyloglucosidase, as the level of this activity in the enzyme used (amyloglucosidase, E-AMGDF, Megazyme International) was negligible (less than 0.01 U per 0.2 ml of amyloglucosidase) and the levels in protease and thermostable α-amylase preparations were undetectable. The low recovery must be due to incomplete precipitation of the pectin in 78% ethanol. The reason for this is not apparent, as both pectins used were quite pure, high-molecular weight materials.

8.2 Specific dietary fibre components

8.2.1 *1,3:1,4 β-D-Glucan (mixed-linkage β-glucan)*

Plant-derived dietary fibre is generally considered to include all plant components except protein, non-resistant starch, lipids and low-molecular weight materials not precipitated by 80% ethanol. The first of this mixture of components to attract individual attention was mixed-linkage β-glucan (β-glucan) from cereal grain sources, particularly barley and oats. The interest in this polysaccharide was catalysed by the numerous health claims concerning the use in human nutrition of oat fibre products rich in β-glucan (Anderson and Bridges 1993). The Food and Drug Administration (USA) has allowed the claim that the inclusion of oat products in the diet may reduce the risk of heart disease.

A specific procedure for the measurement of β-glucan was developed in 1985 (McCleary & Glennie-Holmes) (Fig. 8.4), and then further simplified (McCleary & Codd 1991). In prin-

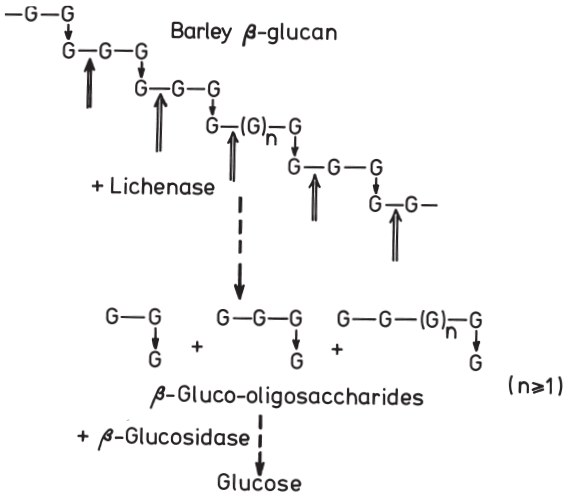

Fig. 8.4 Schematic representation of the measurement of β-glucan with lichenase and β-glucosidase (AOAC Method 995.16).

ciple, β-glucan in the sample is hydrated by cooking a slurry of the flour, followed by depolymerisation with a specific 1,3:1,4 β-glucanase (lichenase) enzyme. This enzyme cleaves the (1→4)-glycosidic linkage of a 3-linked D-glucosyl residue within the β-glucan chain and thus has no action on (1→4) β-linked D-glucans (cellulose). The oligosaccharide reaction products released on hydrolysis of β-glucan by lichenase are then hydrolysed to glucose with a highly purified β-glucosidase, and the glucose is measured enzymatically with glucose oxidase/peroxidase reagent. Since the procedure is used to measure trace levels of β-glucan in cereal and food products, which also contain high levels of starch and other glucose containing oligosaccharides, such as malto-oligosaccharides and sucrose, the enzymes must be very pure. Invertase contamination of β-glucosidase will give glucose release from sucrose, and contamination by α-glucosidase or amyloglucosidase will result in release of glucose from malto-oligosaccharides. In both cases, an inflated estimate of β-glucan content will result. The purity of β-glucosidase can be tested by incubating a range of concentrations of the enzyme with aliquots of lichenase treated barley flour slurry (as per the standard β-glucan assay format). If the enzyme is devoid of interfering activities, the same glucose absorbance value should be obtained. The results of such an experiment are shown in Fig. 8.5. Normally, 0.2 U of β-glucosidase is used per assay, but it is evident that even with a 75-fold excess (15 U/assay) of this enzyme, the determined β-glucan content is only very slightly (~3%) overestimated.

β-Glucosidase from *A. niger* is very effective in catalysing complete hydrolysis, to glucose, of the mixed-linkage β-gluco-oligosaccharides released on hydrolysis of barley or oat β-glucan by lichenase. However, this is not the case for the β-glucosidase from almond emulsin (Fig. 8.5). The almond enzyme, even at very high levels of activity, does not completely hydrolyse these oligosaccharides, leading to an underestimation of the β-glucan content of the sample.

Following extensive international interlaboratory evaluations, this assay procedure has become the International standard method for the measurement of mixed-linkage β-glucan

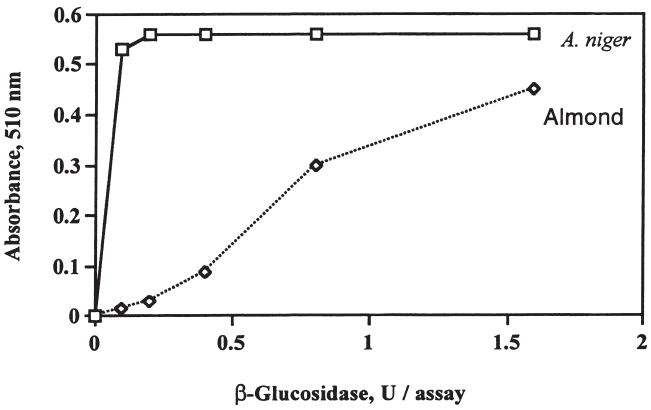

Fig. 8.5 Hydrolysis of β-gluco-oligosaccharides (from lichenase treatment of mixed-linkage β-glucan) by highly purified *A. niger* and almond-seed β-glucosidases.

(AOAC 995.16; AACC Method 32-23; ICC Standard No. 166; EBC Methods 3.11.1, 4.16.1 and 8.11.1). The method has been adapted for the analysis of mixed-linkage β-glucan in cereal and food products and in liquid materials such as wort and beer.

8.2.2 *Resistant starch*

Until recently, it was generally thought that starch was completely digested in the small intestine. However, it is now recognised that there is a portion (resistant starch, RS) which resists digestion, passes into the large intestine and is fermented there. Three types of RS have been identified: (1) physically trapped starch; (2) resistant starch granules; and (3) retrograded starch (Muir *et al*. 1993; Englyst *et al*. 1994). Physically trapped starch granules are locked within the food matrix, such that digestive enzymes are prevented or delayed from having access to them. Resistant starch granules, as are present in raw potatoes and green bananas, resist attack by α-amylase, which is probably due to the crystalline nature of the starch. From an industrial and food technology perspective, the most interesting resistant starches are those which are produced through retrogradation of gelatinised starches. Retrogradation is generally attributed to the amylose portion of the starch, so starches high in amylose are particularly amenable to the production of resistant starch.

Since resistant starch is defined as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals' (Asp 1992; Muir *et al*. 1993), any analytical method for RS should take into account all the starch and α-dextrins covered in this physiological definition. Furthermore, the method should be validated using *in-vivo* data from healthy human subjects. The various procedures for the measurement of resistant starch have been summarised and discussed by Champ *et al*. (1999). The methods can be broadly grouped into two approaches. In one approach (Englyst *et al*. 1992; Muir *et al*. 1993), samples are analysed for total starch and for soluble starch, and the latter is subtracted from the former to give resistant starch. The major inherent problem with this approach, analytically, is that the content of resistant starch is generally a small proportion of total starch. Thus, the resistant starch value is obtained by subtracting one large analytical value (for non-resistant starch) from another similar large value (for total starch), meaning that analytical precision is greatly

reduced. In the second approach (Champ 1992; McCleary *et al*. 1997), the sample is treated with starch-degrading enzymes to remove the non-resistant starch, which is washed away from the residue. The residue is then analysed. By definition, this approach must be analytically more precise. The procedure should be used in conjunction with AOAC Method 985.29 to determine total dietary fibre in the sample.

In most TDF procedures, the hydrolytic conditions are quite severe, resulting in underestimation of resistant starch. If the final total dietary fibre value is to be the sum of TDF determined by AOAC Method 985.29 plus resistant starch determined by a method such as that of Champ (Champ 1992; Champ *et al*. 1999), then the amount of resistant starch measured in AOAC Method 985.29, must be allowed for or removed (otherwise it will be counted twice). A possible way to handle this is to pre-treat samples with hot dimethyl sulphoxide (DMSO) or sodium hydroxide prior to TDF analysis by AOAC Method 985.29. The DMSO treatment should dissolve all of the starch, allowing complete hydrolysis by α-amylase and amyloglucosidase, and subsequent removal in the alcohol treatment step. Of course, such a treatment will alter the ratio of soluble to insoluble fibre as determined by AOAC Method 991.43. A DMSO treatment step is used in AOAC Method 996.11 for the measurement of total starch (McCleary *et al*. 1997) and in the Englyst *et al*. (1994) NDF procedure.

8.2.3 *Oligofructan and inulin*

Fructans are widely distributed in the plant kingdom. They are defined as any compound in which one or more fructosyl–fructose linkages constitute a majority of the linkages. They are generally classified as inulin, levan or graminan types, based on linkage types. In nature, inulin occurs as a series of oligosaccharides with degrees of polymerisation from 2 to 60 (Pontis 1990; Lewis 1993). It consists principally of linear chains of fructosyl units linked α-(2-1), ending with a glucosyl unit. Inulin and oligofructose resist hydrolysis by human digestive enzymes, but are fermented in the large intestine. They thus have the same physiological action as dietary fibre.

In recent years, interest in the measurement of fructans such as inulin and oligofructose has been stimulated by applications made to regulatory authorities for acceptance of fructans as dietary fibre components for food labelling purposes (Coussement 1999).

Since fructans are largely soluble in 80% ethanol, they are not significantly measured in currently used dietary fibre methods. Several methods have now been developed for the specific measurement of fructans, and these rely on the complete hydrolysis of the oligofructose and inulin to fructose and glucose, which are measured either instrumentally (Quemener *et al*. 1993; Hoebregs 1997) or chemically (McCleary & Blakeney 1999; McCleary *et al*. 2000). In some procedures, the separate quantities of fructan, sucrose, glucose and starch are determined by hydrolysis with specific enzymes, followed by chromatographic procedures (AOAC Method 997.08) (Hoebregs 1997). In a procedure developed in the author's laboratory (McCleary & Blakeney 1999), fructan is measured chemically, after removal of sucrose and starch through enzymatic hydrolysis, followed by borohydride reduction (Fig. 8.6). Sucrose is completely hydrolysed to glucose and fructose with a specific sucrose-degrading enzyme (sucrase; α-glucosidase) with no detectable hydrolysis of fructo-oligosaccharides, not even kestose (Fig. 8.7). The relative rates of hydrolysis of sucrose and kestose (under optimal assay conditions (i.e. pH 6.5 and 40°C, and at a substrate concentration of 10 mg/ml) was 3800 : 1. Since starch and maltosaccharides are unstable in the highly alkaline conditions used in the PAHBAH reducing sugar method, it is essential to remove these from the sample

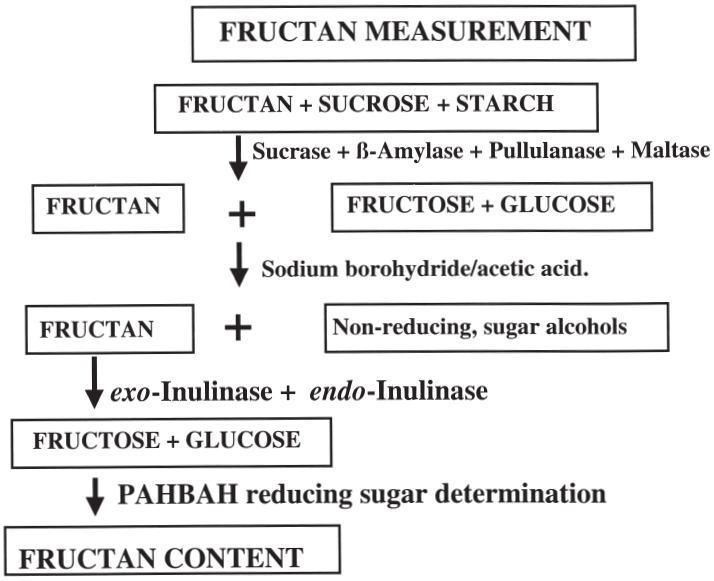

Fig. 8.6 Schematic representation of an enzymic procedure for the measurement of fructan (inulin) (AOAC Method 999.03).

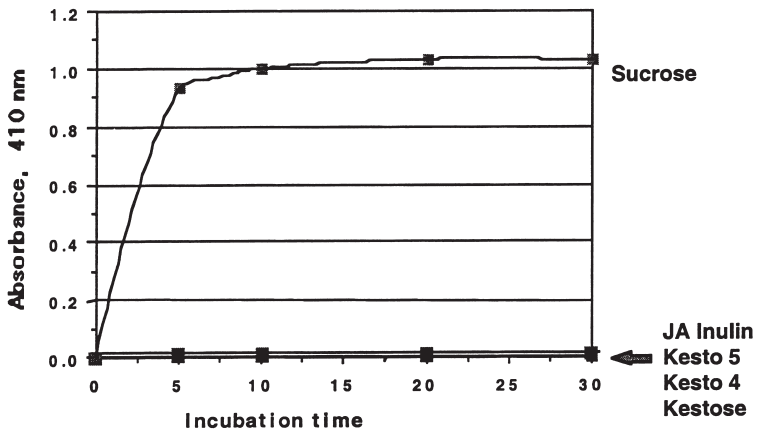

Fig. 8.7 Hydrolysis of sucrose and fructans by sucrase (α-glucosidase). Sugar compound (0.2 ml, 50 μg) was incubated with sucrase (1 unit) in sodium maleate buffer (100 mM, pH 6.5) at 40°C. The reaction was terminated at various time intervals with PAHBAH working reagent, and the colour developed.

extract. This is achieved by hydrolysis to glucose by the combined action of pullulanase, β-amylase and maltase, followed by borohydride reduction. The level of each enzyme used was 10 times that required for complete hydrolysis. This combination of enzymes was chosen to allow starch hydrolysis to be performed concurrently with hydrolysis of sucrose by sucrase (i.e. at pH 6.5 and 40°C).

In the development of the current procedure for the assay of inulin and fructo-oligosaccharides, two inulin-degrading enzymes were purified, *exo*-inulinase and *endo*-inulinase. Thin-layer chromatographic patterns of the products released on hydrolysis of onion, wheat and high degree of polymerisation (DP) chicory fructans by *exo*-inulinase showed that the only reaction products are fructose and glucose (McCleary 1999). Wheat and chicory fructan are hydrolysed less rapidly than onion fructan. The slower rate of hydrolysis of the wheat fructan is probably due to the highly branched nature of this mixture of oligosaccharides. The Bio-Gel P-2 chromatographic patterns of onion fructo-oligosaccharide mixture, before and after incubation with *exo*-inulinase (Fig. 8.8) demonstrate that with the level of enzyme used, hydrolysis to fructose and glucose is complete. Incubation of chicory fructan with *endo*-inulinase produces an accumulation of oligosaccharides of DP 4, 5 and 6.

In the final assay format, the inulin and oligofructose are hydrolysed to fructose and glucose using a highly purified *exo*-inulinase enzyme (to which a small amount of *endo*-inulinase has been added). The released sugars are measured with a reducing-sugar method. In initial work, it was considered that rapid hydrolysis of high-molecular weight inulin by *exo*-inulinase may require the presence of *endo*-inulinase. However, as shown in Table 8.3, addition of *endo*-inulinase (at 10% the level of *exo*-inulinase) has an insignificant effect on the level of measured inulin, when more than 18 U of *exo*-inulinase is used per assay. A major problem with the addition of *endo*-inulinase is the fact that in the purification of this enzyme it is difficult to completely remove β-glucosidase and cellulase. After extensive purification, contamination of *endo*-inulinase with β-glucosidase is about 1%, and with cellulase is 0.001%. When this is mixed with *exo*-inulinase at a ratio of 1 : 10, the final contamination with β-glucosidase is thus just 0.1%. However, this level of contamination is sufficient to give an overestimation of the fructan content of cereal flours (up to 20%) through hydrolysis of β-glucan and release of glucose. For this reason, the level of *endo*-inulinase in the fructanase mixture used in the standard fructan assay procedure has been reduced to just 1% of the *exo*-inulinase. Under these conditions, the level of contaminating β-glucosidase has an insignificant effect.

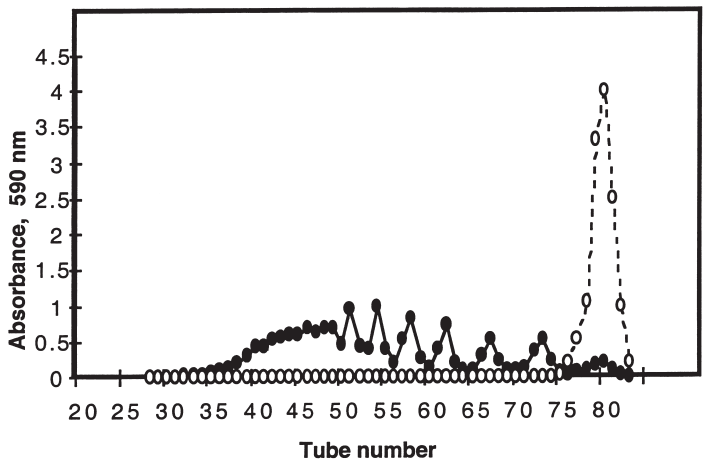

Fig. 8.8 Bio-Gel P-2 chromatography of the sugars produced on hydrolysis of onion fructan by *exo*-inulinase. Column eluates were analysed by the phenol–sulphuric acid procedure. Incubated for 0 min (●) and 60 min (○).

Table 8.3 Effect of concentration of *exo*-inulinase and presence or absence of *endo*-inulinase on the determined fructan content of selected samples.

Sample and amount (U/assay) of exo-inulinase*	Determined fructan content, % w/w (db)	
	exo-inulinase	*exo*- plus *endo*-inulinase
Dehydrated onion		
36	58.8	61.1
18	58.6	58.9
9	57.8	58.0
4.5	57.2	55.6
Wheat stalks		
36	5.3	5.3
18	5.1	5.1
9	4.7	4.6
4.5	4.2	4.2
Chicory		
36	98.7	98.9
18	93.4	97.8
9	90.0	93.4
4.5	81.8	83.5

* The level of *endo*-inulinase in each incubation was 10% of the level of *exo*-inulinase.

exo-Inulinase acts on galactosyl-sucrose oligosaccharides (e.g. raffinose and sucrose) to produce a series of galactosyl-glucose oligosaccharides (e.g. melibiose from raffinose). Thus, the presence of these oligosaccharides in the samples that are being analysed will result in an overestimation of the fructan content. This may be of significance in the analysis of food materials that contain legume seed material as well as fructo-oligosaccharides. With such samples, if an accurate measurement of fructo-oligosaccharides is required, then extracts of the samples being analysed should be pre-treated with α-galactosidase (plus sucrase) to catalyse complete hydrolysis of galactosyl-sucrose oligosaccharides to galactose, glucose and fructose. This is best performed in the pre-incubation step in which sucrose and starch are hydrolysed and removed (by borohydride reduction).

The specific advantages of this assay procedure for inulin and fructo-oligosaccharides are that highly purified enzymes are used and the assay can be performed with basic laboratory equipment. The method has been evaluated on a range of fructan-containing materials, including both natural and compounded food products, and has been subjected to an extensive international interlaboratory evaluation under the auspices of AOAC International (McCleary *et al*. 2000). This study involved 15 laboratories and 18 samples (nine blind duplicates). Samples included a range of native materials as well as a range of commercial food products such as milk powder, chocolate and low-fat spreads (to which inulin had been added). Repeatability relative standard deviations (RSD_r) ranged from 2.3 to 7.3% and reproducibility relative standard deviations (RSD_R) ranged from 5.6 to 11.5%. The mean value obtained for 'pure' fructan was 95.8% (on an as-is basis. On the basis of these results, the method has been recommended by AOAC International for First Action Approval (Method 999.03; McCleary *et al*. 2000).

Fructans comprise a series of oligosaccharides, some of which are precipitated with alcohol in the total dietary fibre assay procedure, but others – the lower DP oligomers – remain

soluble at this concentration of alcohol. This introduces a complication into dietary fibre analyses. A proposed solution to this problem (Quemener *et al*. 1993, 1997) is to treat samples for dietary fibre analysis with a fructanase mixture to catalyse completely depolymerisation of the fructan (and thus complete solubilisation in 80% alcohol). This step ensures that none of the fructan is measured in AOAC total dietary fibre procedure. The fructan is then measured separately and added to the dietary fibre value. For this modification to give reliable results, the fructanase mixture used to hydrolyse the fructan must be devoid of enzymes active on other dietary fibre components. Quemener *et al.* (1997) recommended the use of heat-treated Fructozyme®. Heat treatment was shown to effectively inactivate most of the pectin degrading activities in the preparation, and resulted in quantitative pectin measurement in the AOAC total dietary fibre method 985.29. However, Fructozyme® also contains high levels of α-galactosidase and β-glucanase (cellulase) (Table 8.4). The β-glucanase is not inactivated by the heat treatment step recommended by Quemener *et al*. (1993) (60°C, 2 h) and gives a rapid depolymerisation of β-glucan, resulting in a significant underestimation of this component in AOAC method 985.29 (McCleary 1999). This β-glucanase contamination can, however, be removed chromatographically (Megazyme Data Sheet; E-FRMXLQ) (Table 8.5) allowing the use of the enzyme as proposed by Quemener *et al.* (1997).

8.2.4 *Polydextrose*

Polydextrose is prepared by vacuum thermal polymerisation of glucose, using sorbitol as plasticiser and citric acid as catalyst (Craig *et al*. 1999). This random polymerisation and branching yields various types of glucosidic bonds in the structure with α-1,6 bonds predominating. The compound includes covalent linkages to sorbitol and citric acid, and has an average degree of polymerisation of 12. Polydextrose is an approved food additive in over 40 countries. It is not hydrolysed by normal human digestive enzymes, but it is fermented in the colon like many other fibres.

Polydextrose is not quantitatively measured with AOAC Method 985.29 because, as is the case with other resistant oligosaccharides (RO), it is not completely precipitated with 80% ethanol. Because of the complex chemical nature of the compound, measurement cannot be achieved through specific enzymatic hydrolysis. Consequently, analytical procedures

Table 8.4 Levels of inulinases, cellulase, polygalacturonanase (pectinase) and α-galactosidase in Fructozyme®, heat-treated Fructozyme® and a purified fructanase mixture (ex. Megazyme).

	Enzyme activity (Units/ml)*		
	Fructozyme	HT Fructozyme†	Pure Fructanase Mixture‡
exo-Inulinase	2000*	1200	2000
endo-Inulinase	200	200	200
Pectinase	40	4.1	0.4
Cellulase	4.0	4.0	0.1
α-Galactosidase	284	10.2	0.05

* One Unit of activity is the amount of enzyme required to release 1 μmol product/min under standard assay conditions (40°C, pH 4.5).

† The Fructozyme was pre-heated (HT Fructozyme) at 60°C for 120 min.

‡ This preparation is available from Megazyme (cat. no. E-FRMXLQ).

Table 8.5 Effect of addition of crude and purified fructanases on the recovery of inulin and β-glucan in the AOAC International total dietary fibre assay procedure.

		Recovery (%)	
Enzyme preparation	Quantity	Inulin (Fructan)	β-Glucan
Heat-treated Fructozyme*	0.2 ml	0.2 ± 0.2	11.4 ± 0.4
	0.1 ml	0.2 ± 0.2	52.0 ± 2.0
	0.05 ml	7.2 ± 0.3	80.0 ± 1.2
Pure Fructanase mixture	0.2 ml	0.2 ± 0.2	99.0 ± 1.3
	0.1 ml	0.2 ± 0.2	100.8 ± 0.8
	0.05 ml	3.5 ± 0.2	98.5 ± 1.8

* All samples were analysed in duplicate.
The levels of enzyme activities in heat-treated Fructozyme and the pure fructanase mixture are as shown in Table 8.4. The amount of β-glucan used in assays was 100 mg.

depend on aqueous extraction of the sample being analysed, followed by enzymatic hydrolysis of other oligosaccharide and polysaccharide materials that are likely to interfere in subsequent chromatographic quantitation. Highly purified enzymes active on starch, cellulose, pectins and gums (e.g. galactomannans), have been employed (Craig *et al*. 1999).

8.2.5 *Galacto-oligosaccharides*

Galacto-oligosaccharides [α-D-Glc*p*(1–1)-β-D-Gal*p*-containing oligosaccharides) have been shown to be beneficial to human health through promotion of the growth of bifidobacteria in the large intestine (Fransen *et al*. 1998). The oligogalactosylated glucoses are prepared from lactose through the transgalactosylating activity of β-galactosidase. Various parameters, such as the source of the enzyme, substrate concentration, pH and temperature influence the transgalactosylation and thus, the final yields of the different products. Typically, the reaction products are composed of about 60% transgalacto-oligosaccharides (mainly tri-, tetra- and pentasaccharides) and about 40% of a mixture of lactose, glucose and galactose.

Alternative galacto-oligosaccharides are the galactosyl-sucrose oligosaccharides of plant origin, namely raffinose, stachyose and verbascose (Dey 1978). These oligosaccharides consist of sucrose, to which an -extended chain of 1,6 α-linked galactosyl residues is attached to C-6 of the glucosyl residue. Raffinose is a major by-product of sugar crystallisation from sugarbeet extracts, and it has been shown to promote the proliferation of bifidobacteria in the human intestinal microflora.

Acceptance of galacto-oligosaccharides as soluble dietary fibre components requires the development of analytical procedures that will specifically measure these components in food mixtures. The lactose-derived β-galacto-oligosaccharides are effectively hydrolysed with *A. niger* β-galactosidase, while the galactosyl-sucrose oligosaccharides are rapidly and quantitatively hydrolysed by *A. niger* α-galactosidase. The released sugars can be measured, either chromatographically, or using various colorimetric procedures. In both cases, the specificity of the procedure depends on the purity of the enzyme used. A procedure for the measurement of transgalacto-oligosaccharides is the subject of an upcoming AOAC International, interlaboratory study. A major consideration is the measurement of the transgalacto-oligosaccharides independently of lactose present in the sample.

8.3 Conclusions

In conclusion, success in the introduction of 'new' dietary fibre components will be dependent on identifying a specific health benefit of the component, as well as providing an analytical procedure that will allow accurate measurement of this component in complex food mixtures. Development of such procedures will require the use of highly purified enzymes of known activity and specificity to either hydrolyse the fibre component to components that can be analysed, or alternatively, to remove other fibre components which will interfere in the assay.

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9 *In-vivo* and *In-vitro* Methods for Resistant Starch Measurement

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9.1 Introduction

Resistant starch (RS) is present in a number of foods, and constitutes up to 15% of the dry matter of the product. Several studies indicate that its presence in foods might be beneficial for health (Asp *et al.* 1996). RS is, by definition, not absorbed in the small intestine (Asp 1992), and thus it does not contribute to postprandial hyperglycaemia (Ranganathan *et al*. 1994). Its effect on lipid metabolism has recently been investigated. RS, if added to the meals, might be beneficial for patients with subnormal or abnormal level of lipaemia (Faisant *et al*. 1995b). Moreover, some animal studies indicate a serum cholesterol-lowering effect, but no evidence of similar effect on humans has been demonstrated (Heijnen *et al*. 1996). RS is largely fermented, producing short-chain-fatty acids and bacterial cells. It may be important in determining colonic epithelial cell health through effects on bile acids, butyrate production and moderation of nitrogen metabolism (Cummings *et al*. 1995; Hylla *et al*. 1998). A number of studies of the effect of RS on bowel habit have been reported, but findings are somewhat inconsistent (Cummings *et al*. 1995).

The interest in RS has led to the need for a valid analytical method for its quantification in foods. RS has been defined as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals' (Asp 1992). An analytical method of RS should then take into account all the starch and α-dextrins covering this physiological definition. Furthermore, it should be validated using *in-vivo* data from healthy subjects.

A physiological definition is probably the most intellectually satisfying, but it is particularly difficult in this case. Indeed, there are no analytical differences between digestible starches (DS) and resistant starches (RS). The differences between DS and RS are linked to the structural organisation of the starch and/or to its environment, which affect enzyme accessibility to its substrate. Moreover, starch digestion is dependent on the efficiency of chewing, transit time (in the stomach and small intestine) and enzyme secretion – all factors which may vary from one subject to the next.

Therefore, an *in-vitro* method has to provide a result that should be similar to the average response of the healthy population. It should then be validated with a large range of RS sources on a large number of subjects.

The second difficulty is the choice of the method used to obtain the *in-vivo* RS values, which are necessary for the validation. Two methods are available: (1) the intubation of healthy subjects to collect the residual starch at the end of the ileum; and (2) the involvement of ileostomates. Both techniques are opened to criticism, and the advantages and disadvantages of each will be discussed later in the chapter.

9.2 Classification of resistant starches

The increasing knowledge of starch digestion in humans has allowed a new classification of RS that is commonly approved (Englyst *et al*. 1992, 1996). Three classes of RS are usually identified:

(1) Physically inaccessible starch (RS_1). This is found in partly milled grains and seeds. Legumes such as beans or lentils are known to be one of the main sources of RS. The preparation and cooking process is of great importance in the RS content of the food.
(2) Resistant starch granules (RS_2). B-type starches when uncooked are known to be very resistant to enzymatic hydrolysis. B-type refers to the X-ray diffraction pattern of the starch. Raw starches are classified in three main types: A (e.g. most cereal starches, cassava starch); B (e.g. potato and banana starches) (Englyst & Cummings 1986; Faisant *et al*. 1995a); and C (most legume starches). Banana is the main source of RS_2 in the human diet. Starch is converted into simple sugars and sucrose during ripening of the fruit, so only non-ripe banana still has a significant amount of starch. Most of the cooking procedures are able to gelatinise raw starches, allowing the disappearance of RS_2 in the food.
(3) Retrograded starch (RS_3). This is present in most starchy foods which have been cooked and then cooled and stored from several hours to several months. The retrogradation is a recrystallisation of starch granules that occurs after the gelatinisation, and which implies that mainly the linear fraction of the starch – the amylose – is involved. Amylopectin can also retrograde, though this occurs over a much longer period than that required for amylose. Potatoes that are cooked and then cooled have been shown to contain RS_3 (Englyst & Cummings 1987). Re-heating of starch reduces the RS_3 content of the potato, showing that the retrogradation is partly reversible. Several cycles of heating then cooling allow an increase in the RS_3 levels.

RS_1, RS_2 and/or RS_3 can coexist in a same food. Indeed, for example, a meal of beans contains both RS_1 and RS_3 (Noah *et al*. 1998) whereas RS_1 and RS_2 are present in bananas (Faisant *et al*. 1995a).

A fourth type of RS (RS_4) has recently been described; this includes the fraction of the chemically modified starches (starch ethers and esters as well as cross-bonded starches) which is resistant to small intestine digestion (Baghurst *et al*. 1996; Brown *et al*. 1998).

9.3 *In-vivo* methods

We can consider that three methods are available to obtain *in-vivo* values on the resistant starch content of foods. One of these methods is indirect, but it has been used to estimate RS. The three methods are presented in Table 9.1, together with their advantages and failures. These methods have been described in more detail earlier (Champ *et al*. 1999b). Some of the results obtained with these methods are described in Table 9.2.

9.3.1 *Hydrogen breath test*

Hydrogen is one of the end products of carbohydrate fermentation, and is formed exclusively

Table 9.1 Advantages and shortcomings of the studies performed with humans.

H_2 breath test	
Determination of the increase in hydrogen in the breath after the consumption of malabsorbed carbohydrates	
Advantages	Shortcomings
• Simple and non-invasive	• Semi-quantitative
• Healthy subjects	• Strict standardisation necessary
	• Large intra- and inter-individual variation in H_2 excretion
Ileostomy model	
Patients who have had a colectomy for ulcerative colitis and Crohn's disease	
Advantages	Shortcomings
• Direct collection of the ileal effluent = > quantitative	• Cannot be considered as healthy
	• Physiological adaptation
• Easy to perform	• Water and electrolytes absorption
	• Bacterial overgrowth
	• Transit time (≠ from normal)
Intubation of healthy subjects	
Collection of the ileal content in healthy subjects after intubation using a constant perfusion technique of solution containing an unabsorbable marker	
Advantages	Shortcomings
• Healthy subjects	• Disturbance of the normal physiology by the long triple lumen tube
• Direct collection of the ileal effluent	• Quantification of the flow rate using a liquid phase marker
	• Risk of selectivity of the tube in case of heterogeneous food
	• Expensive and time-consuming

Source: adapted from Bach-Knudsen (1991)

in the colon as a result of bacterial fermentation. The hydrogen gas is partly absorbed and cleared in a single passage of the lungs, and then excreted from the lungs in the expired air. The results of gas perfusion techniques have suggested that a rather constant fraction of the total H_2 production is excreted by the lungs, and that rates of breath H_2 and H_2 production correlate well (Levitt 1969). Flourié *et al*. (1988) used a non-absorbable but rapidly fermented oligosaccharide (lactulose) to 'calibrate' the subject. This was considered to be a good control to allow quantification of malabsorbed carbohydrates. The calculation is usually performed by calculating a ratio between the areas under the curves after the test meal, and again after the lactulose. Knowing the amount of lactulose fermented, then the amount of carbohydrate from the experimental meal – which has in theory been fermented – can be calculated. Although the principle appears quite simple, there are several theories on the best way to perform this quantification (Rumessen 1992).

Although this procedure is quantitative for oligosaccharides, it is only qualitative for insoluble or slowly fermented substrates. Thus the method is not appropriate for the quantification of RS.

9.3.2 *Ileostomy model*

The ileostomy model offers a method for direct and quantitative determination of small-bowel excretion, provided that the bacteriological degradation of the effluent can be minimised. The subjects who take part in these studies have had a conventional ileostomy after

Table 9.2 Malabsorption of starch estimated on humans by different techniques.

Source of starch	Starch ingested (g)	Malabsorption (%)	Reference
H_2 breath test			
HACS, raw	30	9.2	(a)
HACS, complexed	30	9.5	(a)
HACS, retrograded	30	5.5	(a)
Cooked potatoes	60	6.7	(b)
Cooked and cooled potatoes	60	22.8	(b)
Cooked, cooled and warmed-up potatoes	60	5.0	(b)
White bread	120	10.7	(c)
Wholemeal bread	120	8.3	(c)
Wheat flour	50	2.8	(e)
Wholemeal	50	13.6	(e)
Oat flakes (raw)	35	7.8	(e)
Oat flakes (cooked)	35	5.8	(e)
Lentils	120	17.6	(c)
Beans	100	38.0	(d)
Green banana	100	38.6	(f)
Banana + rice	NM	0.0	(g)
Ileostomy model			
Rice + bread	98–127	0.5	(h)
Bread	107	0.8	(i)
White bread	100	13	(c)
White bread	61.9	2.5	(j)
Wholemeal bread	100	11	(c)
Oat flakes	57.8	2.2	(j)
Corn flakes	74.2	5.0	(j)
Cooked potatoes	45.4	3.3	(j)
Cooked and cooled potatoes	47.2	11.9	(j)
Cooked, cooled and warmed-up potatoes	47.6	7.7	(j)
Banana (ripe)	3.0	55.7	(k)
Banana (unripe)	13.9	84.0	(k)
Lentils	100	21	(c)
Intubation of healthy subjects			
HACS, complexed	32.5	21.1	(l)
HACS, retrograded	32.5	50.9	(l)
Banana (unripe)	23.1	83.7	(m)
Banana + rice	20	10.2	(g)
Banana + rice + potatoes + beans	61	8.0	(g)
Bread + pasta + potatoes	100	5.2	(n)
Bread + pasta + potatoes	300	4.1	(n)
Beans	68.5	16.5	(o)

HACS, high-amylose corn starch; NM, not measured.
(a) Bornet *et al.* (unpublished data); (b) Scheppach *et al.* 1991; (c) Wolever *et al.* 1986; (d) Levitt *et al.* 1987; (e) Lund & Johnson 1991; (f) Christl *et al.* 1992; (g) Stephen *et al.* 1983; (h) Sandberg *et al.* 1981; (i) Sandberg *et al.* 1986; (j) Englyst & Cummings 1985; (k) Englyst & Cummings 1986; (l) Champ *et al.* 1998; (m) Faisant *et al.* 1995a; (n) Flourié *et al.* 1988; (o) Noah *et al.* 1998.

proctocolectomy for ulcerative colitis. This surgery is performed by eversion of the distal 5–10 cm of the small bowel, which is pulled onto the abdominal wall as a fistula (Andersson 1992). These people can easily collect their effluent in a bag, which is usually changed every 2 h during the daytime. The ileostomy bags are immediately deep-frozen (on dry-ice when possible). After the experimental meal, the subjects usually fast for 4 h, after which they are given several snacks (plant polysaccharide-free) (Langkilde & Andersson 1995).

9.3.3 *Intubation technique*

Healthy volunteers are intubated with a triple-lumen polyvinyl tube, the transit time of which down the gut is aided by a terminal inflatable bag containing mercury. When the bag reaches the caecum, as confirmed fluoroscopically, it is deflated and the subjects have to remain in a semi-recumbent position. One lumen is used to sample ileal content 5 cm above the ileocecal junction, and the other lumen (25 cm proximal to the aspiration port) is used for perfusion. The perfusate contains NaCl and polyethylene glycol 4000 as a recovery marker to estimate water flow through the distal ileum (Flourié *et al.* 1988). The solution is maintained at 37°C and stirred until the end of perfusion. The day after the intubation, the subjects are given the experimental meal as a breakfast. The intestinal contents are aspirated for 14 h, during which time subjects do not eat or drink. Intestinal contents are collected continuously on ice by manual aspiration, the aim being to aspirate as much fluid as possible. Samples are divided into 30-min aliquots that are frozen in liquid nitrogen and then freeze-dried. Maintenance of tube position is confirmed fluoroscopically at the end of all experiments.

9.3.4 *Comparison of ileostomy model and intubation technique*

It is difficult to compare data obtained within different studies with different types of meals and different starch intakes. However, one comparison can be made between two studies performed with the ileostomy model (Langkilde *et al.* 1995) and the ileal intubation in healthy subjects (Faisant *et al.* 1995a) with the same meal containing 30 g of starch from green banana. The ileal excretions of starch (= RS) were respectively 15.8 ± 0.7 and 19.3 ± 0.7 g/day for the ileostomates and the healthy subjects.

Such a difference can be explained by an underestimation of RS in ileostomates and/or by an overestimation of RS when intubation techniques are used. Indeed, on one hand, there is evidence of a bacterial overgrowth in the distal ileum of the ileostomates as well as in the collection bags. On the other hand, the intubation is thought to be the cause of a decrease of the oro-ileal transit time as a result of the tube perhaps decreasing the efficiency of the intestinal digestion.

9.4 *In-vitro* methods

During the past ten years, much effort has been expended in the evaluation – and then in the improvement – of the existing methods for the measurement of RS. A review presenting most of these methods has been published recently (Champ *et al*. 1999a). Several analytical methods are now proposed in the literature, some of these were developed during the European Research Program EURESTA (Champ 1992; Englyst *et al.* 1992). When comparisons have been made, the different methods seemed to provide very similar data for most

starch samples, although most of them probably underestimate RS, as defined above. Indeed, RS collected *in vivo* in humans (ileostomates or healthy subjects using the intubation technique) is composed of oligosaccharides (including glucose), α-glucans of high molecular weight (mainly starch granules) and a crystalline fraction, the size of which depends on the origin and the treatment of the starch. None of the analytical methods for RS takes into account the potentially digestible starch (oligosaccharides and part of the high-molecular weight fraction) (Faisant *et al*. 1993a, b).

9.4.1 *General principle*

In order to quantify RS, the main step is to remove the digestible starch from the sample. This is performed using a pancreatic α-amylase. In some of the methods, an amyloglucosidase is added in order to avoid a possible inhibition of the α-amylase by the products of the digestion (mainly maltose and maltotriose). However, this enzyme – which is of fungal origin – seems to be very active on some specific crystalline structures. The question is raised of a possible 'overdigestion' of some RS structures by the cocktail 'α-amylase-amyloglucosidase'.

The amylolysis is also sometimes preceded by a proteolysis (often, pepsin hydrolysis) which is supposed to reflect the action of the pepsin inside the stomach and of the trypsin which is secreted in the pancreatic juice together with the α-amylase.

After hydrolysis of the digestible starch, RS is quantified directly in the residue (usually isolated by 80% ethanolic precipitation) (Champ 1992; Faisant *et al*. 1995c; Champ *et al.* 1999a) or by difference between total starch and digestible starch which are quantified separately (Englyst *et al*. 1992).

9.4.2 *Main analytical methods*

The main methods proposed in the literature will not be described in detail, but the most recent are summarised in Table 9.3.

Method of Björck et al. *(1986)*

Principle: This method quantifies part of the starch present in the dietary fibre residue obtained after enzymatic solubilisaion according to Asp *et al.* (1983), then final digestion of protein and starch (using Termamyl), then pancreatin. The soluble dietary fibre components are precipitated with 95% ethanol and the total dietary fibre components are then recovered by filtration. Resistant starch is calculated as total starch remaining in the fibre residue after solubilisation in KOH minus the value obtained without prior KOH treatment.

Advantage and drawbacks:

- can be performed together with total dietary fibre analysis
- the main component measured is retrograded starch
- has been validated with a rat model (antibiotic-treated rat) (Björck and Asp 1991).

Method of Englyst et al. *(1992) (Table 9.3)*

Principle: The various types of starch are determined by controlled enzymatic hydrolysis

Table 9.3 Main *in-vitro* methods to quantify resistant starch (RS).

	Englyst *et al.* (1992)	Muir & O'Dea (1992, 1993)	Goñi *et al.* (1996)	Åkerberg *et al.* (1998)	Champ *et al.* (1999a)
Sample size	0.8–4.0 g depending on water and starch content	≈0.1 g carbohydrate basis	100 mg dry sample	1 g, total starch basis	50 mg, total starch basis
Sample pre-treatment	Minced (9 mm Ø holes)	Chewing	Dry samples: milled (Ø ≤ 1 mm)	Chewing (15 times, 15 s)	Minced (9 mm Ø) Fresh samples: homogenised
Protein * hydrolysis	No protein hydrolysis	Pepsin treatment (pH 2, 37°C, 30 min)	Pepsin treatment (pH 1.5, 40°C, 60 min)	Pepsin treatment (pH 1.5, 37°C, 30 min)	No protein hydrolysis performed
Digestible starch * hydrolysis	Pancreatin (Pancrex V) + amyloglucosidase + invertase + glass balls + guar gum (pH 5.2, 37°C) Time: 20 min → RDS Time: 120 min → SDS Samples collected in ethanol (64% final concentration)	α-amylase (Speedase PNA-8)+ amyloglucosidase (pH 5.0, 37°C, 15 h)	Pancreatic α-amylase (pH 6.9, 37°C, 16 h)	Pancreatin + amyloglucosidase (pH 5.0, 40°C, 16 h)	Pancreatic α-amylase + amyloglucosidase (pH 5.25, 37°C, 16 h)
Removal of starch hydrolysis products	No	No ethanolic precipitation Centrifugation (10 min, 1200 *g*)	No ethanolic precipitation Centrifugation (15 min, 3000 *g*)	Precipitation 76% EtOH Filtration	Precipitation 80% EtOH Centrifugation (10 min, 3000 *g*)
Dispersion of RS	No	Boiling (20 min) + 2 M KOH (0°C, 30 min)	2 M KOH (room temperature, 30 min)	2 M KOH (30 min)	Boiling (20 min) +2 M KOH (0°C, 30 min)
RS hydrolysis*	No	Amyloglucosidase	Amyloglucosidase (60°C, 45 min)	Termamyl + amyloglucosidase	Amyloglucosidase (14 AGU/ml; 70°C, 30 min + 100°C, 10 min)
Glucose determination	Enzymatic, GOD-PAP	Enzymatic, GOD-PAP	Enzymatic GOD-PAP	Enzymatic, GOD-POD	Enzymatic, GOD-PAD
Validation	*In-vivo* ileostomy data	*In-vivo* ileostomy data	No	With *in-vivo* ileostomy data (partly from literature)	*In-vivo* ileostomy and intubation data
Specificity	RS = TS – (RDS + SDS)				

Further details on the enzymes used can be found in the text.

with measurement of the released glucose using glucose oxidase. Resistant starch is defined as the starch which is not hydrolysed after incubation with pancreatin (Pancrex V, Paines and Byrne, Greenford, UK) and amyloglucosidase (Novo Nordisk, Bioindustries, Copenhagen, Denmark), after 120·min at 37°C. Due to a contamination of amyloglucosidase with an invertase activity, invertase is also added; sucrose has thus to be determined separately. RS is calculated by deducting the Rapidly Digestible Starch (RDS) plus the Slowly Digestible Starch (SDS) contents (i.e. after 20 and 120 min incubation, respectively) of the sample hydrolysed, from Total Starch content.

Advantages, drawbacks and characteristics:

- the conditions of hydrolysis have been optimised
- has been adapted to a large variety of substrates
- a laborious method giving poor reproducibility without extensive training
- need for very specific equipment (mincer, shaking water-baths)
- enzymes seem to be no more available from Novo Nordisk Bioindustries, but must be purchased from Englyst Carbohydrate Services Ltd.
- RS is not measured directly, but calculated by difference between total starch and RDS + SDS
- no pepsin step in this version of the method.

Method of Muir and O'Dea (1992, 1993) (Table 9.3)

Principle: The chewed food sample is incubated with pepsin and then hydrolysed by α-amylase (Speedase PNA-8, Halcyon Chemicals, Sandrigam, Australia) and amyloglucosidase (Sigma, St Louis, MO, USA). The insoluble residue, collected by centrifugation, contains RS.

Advantages, drawbacks and characteristics:

- This method has been validated with *in-vivo* studies on ileostomates (Muir and O'Dea 1993; Muir *et al*. 1995). From the seven comparisons available, it seems satisfactory. However, no comparisons with other *in-vitro* methods have been published. Until now, it has been used mainly by the authors; thus difficulties and reproducibility cannot be evaluated.
- No alcoholic precipitation to isolate RS after α-amylase digestion.

Method of Goñi et al. *(1996) (Table 9.3)*

Principle: The food sample is milled (dry samples) or homogenised (wet samples), defatted when fat content is ≥5%, incubated with pepsin, then hydrolysed by pancreatic α-amylase. The insoluble residue, collected by centrifugation, contains RS.

Advantages, drawbacks and characteristics:

- This method has not been validated on human subjects.
- No use of amyloglucosidase
- No alcoholic precipitation to isolate RS after α-amylase digestion.

Method of Åkerberg et al. *(1998) (Table 9.3)*

Principle: The analytical procedure was chosen to mimic physiological conditions, and included chewing as a pre-step before incubation with pepsin, pancreatin (Sigma)and amyloglucosidase (Boehringer, Mannheim, Germany). The non-digestible polysaccharide, including RS, is recovered by ethanol precipitation and subsequent filtration. RS is analysed as total starch in the filter residue.

Advantages, drawbacks and characteristics:

- RS values obtained from *in-vivo* and/or *in-vitro* analyses have been compared for identical products. The pooled standard deviation for the method is 2.9%. The correlation obtained with *in-vivo* values from the literature (19 foods) was: $r = 0.97$; $y = 0.77x + 0.45$). According to the authors, the method allows parallel determination of the potentially available starch fraction and dietary fibre.

Method of Champ et al. *(1999a)*

The method is derived from the one published in 1992 within the EURESTA project [Champ 1992 (Method A)].

Principle: *In vitro*, RS was defined as the starch not hydrolysed by incubation with α-amylase and amyloglucosidase. Amyloglucosidase (Novo Nordisk Bioindustries) is added to the pancreatic α-amylase (Sigma) to avoid inhibition of the amylase by the products of the digestion. Hydrolysis products are extracted with 80% ethanol and discarded. RS is then solubilised with 2 N KOH and hydrolysed with amyloglucosidase.

Advantages, drawbacks and characteristics:

- simple and relatively rapid. Ten samples can easily be analysed (in duplicate) in a normal day of work. No particular training is needed.
- the procedure has been validated in collaboration with Dr Langkilde and Prof. H. Andersson using *in-vivo* values obtained from ileostomates and with intubation studies (Faisant *et al*. 1995a; Champ *et al*. 1998; Noah *et al.* 1998). However, more comparisons should be performed between *in-vitro* and *in-vivo* values to check the validity of the method on a large range of starches and foods.
- amyloglucosidase is no longer available from Novo Nordisk Bioindustries
- no proteolysis.

9.4.3 *Comparison of the main* in-vitro *methods*

Few comparisons have been performed using exactly the same samples. However, one such study has been published by Champ (1992) and Englyst *et al.* (1992), within the EURESTA program (Table 9.4).

As expected, the method of Björck *et al.* (1986) gave the lowest values. However, the significant underestimation was observed only for native and treated pure starches. The Englyst *et al.* (1992) and Champ (1992) methods gave very similar values. The method modified by Faisant *et al.* (1995c) give a significantly higher value of RS for the raw potato starch. This value is closer to the *in-vivo* value obtained with ileostomates. This result was later confirmed

Table 9.4 Analysis of resistant starch (RS; g/100 g dry matter) in different sources of starch with different in-vitro methods.

Source of starch	Björck *et al.* (1986)	Englyst *et al.* (1992)	Champ (1992) (method A)	Faisant *et al.*(1995)	Champ *et al.* (1999a)
Potato starch, raw	0.2	64.9	66.2	85.3	
HACS, raw		68.8		67.3	
HACS, retrograded		30.0		35.3	
HACS, pre-gelatinised	9.6	16.3	15.7		
Bean flakes	4.3	5.0	5.0	4.5	
Corn flakes	2.3	2.4	2.8	2.8	
Beans (fresh)		7.3			7.3

HACS, high-amylose corn starch.

by the data published by Langkilde and Andersson (1995). The new method from our group (Champ *et al.* 1999a) has been compared with the method of Englyst on samples of fresh and mashed beans. Both methods provided exactly the same results: 7.3 g RS/100 g dry matter.

9.4.4 *Comparison between* in-vitro *and* in-vivo *measurements*

Some of the *in-vitro* methods have been validated using *in-vivo* data that were, in most cases, obtained with ileostomates. However, Björck and collaborators compared their *in-vitro* values to data obtained in rats (nebacitin-treated rats). The same group recently validated their latest method (Åkerberg *et al*. 1998) on the basis of *in-vivo* results published in the literature and obtained on ileostomates.

In-vitro *and* in-vivo *data from Englyst* et al. *(1992) (Table 9.5)*

This comparison appears to be relatively satisfactory. The RS in potato biscuit is probably, as expected, underestimated (see earlier comments). However, it is unexpected that the same discrepancy is not seen with banana biscuits, if these are made from green banana as the main ingredient.

Table 9.5 *In-vivo* recovery of resistant starch (RS; g/100 g dry matter) from different sources of RS in ileostomates.

	Resistant starch		
Source of starch	Fed (g)*	Recovered (g)	Mean recovery (%)
Wheat biscuit	0	0.3	–
Wheat RS biscuit	8.5	9.0	106
Maize RS biscuit	8.5	8.6	101
Potato biscuit	11.7	13.7	117
Banana biscuit	15	13.7	91

*Determined according to the *in-vitro* method described by Englyst *et al.* (1992).
Source: data from Englyst *et al*. 1992.

In-vitro *and* in-vivo *data from Muir and O'Dea (1993) and Muir* et al. *(1995) (Table 9.6)*

The predicting power of the *in-vitro* method is satisfactory. However, a slight discrepancy appears with some of the samples, e.g. baked beans, low- and high-RS meals.

In-vitro *data (Champ* et al. *1999a) and* in-vivo *data (Langkilde and Andersson 1995)*

When compared to *in-vivo* data obtained on ileostomates with the same starchy foods (Table 9.7), the method appears to be satisfactory. However, when compared to data obtained from healthy subjects by the intubation technique, it underestimates the RS values, except in the case of canned beans. Indeed, the intubation technique always provides higher RS values than the ileostomy model; the exception in the case of canned beans is probably due to a partial

Table 9.6 *In-vivo* recovery of resistant starch (RS; g/100 g dry matter) from different sources of RS in ileostomates.

	Starch (g)		%RS	
Source of starch	Ingested*	Recovered	*In vivo*	*In vitro*
High-RS meal	52.7	19.9	37.8	35.1
Baked beans	22.7	1.3	5.7	6.9
Pearl barley	41.0	2.3	5.5	5.5
Low-RS meal	51.8	2.4	4.6	3.1
Corn flakes	44.1	1.4	3.1	2.9
Whole rice	48.9	1.5	3.1	2.7
Ground rice	46.1	0.3	0.7	0.8

*Determined according to the *in-vitro* method described by Muir & O'Dea (1993).
Source: data from Muir & O'Dea 1993; Muir *et al.* 1995.

Table 9.7 Comparison of resistant starch (RS; % total starch) data determined *in vivo* and *in vitro.*

	In-vitro RS			
Source of starch	Englyst *et al.* (1986#, 1992)	Faisant *et al.* (1995)	Champ *et al.* (1999a)	*In-vivo* RS
Potato starch, raw	66.5	83.0	77.7	78.8*
HACS, raw	71.4	72.2	52.8	50.3*
HACS, retrograded	30.5	36.4	29.6	30.1*
Bean flakes	10.6	12.4	11.2	9–10.9+
Corn flakes	3.9	4.9	4.3	3.1–5.0‡
Beans	17.1#	–	17.1	16.5§

HACS, high-amylose corn starch.
Ileostomy model: *Langkilde *et al.*(pers. commun.); +Schweizer *et al*. (1990); ‡Muir & O'Dea (1992); Englyst *et al*. (1992).
Intubation technique: §Noah *et al*. (1998).

collection of the bean residues in the ileum, and the presence of large particles of beans (Noah *et al*. 1998).

9.5 Conclusions

Two main methods are available to evaluate RS *in vivo* in humans – the intubation and ileostomate methods. The intubation technique is the only *direct* technique available for use in healthy volunteers, but it has a major drawback in that the tube, when present in the small intestine, may influence food transit. Thus, the intubation method may slightly overestimate the RS content of foods. In the ileostomate method, RS is quantified directly by collecting ileal samples. The determination of small bowel excretion is quantitative, provided that the bacterial degradation of the effluent can be minimised; however, this technique probably slightly underestimates RS content of the foods. The hydrogen breath test is also used to quantify malabsorption of starch; the method is rapid and simple, though indirect and consequently semi-quantitative. However, the use of an appropriate standard for 'calibration' of the subject might improve the method's quality.

In conclusion, the two direct methods performed on humans undoubtedly are the best means of quantifying RS as it has been defined in 1992 as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals' (Asp 1992).

Resistant starch, as identified in this physiological definition, is composed not only of strictly resistant starch but also of potentially digestible fractions. None of the RS methods presently available has been shown to analyse all RS as defined. Several methods have been proposed for the measurement of RS, and some of these have been validated on a quantitative basis with *in-vivo* data obtained from healthy subjects (using the intubation technique) or ileostomates.

The main methods that have been validated with digestibility values obtained from human volunteers (mainly with ileostomates) have provided acceptable values, but none of these methods is exempt from criticism.

The method proposed by Englyst *et al*. (1992) has been optimised to be applicable to most foods, although it is laborious to perform and offers poor reproducibility unless technicians undergo specific training.

Muir and O'Dea (1993) developed a new method based on the method of Englyst. Unfortunately, no comparison with other methods is yet available and it has apparently not been used outside Australia. The number of comparisons with *in-vivo* data is still too limited to make conclusions on its validity.

Our own method (Champ *et al.* 1999a) provides similar values to those obtained with the method of Englyst, but is much simpler and easy to use by laboratory technicians. Some comparisons have been performed with *in-vivo* values obtained in ileostomates and in healthy subjects, but more comparisons are necessary.

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Part 4

Regulatory Issues

10 Analytical Issues Regarding the Regulatory Aspects of Dietary Fibre Nutrition Labelling

Jonathan W. DeVries

10.1 Introduction

The objective of ethical nutrition research is to understand nutrition factors that result in increased life span and optimised quality of life through improved health and body function. To realise maximum benefit, the results of significant and reproducible research must be translated into information for general public use. This often requires public policies that also minimise the opportunity for abuse in using the information. Nutrition labelling on the package of the product and truthful advertising in the various media are two ways of achieving this.

Decisions that are made regarding the regulation of food labelling is a societal function *per se.* Once regulations are established, the impact is widespread upon consumers, food companies, regulatory enforcement personnel, analytical laboratories, ingredient companies and the legal community. The impact becomes particularly intense when health improvement claims and nutrition labelling claims are involved.

Dietary fibre labelling requires a clear concise definition of the relevant food component(s). For a multi-component, non species-specific nutrient, enforcement of the regulations must necessarily depend upon proximate assays, i.e. systems where the quantity of the analyte is defined, in part, by the assay. Such a proximate assay must accurately reflect the quantity of the food component meeting the physiological definition.

Dietary supplements require additional considerations. In a world where miracles have been accomplished by drugs delivered in pill and powder forms, supplements are often perceived as having enhanced value over traditional food sources for the same nutrient. This can lead to exaggerated claims, excess consumption, unbalanced nutrition in the diet, and safety risks.

10.2 Why regulate?

10.2.1 *Nutrition research and discoveries*

Whether due to instinct, or due to thoughtful observation, humans (and other animals) learn at a very early stage in life what foods are available to them and, of the foods available, which are best for their health. For centuries, mothers and fathers have watched their children grow and have appreciated the correlation between a good diet and healthy, normal children. As individuals advance in years, they recognise and appreciate certain factors in their diet, or in others' diets that appear to result in better states of health than do other alternative factors. Scientists take these observation powers to a higher level, recording and correlating their observations and setting up controlled experimentation to induce measurable changes in subjects with corresponding changes in diet. The objective of ethical nutrition research

is to understand those nutrition factors that can be changed to give a result of increased life span. Further, the quality of life associated with that increased life span should be improved due to better health and better body function(s). In the case of dietary fibre, the original observational research indicated that diets high in fibre provide improved body function and reduced occurrence of certain diseases. Trowell *et al*. defined dietary fibre as 'Dietary fibre consists of the remnants of edible plant cells, polysaccharrides, lignin and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans' (Burkitt *et al.* 1972; Trowell 1972a, b, 1974; Painter 1975; Trowell *et al.* 1976; DeVries *et al.* 1999). These researchers hypothesised that increasing the intake of this component of the diet to an adequate level would reduce the risks of constipation, diverticular disease, hiatus hernia, appendicitis, varicose veins, piles (haemorrhoids), diabetes, obesity, coronary heart disease, large-bowel cancer and gallstones (Burkitt 1983a). There was also an association implicated for duodenal ulcers, breast cancer and blood clotting (Burkitt 1983b). Subsequent research has borne out the validity of many – but not all – of these hypotheses with regard to the reduction in disease or disease risk. In 1985, the Canadian Expert Advisory Committee on Dietary Fiber listed laxation, blood cholesterol-lowering, or blood glucose attenuation as necessary attributes that novel fibre sources (sources beyond that intrinsic to foods) must exhibit (Canadian Food Inspection Agency 2000). The positive nutritional effects of dietary fibre were reaffirmed by the American Association of Cereal Chemists' (AACC) Expert Committee on the Definition of Dietary Fiber in 1999 (AACC 1999; DeVries and Faubion 1999). The expert committee determined that 'Dietary fiber is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber exhibits one or more of either laxation (fecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation.' This definition was submitted to the AACC Board of Directors for endorsement in November, 1999. After a brief review, the AACC Board of Directors expressed concern with the particular wording of the definition, while agreeing it was scientifically correct. The expert definition committee revised the verbiage, but not the intent of the definition. The rewritten definition submitted to the AACC Board of Directors and currently up for consideration is 'Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation'.

10.2.2 *Utilizing the benefits of nutrition research*

To realise maximum benefit, the results of significant and reproducible research must be translated into information for the general public's use. Generally speaking, simply recommending that consumers eat a good diet, or select proper foods for their diet, is insufficient in cultures where processed and prepared foods abound. To consumers, the recommended selection and their subsequent purchase and consumption of some foods, i.e. whole grains, fruits and vegetables is obvious. Nutrition research certainly aids in defining the nutrition characteristics of whole grains, fruits and vegetables, but once established, these nutrition parameters vary little and consumers recognise the food for the purposes of diet selection.

However, when foods are processed into alternative forms that differ significantly from their original source, the consumer can no longer select diet items based on their appearance, and nutrition labelling becomes necessary. This often requires public policies to educate the consumer and minimise the opportunity for abuse in using the information. Regulations regarding accurate nutrition labelling on the package of the product and truthful media advertising are a means of achieving this.

10.3 Labelling of dietary fibre on food products

Establishment of regulations designed to inform consumers is a societal decision *per se.* Sound public policy needs to be based on relevant scientific input, consumer need, and must weigh the cost versus the potential benefits. It is generally insufficient to recommend that consumers eat good foods as part of a good diet. Standardised procedures and policies are necessary to differentiate the various qualities of foods. Even when the positive health effects of a particular food have been established, standards are necessary.

Oat bran provides an excellent example. Upon establishment of the positive physiological effects of oat bran consumption, consumer use of oat bran and oat bran-containing products increased dramatically. However, the characteristics of the oat bran available for consumption was highly variable, dependent upon production procedures. Responding to the need for a standard definition for oat bran, the American Association of Cereal Chemists, utilising a committee of expert scientists, established a definition for oat bran (AACC 1989). This definition formed the essence of the oat bran definition applicable to the health claim relating the consumption of oat products to reduced risk of heart disease (Anon. 1999a). Standard characteristics that can be quantitated analytically are necessary to assure that consumers have adequate and accurate information. Therefore it is apparent that regulations are necessary to:

(1) Optimise the value of nutrition research.
(2) Assure the public of the value and nutrition of the products they are consuming.
(3) Minimise abuse of nutrition information through misleading labels.
(4) Provide an equal basis for producers of products to compete.

Once regulations are established, the impact is generally widespread, and affects consumers, food companies, regulatory enforcement personnel, analytical laboratories, ingredient companies and the legal community. Consumers are provided with information to aid them in food selection directly applicable to improving their diet. Food companies incur the responsibility of generating the nutrition data on their products and ensuring that it is accurately reflected on the label. Regulatory enforcement personnel need to increase their analytical capabilities and be willing to enforce the regulations for the programme to be fully effective. Ingredient companies can introduce new, or modify current ingredients to effect improvements in the nutrition profile of the ingredient. Finally, the legal community will become involved in ensuring that regulations are fairly enforced and that competition is held on a level playing field. Activity within all these segments of society becomes particularly intense when health and nutrition labelling claims are involved, especially when the quantity of sales of a particular food item or items depends upon the claims made on the label and in the advertising of a product.

10.3.1 *Labelling regulations, policies and/or practices*

Throughout the world, numerous countries have adopted the AOAC International Official Methods of Analysis as the basis for dietary fibre labelling regulations, utilise them for enforcement of labelling policy, or have them as suggested practices for dietary fibre labelling. Examples are listed below.

Australia

Australian regulations stipulate the labelling of Total Dietary Fibre (TDF) and stipulate the use of AOAC 985.29 for determination of the quantity of TDF on the label (Mugford 2000). Currently there is a proposal to allow the use of AOAC 991.43 for the analysis, provided it is used for TDF and not for Insoluble and Soluble Dietary Fibre. As with the United States, Australia is currently giving consideration to the question of including inulin in the dietary fibre quantity.

Canada

Canadian dietary fibre labelling is based on the definition of Trowell *et al*., and reads specifically: Dietary fibre is defined as the endogenous components of plant material in the diet which are resistant to digestion by the enzymes produced by humans. They are predominantly non-starch polysaccharides and lignin. The composition varies with the origin of the fibre and includes soluble and insoluble substances Quantity of dietary fibre in food products to be determined using AOAC 985.29, 992.16, or 994.13. Food ingredients that have been manufactured to be sources of dietary fibre are defined as 'novel fibres'. Foods or ingredients that are identified as novel fibre must be demonstrated to be as efficacious as fibre by clinical studies that show they have a positive effect on one of three well-established and measurable functions of dietary fibre: laxation, blood cholesterol-lowering and blood glucose attenuation. (Canadian Food Inspection Agency 2000).

European Union

Within the European Union, the methodology outlined in AOAC International Official Method of Analysis 985.29 determines the quantity of dietary fibre on the food labels. An exception has been the United Kingdom which is now, however proposing to utilise the AOAC methodology as well (Hignett 1999b). The countries of Denmark, Finland, Italy and Sweden specifically reference AOAC 985.29, while the Netherlands reference the enzymatic-gravimetric methods of Asp (Asp *et al.* 1983; Asp and Johansson 1984), one of the forerunners to AOAC Internationals Official Method of Analysis 985.29. The remaining countries have not specified policies (Craig 2000).

Japan

Dietary fibre is defined as the component of food which is determined by the Prosky method(s), i.e. AOAC International Official Method of Analysis 985.29 for total dietary fibre combined with AOAC International Official Method of Analysis 991.42 if soluble and insoluble fibre are to be determined. In addition, for low-molecular weight dietary fibre that

resists enzymatic digestion, but does not precipitate in the 78% alcohol solution of the Official Method, an HPLC method can be used for quantitation. This quantity is added to the quantity determined by the Official Method. The energy value for dietary fibre is counted as 0 kcal/g (Sato 2000a). In addition to the dietary fibre labelling regulations, a regulatory programme (FOSHU-Foods for Specific Health Use) is in place for products with scientific evidence of effectiveness for a specific purpose. Scientific evidence is established on the effective ingredient and on the product containing the ingredient. As of December, 1999, dietary fibres approved as effective ingredients in the FOSHU programme are an indigestible dextrin (Fibersol-2®), hydrolysed guar gum, Polydextrose, psyllium, wheat bran, chitosan and depolymerised sodium alginate.

Korea

Dietary fibre is defined as the (food) component which is hardly digested by human digestive enzymes, or high-molecular weight indigestible components derived from living organisms (Sato 2000b). Analysis is by AOAC International Official Method of Analysis 991.42 and 985.29. In addition, for low-molecular weight dietary fibre that resists enzymatic digestion, but does not precipitate in the 78% alcohol solution of the Official Method, an HPLC method can be used for quantitation. This quantity is added to the quantity determined by the Official Method. The energy value for dietary fibre is counted as 0 kcal/g.

Mexico

Nutrition labelling is optional, and dietary fibre labelling is optional when other nutrients are labelled (USDA, FAS 1999). Method of analysis is not specified, use of AOAC 985.29 is accepted.

United Kingdom

The current policy of the United Kingdom is to measure and label non-starch polysaccharides rather than dietary fibre on food products. Action is currently underway to adopt a proposal to change labelling requirement to dietary fibre by AOAC Official Methods of Analysis 991.43 and 997.08 (Hignett 1999a). Under the proposal, food labels will reflect the dietary fibre content as defined by Trowell *et al.* (1976). Inulin and oligofructans will be included as part of the dietary fibre content.

United States

Prior to 1987, US food labelling regulations only allowed for an adjustment to calories for a serving of food on the basis of the crude fibre content of the food. Fibre was rarely declared as part of the nutrition label, nor was it emphasised on product labels. An exception was products containing high levels of crude fibre that were intended for improvement of laxation. In 1987, the United States Federal Code of Regulations was modified to allow the inclusion of the TDF content of a product as part of its nutrition label. The code change further allowed an adjustment in the calculated calories of a serving of the product by subtracting the *total dietary fibre* content of the food from the total carbohydrates present before multiplying the carbohydrate content by the 4 kcal/g energy conversion factor (Anon. 1987). The Code specified the use of

the then recently adopted AOAC Official Method of Analysis 985.29, Total Dietary Fiber in Foods, Enzymatic-Gravimetric Method (AOAC 1985). This method is based on the definition delineated by Trowell *et al.* (1976). Use of the method served the purpose of indicating the dietary fibre content on the label and making the appropriate adjustment for non-digestible food portion to the calories listing on the label.

In 1993, the US adopted regulations to enforce the Nutrition Labeling and Education Act of 1990 (USDA 1993; USFDA 1993). Under these regulations, TDF content labelling is required on all food products, but Soluble and Insoluble Dietary Fibre content labelling is optional. Total, Soluble and Insoluble Dietary Fibre are to be determined by the applicable AOAC International Official Methods of Analysis. The applicable AOAC Official Methods and some of their key characteristics are listed in Table 10.1. The labelling of the caloric content of the food product is adjusted based upon the insoluble dietary fibre content of the food, not the total dietary fibre content. The energy values attributed to dietary fibre are 0 cal/g for insoluble dietary fibre, and 4 cal/g for soluble dietary fibre. The rationale for this is based on the fact that a large percentage of the soluble dietary fibre consumed is fermented in the large intestine, and the calories thus released are captured by the body in the form of short-chain fatty acids, i.e. acetic, propionic and butyric acids. Proposals have been made to the US Food and Drug Administration to have inulin specifically included in quantity of dietary fibre listed on the label of foods containing the polyfructan. While the regulations do not specifically exclude inulin from being included in the dietary fibre content, traditional reliance on AOAC 985.29 and 991.43 (methods which do not isolate and quantitate the inulin as part of the total dietary fibre quantity) has resulted in a reluctance to include inulin. From a scientific point of view, the functionality of inulin has been shown to be characteristic of dietary fibre, and it should be included in the label quantity, otherwise the consumers are not aware of the actual amount of dietary fibre that they are consuming.

Codex Alimentarius

The Codex Committee on Methods of Analysis and Sampling has endorsed the use of AOAC International Official Method of Analysis 985.29 for defining dietary fibre (ALINORM 1993). The Codex Alimentarius Committee on Nutrition and Foods for Special Dietary Uses through its Consultation of an Informal Working Group on Dietary Fibre is currently deliberating on the definition of dietary fibre and the appropriate criteria for 'source' and 'high' dietary fibre contents of foods (Hignett 1999b). There is general agreement that dietary fibre reduces constipation, prevents bowel cancer, increases stool bulking, reduces stool transit time, and provides bifidogenic effects as a result of its ability to act as a substrate for fermentation in the large intestine. The Informal working group recommended that fibre content claims be developed based on role dietary fibre plays in the above. The working group members further concluded that sufficient evidence exists that cellulose, hemicellulose and pectin play a role in these effects.

A brief summary of labelling regulations, policies and/or practices is listed in Table 10.2. It should be noted that the regulations and policies in some constituencies allow for special health claims with a component of dietary fibre being the analyte of concern for assuring claim compliance. Such is the case for the oat health label claim in the United States where β-glucan soluble fibre is measured in the oats or oat bran and a level of oat flour or oat bran containing greater than a minimum level of β-glucans must be used in the product to justify the claim. Some constituencies also allow the polyfructan fibres (inulin and/or oligofructans)

Table 10.1 AOAC International official methods of analysis for dietary fibre.

AOAC Official Method	Title	Principle	Comments
985.29	Total Dietary Fibre in Foods, Enzymatic-Gravimetric Method	Gravimetric determination of dietary fibre quantity after enzymatic digestion simulating human digestion	Designed for food labelling purposes. Uses an inorganic phosphate buffer system. Practical for all foods. Does not quantitate inulin or other fibres soluble in 78% ethanol in food samples.
991.42	Insoluble Dietary Fibre in Foods and Food Products, Enzymatic-Gravimetric Method, Phosphate Buffer	Gravimetric determination of insoluble dietary fibre quantity after enzymatic digestion simulating human digestion	Designed for food labelling purposes. Uses an inorganic phosphate buffer system. Can be combined with AOAC 985.29 to determine soluble dietary fibre. Practical for all foods
993.19	Soluble Dietary Fibre in Foods and Food Products, Enzymatic-Gravimetric Method, Phosphate Buffer	Gravimetric determination of soluble dietary fibre quantity after enzymatic digestion simulating human digestion	Designed for food labelling purposes. Uses an inorganic phosphate buffer system. Practical for all foods
991.43	Total, Soluble, and Insoluble Dietary Fibre in Foods, Enzymatic-Gravimetric Method, MES-Tris Buffer	Gravimetric determination of Total, Insoluble, and Soluble dietary fibre quantity after enzymatic digestion simulating human digestion. Meets same enzyme requirements of 985.29	Designed for food labelling purposes. Uses an organic buffer system (reduces analytical time). Practical for all foods. Does not quantitate inulin or other fibres soluble in 78% ethanol in food samples.
992.16	Total Dietary Fibre, Enzymatic-Gravimetric Method	Gravimetric determination of total dietary fibre quantity after enzymatic digestion simulating human digestion. Meets same enzyme requirements of 985.29	Sum of soluble fibre release by autoclave plus insoluble fibre determined by neutral detergent fibre (quantity may be affected by residue overlap).
993.21	Total Dietary Fibre in Foods and Food Products with ≤2% Starch, Non-enzymatic-Gravimetric Method.	Gravimetric determination of Total dietary fibre quantity after precipitation of fibre from aqueous suspension/solution using 4 parts alcohol. No enzymatic treatments are involved.	Applicable to samples with ≤2% starch. High protein samples may be an issue.
994.13	Total Dietary Fibre (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin) Gas Chromatographic-Colorimeteric-Gravimetric Method.	Gas chromatographic, colorimetric, gravimetric determination of total dietary fibre quantity after enzymatic digestion simulating human digestion.	Provides data on saccharide composition of fibre and limited data on uronic acid and lignin content.

Table 10.2 Regulations, policies and practices regarding the labelling of dietary fibre in foods

Country or Political Entity	Regulations	Nutrient Content Claims	Comments
Australia	Total Dietary Fibre – required Insoluble Dietary Fibre – not allowed Soluble Dietary Fibre – not allowed		Analysis by AOAC Official Method of Analysis 985.29. Official Method of Analysis 991.43 under consideration. Inulin as fibre under consideration. Requirement of ≥80% of label under consideration.
Canada	Total Dietary Fibre labelling required. Dietary fibre is defined as the endogenous components of plant material in the diet which are resistant to digestion by the enzymes produced by humans. They are predominantly non-starch polysaccharides and lignin. The composition varies with the origin of the fibre and includes soluble and insoluble substances	Source of fibre is 2 g per serving. High fibre is 4 g per serving. Very high fibre is 6 g per serving.	Analysis by AOAC Official Methods of Analysis 985.29 or 992.16. Also may be determined as non-starch polysaccharides, although this will typically underestimate the dietary fibre content. 'Novel fibres' allowed provided they are demonstrated to be as efficacious as fibre by clinical studies that show they have a positive effect on one of three well established and measurable functions of dietary fibre: laxation, blood cholesterol-lowering, and blood glucose attenuation. Rounding to nearest gram for >10 g/serving, rounding to nearest g/10 g for <10 g/serving.
European Union	Dietary Fibre as defined by AOAC 985.29 if specified, except for The Netherlands which specifies Method of Asp et al. (See United Kingdom below)		Analysis by AOAC International Official Methods of Analysis.
Japan	Total, Soluble, and Insoluble Dietary Fibre defined as the component determined by AOAC 991.42. (In addition, enzyme resistant, low-molecular weight soluble dietary fibre may be added).	Source of (Supplies, Contains, Using, Added) fibre is 3 g per 100 g food [beverages 1.5 g per 100 g] or 1.5 g per 100 kcalHigh (Much, Rich) fibre is 6 g per 100 g food [beverages 3 g per 100 g] or 3 g per 100 kcal	Analysis by AOAC Official Method of Analysis 991.42 and related methods. Additionally, a differential refractometry HPLC method can be used to determine enzyme-resistant, low-molecular weight soluble dietary fibres which do not precipitate in the 78% ethanol solution used in the Official Method. Quantity thus determined is added to the quantity obtained using the Official Method. Provision made for functional Foods for Special Health Use (FOSHU). The energy value of fibre is 0 kcal/g

Korea	Dietary fibre is defined as the (food) component which is hardly digested by human digestive enzymes, or high-molecular-weight indigestible components derived from living organisms.	Source of (Supplies, Contains, Using, Added) fibre is 3 g per 100 g food or 1.5 g per 100 kcalHigh (Much, Rich) fibre is 6 g per 100 g food or 3 g per 100 kcal	Analysis by AOAC Official Method of Analysis 991.42 and related methods. Additionally, a differential refractometry HPLC method can be used to determine enzyme-resistant, low-molecular weight soluble dietary fibres which do not precipitate in the 78% ethanol solution used in the Official Method. Quantity thus determined is added to the quantity obtained using the Official Method. The energy value of fibre is 0 kcal/g
Mexico	Dietary fibre labelling is optional		Round to nearest gram per serving, per 100 g, or per contained for single servings. Carbohydrates, when quantity is indicated on label, exclude dietary fibre.
United Kingdom	Currently using non-starch polysaccharides in place of dietary fibre on food label. Proposal in place to adopt policy consistent with the rest of the European Union		Non-starch polysaccharides determined by one of the methods of Englyst et al. Proposed change in regulations would require use of AOAC International Official Methods of Analysis and include inulin and oligofructans in fibre quantity.
United States	Total Dietary Fibre – required Insoluble Dietary Fibre – voluntary Soluble Dietary Fibre – voluntary		Round to nearest gram per serving. Actual dietary fibre content must exceed 80% of the quantity labelled. Inulin as fibre under consideration. The energy value of Insoluble dietary fibre is 0, the energy of soluble dietary fibre is 4 kcal/g
Codex Alimentarius Committee on Methods of Analysis and Sampling	Dietary Fibre as determined by AOAC 985.29		
Codex Alimentarius Committee on Nutrition and Food for Special Dietary Uses	Deliberating on definition of dietary fibre and commensurate methodology.	Deliberating on appropriate criteria for ‘source’ and ‘high’ dietary fibre contents.	

to be included in the total dietary fibre declared as the fibre quantity on the label. AOAC International has adopted and published as of the latest edition, two Official Methods of Analysis for β-glucans [AOAC Official Methods of Analysis 992.28 (1→3)(1→4)-β-D-glucans in Oat and Barley Fractions and Ready-To-Eat Cereals; 995.16, β-D-Glucans in Barley and Oats-Streamlined Enzymatic Method.] and two Official Methods of Analysis for polyfructans [AOAC Official Method of Analysis 997.08, Fructans in Food Products-Ion Exchange Chromatographic Method and 999.03, Measurement of Total Fructan in Foods]. Additional methods are in the process, or have been adopted as Official Methods of Analysis for Fibersol-2® (a hydrothermally modified indigestible starch), and Polydextrose (a synthetic glucose polymer of random linkages plasticised with sorbitol). These techniques, when and if adopted as official methods, will appear in future editions of the Official Methods of Analysis.

10.3.2 *Enforcement of labelling regulations*

Once a societal decision has been made to disseminate nutrition information to consumers via the nutrition label of the food product, adequate analytical methodologies must be in place, or be put in place. This allows food producers to label their products correctly, and enforcement personnel adequately to enforce labelling regulations. Obviously, the ideal methodology will be very simple, inexpensive and rugged so that it can be carried out in all competent laboratories, it will be inherently accurate, and it will have a sufficiently high precision so that minimal or no replicates need to be carried out on any given sample. For the majority of food assays, this ideal has not been achieved, and dietary fibre is no exception.

Grouping of food components into a nutrient category (such as dietary fibre) means that the category must be defined, and the methodology then put in place must meet that definition as closely as possible. Having said that, the methodology used will influence the final quantity obtained; therefore, the method will be 'proximate' in that it will now define the analyte. In an ideal situation, the simplest enforcement scenario would have all regulatory bodies, research scientists, and those regulated accept a *single* concise definition and a *single* proximate method best suited to fit that definition. Then, by virtue of this acceptance, there would be universal agreement on enforcement of labelling regulations and the results obtained using the accepted method. However, the nutrition and analytical sciences are a continually evolving field, and nutrition and analytical scientists continue to discover new aspects regarding dietary fibre. Because of this, they differ in their scientific opinions on the optimum approach to quantitation, so a number of methods evolve, with a variety of virtues, such as increased speed, more detailed data, applicability to specific matrices, etc. AOAC International, in its role of validating Official Methods of Analysis serves the scientific and enforcement community by assuring that methods adopted as Official Methods of Analysis give equivalent results for dietary fibre, in accordance with the applicable definition. Should the accepted definition for dietary fibre change, then AOAC International has the framework in place to ensure that the methods are updated and/or modified to assure alignment and compliance with the updated definition.

10.4 Analytical aspects of regulatory enforcement

10.4.1 *Accuracy*

The key to accuracy in determining the dietary fibre content of a food lies in how well the method quantitates the portion of food meeting the dietary fibre definition. Because dietary fibre does not consist of a single, concise chemical entity, any method devised to measure it will be a proximate method. A proximate method, by definition, defines the analyte. Careful consideration is necessary to assure that the method includes all of the dietary fibre, while excluding any extraneous, non-dietary fibre components. Further, it behoves standardisation and methods validation organisations to adopt only those methods that meet the dietary fibre definition. Careful scrutiny and scientific peer review by those knowledgeable and skilled in the dietary fibre arena is necessary to assure methods that are accurate. If the proximate entity being quantitated is quite diverse, a combination of methods may be necessary for accurate quantitation.

A very important aspect of the accuracy of a dietary fibre method is to assure the method actually measures what is intended by the dietary fibre definition. In the case of methodology to meet the Trowell *et al.* definition, this means that the sample must be digested in a manner that simulates (as closely as possible) the human digestive system while still being practical for *in-vitro* testing at the laboratory bench. The enzymes used for digestion as part of the AOAC International Official Methods are set up to meet a strict set of enzymatic digestion requirements to assure proper performance. The requirements are listed in Table 10.3.

An analytical method that is set up in alignment of the dietary fibre definition, must treat the sample in a way that will result in a dietary fibre quantity most closely approximating the amount of indigestible food component the consumer will eat. The method must also allow for consistent regulatory compliance. Therefore, the AOAC International Official Methods incorporate a high-temperature starch gelatinisation step to assure that the fibre level of a particular product will not be overestimated. This is particularly important with respect to resistant starch (RS) which, by definition, resists digestion in the small intestine and ferments in the large intestine in the same manner that dietary fibre does. However, all types of RS do not behave similarly with regard to analysis or digestion. For example, starch that is not gelatinised is typically resistant to digestion. Potato starch that is gelatinised and then cooled will retrograde to become digestively resistant to some extent. The starch in green bananas has a higher percentage of starch that resists digestion than does the starch in ripe bananas. Finally, there is some starch that is transformed during food processing that becomes digestion resistant due to structural modification. RS has been divided (on perhaps a somewhat

Table 10.3 Enzyme performance characteristics in AOAC international dietary fibre methods to assure simulation of human digestion (see AOAC 985.29).

Enzyme activity	Enzyme activity found in human small intestine	Test substrate for AOAC Official methods	Recovery required of enzyme per method (%)
Pectinase	No	Citrus pectin	95–100
Hemicellulase	No	Stractan (larch gum)	95–100
β-Glucanase	No	Barley gum	95–100
α-Amylase	Yes	Wheat starch	0–1
α-Amylase	Yes	Corn starch	0–2
Protease	Yes	Casein	0–2

arbitrary basis) into categories based on their source and chemical make-up, as opposed to their digestive behaviours. From a food labelling perspective, much of this RS becomes irrelevant. The producer and merchandiser cannot control the ripeness of the banana at the time the consumer eats it; nor can they control whether a product is eaten raw (starch ungelatinised) or cooked (starch gelatinised), nor the extent to which potatoes are allowed to cool before eating (starch partially retrograded). However, the manufacturer can control the modifications to starch that occur in processing. It is well known that not only RS but also additional portions of the starch in food manage to escape hydrolysis and absorption in the small intestine. These starch portions then pass into the large intestine where they are fermented, providing energy for bacterial growth and transferring energy back to the body when the fermented material forms short-chain fatty acids that are absorbed through the colon wall (Hill 2000). However, the actual proportion of starch reaching the large intestine is highly variable, depending among other things on the make-up of the meal with which it is eaten. For food labelling purposes, producers and regulators need methodology that will indicate that portion of the RS which is certain to be digestively resistant. Therefore, the enforcement personnel and producers need a method that gelatinises the starch before the sample is quantitated with regard to dietary fibre, so that digestible starch is not inadvertently included in the dietary fibre quantity. The type of starch that resists digestion, no matter the form of the food it is in, or the treatments given the food by the consumer, is the structurally modified starch produced by processing. The AOAC International Official Methods of Analysis include this starch in the dietary fibre quantity.

The current AOAC International Official Methods of Analysis for dietary fibre, based on simulation of physiological digestion of the food sample, are simple, inexpensive, reasonably rugged, and can be carried out in competent laboratories world-wide. Because the methods are based on simulating human physiological digestion, they are proximate methods (i.e. the method defines the result) and therefore are accurate by definition. Future Official Methods Technical committees will need to assure alignment of methods and definition as additional methods are given consideration for adoption as Official Methods.

Because the methods of analysis for dietary fibre are proximate methods, it is incumbent upon the laboratories performing the methods to perform them exactly as they were developed, tested, validated and adopted. Often, minor deviations in performing a proximate method can result in shifts in the results achieved, resulting in a loss of accuracy. Laboratories must be sure they are performing the analysis correctly. One of the means to assure correct performance is through the use of reference materials (samples prepared using representative matrices at defined dietary fibre levels, characterised in a variety of ways, typically by multilaboratory assay). Reference materials for dietary fibre and related analyses are available from the American Association of Cereal Chemists (dietary fibre and β-glucans in oat bran), the National Institute of Standards and Technology (dietary fibre in composite baby food), and the Bureau of Community Reference (dietary fibre in haricot beans, rye flour and wheat flour). Laboratories attempting to upgrade methodologies by investigating new technologies, for example, can utilise the reference materials to assure the upgrades being investigated indeed provide an accurate result.

10.4.2 *Precision*

While the AOAC International Official Methods of Analysis for dietary fibre are simple, inexpensive and relatively rugged, the methods entail enzymatic digestions and numerous

manual manipulations of the sample. Because of this they have a typical within-laboratory precision of approximately ± 0.4% of the sample expressed as the within-laboratory standard deviation. For high-fibre samples, this presents relatively little problem, i.e. a standard deviation of ± 0.4% for a fibre content of 50% gives a relative standard deviation of ± 0.8% of the actual fibre content. However, when the fibre content of the sample is 1%, a standard deviation of ± 0.4% gives a relative standard deviation ± 40%. Therefore, numerous replicates need to be run on samples with low dietary fibre levels to assure that an accurate mean dietary fibre level determination has been achieved. Recent recommendations (Anon. 1999b) regarding dietary fibre intake are in the range of 20–40 g of fibre per day. Therefore, a 100 g portion of a food with 1% dietary fibre will provide 2.5–5% of the recommended daily consumption quantity. Thus, it is important that analysis of samples containing as little as 1% total dietary fibre be adequately performed.

Compensation for much of the inherent natural variability in dietary fibre content of foods (and to some extent the variability in analysis) is handled by regulatory agencies by the use of rounding of results applied to the food label. This provides a convenient means of assuring the accuracy of the label, but in the long term may be a poor reflection of the actual fibre provided by the food, if the true fibre content is at either end of the rounding range. For example, assume the recommended daily dietary fibre intake is 25 g, and that the labelling rule requires rounding to the nearest gram. Then, a food containing 1.51 g of dietary fibre and a food containing 2.49 g of dietary fibre per serving will both be labelled as containing 2 g. The food containing 1.51 g of dietary fibre per serving will provide 6% of the recommended daily intake, while the sample containing 2.49 g will provide 10% of the recommended daily intake. Both will be labelled as having 8% of the recommended daily intake. It might be advantageous in the case of dietary fibre labelling to have results rounded to the nearest 0.1 g per serving, with the labelled quantity being determined on the basis of the average dietary fibre content as measured using a statistically significant sampling plan. Taking such an approach, would however, require that policies emphasising that dietary fibre content exceed a defined relative minimum to be considered acceptably labelled, be replaced by policies that emphasise accuracy of the mean analytical result to be acceptably labelled. The public health impact of such a policy change would have to be evaluated.

The analytical precision of the method(s) used for dietary fibre labelling and enforcement has a significant effect on the amount of effort necessary to produce an accurate nutrition label. For example, assume a product truly contains 3.0 g of dietary fibre per 100 g serving of product. Assume that the rounding rules require rounding to the nearest 1 g increment of fibre – in this case to 3 g as opposed to 2 g. How many replicate assays of the dietary fibre in the product need to be run to be 95% confident that the correct label value is 3 g rather than 2 g? For this example, a within-laboratory standard deviation typical of that for the AOAC International method 985.29 will be used, in this case a standard deviation of ± 0.4% for the fibre analysis.

As can be seen in Table 10.4, only duplicate assays are required to establish that the label value should be 3 g rather than 2 g or 4 g with 95% confidence. On the other hand, if a product truly contains 2.8 g of fibre, five replicate assays will be required for 95% confidence in a 3 g declaration. Finally, as the true value of the fibre approaches the rounding point of 2.5 g of fibre, i.e. say 2.6 g of fibre are actually present, it takes 40 replicate analyses to be 95% confident of rounding to 3 g versus 2 g.

In contrast, if a laboratory is confident that it has reduced the variability of the assay by half, i.e. to a within-laboratory standard deviation of ± 0.2%, the impact on replicate assays is

Table 10.4 Statistical 95% confidence limits of analysis (standard deviation of assay = ± 0.4 g).

Number of Replicates	3.0 g	2.8 g	2.6 g
1	2.34–3.66	2.14–3.46	1.94–3.26
2	**2.53**–3.47	2.33–3.27	2.13–3.07
3	2.62–3.38	2.42–3.18	2.22–2.98
4	2.67–3.33	2.47–3.13	2.27–2.93
5	2.71–3.29	**2.51**–3.09	2.31–2.89
6	2.73–3.27	2.53–3.07	2.33–2.87
7	2.75–3.25	2.55–3.05	2.35–2.85
8	2.77–3.23	2.57–3.03	2.37–2.83
9	2.78–3.22	2.58–3.02	2.38–2.82
10	2.79–3.21	2.59–3.01	2.39–2.81
40	2.90–3.10	2.70–2.90	**2.50**–2.70

tremendous. Table 10.5 illustrates the effect of this improved precision. In this case, a single assay is sufficient for the product containing 3 g of dietary fibre, only duplicates are required for a product containing 2.8 g, and 10 replicates are necessary for a product containing 2.6 g of dietary fibre. Obviously, it behoves laboratory personnel to improve the precision of their assays to every extent possible to reduce the replication workload.

All these determinations of required replicates are based strictly on a statistical point of view. Perhaps the analyst or the product formulator has additional information on the product to make a reliable determination without running all of the 40 replicates in the first case, or the ten replicates in the second case.

Current US regulations allow for consideration of analytical variability in the enforcement of labelling regulations. Although at first blush, this might appear to offer an advantage to those preparing the nutrition label, in fact it does not. The analyst needs always to be cognisant of the fact that analytical variability is indeed that, variability. For example, consider a food with a dietary fibre content near the division line for rounding. From a strictly statistical point of view, there is equal probability that the regulatory laboratory will determine a quantity of

Table 10.5 Statistical 95% confidence limits of analysis (standard deviation of assay = ± 0.2 g).

Number of Replicates	3.0 g	2.8 g	2.6 g
1	**2.67**–3.33	2.47–3.13	2.27–2.93
2	2.77–3.23	**2.57**–3.03	2.37–2.83
3	2.81–3.19	2.61–2.99	2.41–2.79
4	2.84–3.16	2.64–2.96	2.44–2.76
5	2.85–3.15	2.65–2.95	2.45–2.75
6	2.87–3.13	2.67–2.93	2.47–2.73
7	2.88–3.12	2.68–2.92	2.48–2.72
8	2.88–3.12	2.68–2.92	2.48–2.72
9	2.89–3.11	2.69–2.91	2.49–2.71
10	2.90–3.10	2.70–2.90	**2.50**–2.70
40	2.95–3.05	2.75–2.85	2.55–2.65

dietary fibre in the sample that is lower than the quantity the labelling laboratory found as that they will determine a higher quantity – an additional reason for the labelling laboratory to be utilizing the methodology specified in the regulations, and to be certain that the laboratory is performing the method accurately.

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11 Regulatory Issues Relating to Dietary Fibre in the European Context

Paul Coussement

Abstract

Nutrition labelling in Europe is regulated by the Nutrition Labelling Directive. While this directive does mention dietary fibre, it does not provide a definition for fibre, and consequently Member States can use their own definitions. In many countries, there is no official definition of fibre, but in others there are guidelines or even legal definitions or accepted methods. The United Kingdom, with its preference for the Englyst methods (Englyst *et al*. 1994) has always taken a position that was different from the other countries.

In 1994, the European Scientific Committee for Foods attempted to create a new definition for dietary fibre, but no conclusion could be reached. Consequently, the Standing Committee on Foods has released a statement according to which several methods of analysis should be allowed, and differences should not lead to barriers to free trade.

Currently, the last differences between Member States are disappearing – a situation that may finally lead to a harmonised approach to dietary fibre in Europe.

11.1 The EU nutrition labelling directive

Directive 90/496/EEC sets out standardised rules on nutrition labelling, and these exclude any other form of such labelling. Food producers are bound by these rules if they decide to include a nutrition claim on their labels. Foodstuffs that do not bear any nutrition claim may circulate freely. The regulated items contain energy values, amounts of proteins, carbohydrate, sugars, fat, saturates, fibre and sodium.

'Fibre' falls into 'Group 2' labelling, which is required where a nutrition claim is made for sugars, saturates, fibre or sodium. In this group, although it is required to label the energy value and the amounts of protein, carbohydrate, sugars, fat, saturates, fibre and sodium, no definition for dietary fibre was provided. Article 1 in Directive 90/496/EEC refers to:

> '"fibre" means the material to be defined in accordance with the procedure laid down in Article 10 and measured by the method of analysis to be determined in accordance with that procedure.'

However, no definition for 'fibre' – and no method for fibre analysis – has ever been agreed upon at a European level.

It is remarkable to see that the word 'fibre' was chosen, rather than 'dietary fibre'. Moreover, no provisions are made for the use of either 'soluble' or 'insoluble' fibre.

11.2 National variations

As with all directives, the nutrition labelling directive was transposed into national law by all the European Member states. In some cases, this transposal was made almost literally, and no changes were made to the status or definition of dietary fibre. In some other cases – as in Belgium and Italy – the national law or regulation has added a definition of dietary fibre. In these countries, the legislator decided that a labelling law without clear definitions could not be used to control food labelling efficiently. In Belgium, the official definition is, 'The components of the foods that are normally not broken down by the body's own enzymes of humans' (Anon. 1992a), while in Italy, the definition is, 'Edible substance of vegetable origin which normally is not hydrolysed by the enzymes secreted by the human digestive system'. (Anon. 1993a).

In countries such as the United Kingdom and Germany, the inspection authorities will refer to dietary fibre (DF) definitions that have been put forward by official or semi-official bodies. In the UK, the reference definition has long been the COMA (Committee on Medical Aspects of Food Policy) definition of 'non-starch polysaccharides', measured using the Englyst method (Englyst *et al*. 1994), though MAFF (Ministry of Agriculture, Fisheries and Food) has always confirmed that these definitions were not 'legal' definitions. Recently, MAFF has modified its position, by also accepting the AOAC methods for the determination of the amount of fibre in food (Anon. 1999a).

In Germany, reference is often made to the GDCh (German Society of Chemists), which (when translated) has defined 'Ballaststoffe' as 'Substances of plant origin, that cannot be broken down to resorbable components by the body's own enzymes in the small intestine. Included are essentially soluble and insoluble non-starch polysaccharides (cellulose, hemicellulose, pectin, hydrocolloids) and lignin and resistant starch. Substances like some sugar substitutes, organic acids, chitin and so on, which either are not or are incompletely absorbed in the small intestine, are not included'. (Anon. 1989).

11.3 Carbohydrates, polyols and dietary fibre

There are only two possibilities for the classification of carbohydrates in the European Nutrition Labelling directive. The classes 'polyols', 'protein', 'fat', 'alcohol', 'organic acid', 'sodium', ... are excluded for obvious reasons, and only the classes of 'carbohydrate' and 'fibre' are appropriate at first sight.

The definition of 'carbohydrate' is: 'any carbohydrate which is metabolised in man, and includes polyols'. Chemically speaking, 'carbohydrate' includes most dietary fibres, e.g. cellulose, hemicellulose, inulin and oligosaccharides (but does not include lignin); thus it is the section of text relating to 'metabolised in man' that makes the difference in this definition.

'Metabolisable by man' is not clearly defined, and may be interpreted in two ways:

(1) '... used in the metabolism of man, **not including** the fermentation by the microflora in the colon': meaning that the carbohydrate should be hydrolysable in the small intestine (or absorbable in the case of monosaccharides). Carbohydrates that are not hydrolysed in the small intestine but fermented in the colon are in this context not metabolised by *man*, but by the *microflora* present in the colon.

(2) '... used in the metabolism of man, **including** the fermentation by the microflora in the colon': meaning that carbohydrates which are not hydrolysed in the small intestine but fermented in the colon would also be 'metabolised by man'.

Dietary fibre is, by definition, not hydrolysed in the small intestine. In fact, most fibres are fermented to a large extent in the colon (e.g. cellulose 7–40%, non-starch polysaccharides 85–99%, pectin 99%). Therefore, interpretation (2) cannot be maintained, because in this situation a large part of most dietary fibres would be classified as 'metabolisable carbohydrate'. Moreover, there would be a significant overlap between 'carbohydrate' and 'fibre'. As all this would conflict strongly with the available scientific evidence, only interpretation (1) can be valid.

11.4 The 1994 attempt to come to a definition

In 1993 and 1994, the European Scientific Committee for Foods (SCF) attempted to derive a new definition of dietary fibre. A draft document was prepared which basically proposed the use of the term 'Non-Starch Polysaccharides of plant cell walls' as a definition and – obviously – the Englyst method (Englyst *et al*. 1994) for analysis. This proposal was totally rejected by the SCF, and they proposed no alternative definition. Consequently, the European Standing Committee on Foodstuffs released the following important statement:

> 'Dietary fibre.
> 'The Committee is aware that the subject of the definition of dietary fibre for the purpose of nutrition labelling is a controversial one. It recognised that apart from non-starch polysaccharides of plant cell wall, the traditionally accepted as fibre material, other substances were considered as fibre, in many Member States, like resistant starch, lignin, inulin, polydextrose.
>
> 'Different methods were also used for measuring the material declared as fibre on the label. The AOAC method is widely accepted and used while the Englyst method can also be used in some Member States. Discrepancies in the results given by the two methods are not significant for the vast majority of the products. Some of the substances (e.g. inulin, polydextrose) cannot be measured by either of the above methods and need to be measured with specific methods which have been developed. It is noted that the use of these substances in the manufacture of foodstuffs is rather limited.
>
> 'The Standing Committee is convinced that the differences which would arise from the use of the different methods for the determination of the material to be labelled as fibre would not be significant and therefore not detrimental to consumer information. Therefore they should not be evoked for impeding the free movement of goods within the European Union.
>
> 'The Standing Committee also notes that the Scientific Committee for Food will look again into the question of the definition of dietary fibre in the future.' (Anon. 1994).

This text now has become the reference for labelling, and serves as guideline for any evaluation and development of dietary fibre definition in the European Union. Despite the last

sentence, the subject of Dietary Fibre has not been approached since this last treatment by the SCF.

11.5 Caloric value

The European Nutrition Labelling Directive does not specify a calorie factor to be used for dietary fibre; consequently, the caloric value of dietary fibre can be considered to be zero. This was confirmed in letters from the European Commission which '… confirm your interpretation of the current Community rules on nutrition labelling on this point, namely that energy value of dietary fibre should be considered as 0 kcal'. (Anon. 1992b).

The main driver behind this position is that nutrition labelling should be simple, easily understood, and comparable. Counting dietary fibre at 0, 1 or 2 kcal/g would make practically no difference on most food labels. However, in practical cases where high amounts of dietary fibre are used, most companies do not use a caloric value of zero, but have chosen values between 1 and 2 kcal/g, depending on the compounds.

11.6 Official methods

In many (but not all) European countries, the authorities have advised the use of specific methods for dietary fibre analysis. With the exception of the UK, all these methods are gravimetric methods, and are mostly based on the AOAC methodologies (Prosky *et al*. 1988).

11.7 Nutrient content claims on fibre

There are no harmonised European definitions for nutrient content claims, nor for fibre-related claims. In 1992, a proposal was made for a EU Directive (on Claims) which was later abandoned (Anon. 1993b). In this proposal, the following definitions were used:

- Rich in fibres: >6 g/100 g.
- Increased in fibres : >3 g/100 g and at least 25% more.
- Contains x% more fibre: contains at least 2 g/100 g more, and at least 15% more.

As far as we have been able to determine, there are no specific rules in Belgium, Luxembourg, Italy or Spain. In some countries there are guidelines of various origin, some of which are detailed below.

11.7.1 *United Kingdom*

The Food Advisory Committee issued in June 1999 a sensibly simplified version of their previous guidelines, called the 'Nutrition Claims in Food Labelling and Advertising' guidelines. The following claims are defined (Anon. 1999b).

- Source of fibre: either 3 g per 100 g or 100 ml or at least 3 g in the reasonable expected daily intake of the food. In the case of a food naturally high in fibre, the claim must take the form of 'a high-fibre food'.
- Increased in fibre: at least 25% more than a similar food for which no claim is made, and at least 3 g in either the reasonable daily intake of a food for which this is lower than 100 g or 100 ml *or* in 100 g or 100 ml.
- High in fibre: either at least 6 g per 100 g or 100 ml or at least 6 g of the reasonably expected daily intake of the foods.

Recently, MAFF (Anon. 1999a) has modified this system by proposing:

- For 'source of fibre': either at least 4 g per 100 g or 100 ml or at least 4 g in the reasonable expected daily intake of the food.
- For 'increased in fibre': at least 25% more than a similar food for which no claim is made and meets the criterion for source of fibre.
- For 'high in fibre': either at least 8 g per 100 g or 100 ml, or at least 8 g in the reasonable expected daily intake of the food.

11.7.2 *The Netherlands*

The 'Warenwetbesluit Voedingswaarde-informatie levensmiddelen' (Anon. 1993c) describes:

- 'High in fibre': if the product, calculated on the dry matter, contains at least 10% of dietary fibre, to such extent that if the product is a soup, drink or liquid milk product, this content is to be calculated on the final product.
- 'Increased in fibre': if the product contains at least 33% more dietary fibre than the usual content in such products.

11.7.3 *France*

In France, there are no specific regulations regarding minimum doses for specific claims.

Foods which are destined for diets with specific fibre content are considered to be dietetic foods ('Aliments à teneur particulière en son de blé' and 'Aliments à teneur particulière en fibres alimentaires diverses'). Such foods are authorised only upon approval of a file to be submitted, which substantiates the claims which are made. For wheat bran (son de blé), there are specific rules with regard to the claims that can be made for these types of food. A list of acceptable and non-acceptable claims exists.

Recently, the CEDAP (Commission d'étude des produits destinés à une alimentation particulière) (Anon. 1998) has proposed that:

- 'source' requires at least 3 g/100 g or 1.5 g/100 kcal;
- 'rich' requires at least the double of the previous values; and
- 'more' ('teneur accrue en…') requires at least 25% difference in weight, one should obtain at least 5%/100 kcal.

11.7.4 *Germany*

In Germany, there are no specific regulations regarding minimum doses for specific claims. There is an opinion document by the GDCh (Gesellschaft der Deutsche Chemiker), which is considered as a reference for legal evaluations in this field. This document, among others, specifies:

- to make any claim on dietary fibre presence, a minimum of 3 g should be present in a daily portion;
- to make a 'high', 'rich' or 'increased' fibre claim, a minimum of 6 g should be present in a daily portion, with some exceptions (Anon. 1989).

11.8 The future

The update of the Nutrition Labelling Directive has for a long time been on the 'to do' list of the European Commission. However, developments on this are very slow, and expert observers do not expect a significant change or progress in the coming years.

'Dietary fibre' is still a controversial subject, and will remain so for quite some time. It is becoming clear to all experts involved that it is practically impossible to arrive at one perfect definition for dietary fibre, as it is simply not possible to have general agreement on one single definition that takes everything into consideration. So, pushing this line will not quickly lead to success.

However, who needs a definition? The Nutrition Labelling Directive has been working for many years without one, and 'novel' dietary fibres such as inulin and oligofructose have now been independently accepted for dietary fibre labelling by all European countries. The process for obtaining such National authorisations is long and inefficient, but not impossible. We have followed this procedure to obtain acceptance for inulin and oligofructose for 'dietary fibre' labelling in each and every European member state separately. In almost every case, we have submitted a file with all relevant nutritional data, and today – after more than eight years of work – all countries are in agreement. This was most probably successful because we had a very good case – including the scientific data, the analytical methodology and the support of leading scientists.

What is required first is not so much a definition of dietary fibre, but an outline of the 'dietary fibre concept' in nutritional terms, i.e. what should all substances that are classified as 'dietary fibre' bring to the consumer ? Based on this concept, new substances can be evaluated in the same way as 'health claims' or 'structure–function claims' are. This means that the producers should be able to present a good scientific case, based on which a decision can be made as to whether or not the substance should be included in the 'dietary fibre' group. Based on the same concept, recommended daily intakes and dietary guidelines can also be established. Thus, it seems that a good definition of the 'dietary fibre concept' is the first step required.

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Part 5

Health Benefits of Dietary Fibre

12 Dietary Fibre in Health and Disease

David Kritchevsky

12.1 Introduction

The material that we identify as dietary fibre has for centuries been recognised as having health benefits. Burkitt (1986) traced the history of fibre from Hippocrates to Galen, to Atkinson, Lane and McCarrison in England, and to Graham and the brothers Kellogg in the United States. Early work on fibre concentrated, generally, on its laxative properties. Several centuries ago, Elyot (1541) pointed out that increasing the intake of fibre led to improved bowel habits and increased faecal bulk. Burkitt (1983) reminds us that Hippocrates commented on the laxative effects of coarse flour, as did the Persian physician Hakim in the ninth century, who observed that chuppatis made from wheat flour containing little bran take a long time to be excreted. Probably the earliest dietary fibre trial is reported in the Book of Daniel (1:8–16); this describes how Daniel and his men who eschew a rich meal for a diet of pulses appear healthier than a group of Belshazzar's men who subsist on the richer diet. The term 'dietary fibre' was coined by Hipsley (1953).

The modern fibre era dates to an article by Surgeon Captain T.L. Cleave (1956), who suggested that the major ailments of modern man could be due to his use of refined sugar and refined flour. Cleave later summarised his views in books relating to, what he referred to as, 'The Saccharine Disease' (Cleave & Campbell 1966; Cleave 1974).

In the modern era, much of the data relating to fibre and health was generated in Africa by Burkitt, Trowell and Walker, all of whom were physicians at various African venues and all of whom had keen intelligence coupled with great powers of observation. Their studies initially centred on differences in stool weight between Africans and Caucasians, and they were able to reinforce their observations and hypotheses by citing published studies relating to different types of fibre and laxation (Cowgill & Anderson 1932; Williams & Olmstead 1936; Dimock 1937). Walker (1997) summarised a half-century of his research and observations relating to comparisons of diet and lifestyle in Africans and Europeans and how these account, in part, for differences in disease states in these populations.

Following Cleave's lead, the investigations led to studies of the effects of refined carbohydrate foods. The investigators soon recognised the fact that the foods under discussion were those containing carbohydrate which was unavailable to the host. Trowell (1976) eventually offered a definition of dietary fibre which we still use as a reference point. Trowell suggested that dietary fibre was the sum of lignin and polysaccharides that are not digested by the endogenous secretions of the human digestive tract. This definition permits the inclusion, under the inclusive term 'dietary fibre,' of other indigestible polysaccharides that may occur in the diet, namely, pectin, gums, mucilages, algal polysaccharides and modified celluloses.

The substances which we describe as dietary fibre differ in chemical and physical properties and exert a variety of effects; chemical, physical and metabolic. It is not logical to believe

that all the substances we designate as fibre can affect all areas of nutrition and physiology. Water binding and gelling are physical manifestations of fibre action and influence gastric emptying, faecal flow and absorption of nutrients. Structural features of dietary fibre may influence binding of bile acids and inorganic ions. Bulking in the large intestine may increase excretion of those substances trapped within the faecal mass. The fibres themselves, while not subject to digestion in the mammalian digestive tract, serve as substrates for the intestinal microflora with subsequent production of energy and short-chain fatty acids (SCFA), particularly acetate, propionate and butyrate, all of which can influence the metabolism of the host. Hellendoorn (1978) predicted that much of what we describe as fibre action is due to the actions of the SCFA. Focusing of professional and public interest on dietary fibre may be attributed to Burkitt's (1971) argument linking fibre intake with susceptibility to cancer of the colon and rectum, and to a paper by Burkitt *et al*. (1974), which compared eight disease conditions prevalent in the United States and virtually non-existent in African black people, and suggested that those differences were due to the fibre content of their respective diets.

Eventually, we came back to discussing influences of dietary fibre on 'Western' diseases. A compilation of Western diseases has been presented by Trowell (1979), and represents the contributions of a number of authors including Donnison (1937), Trowell (1960), Burkitt (1973), Walker (1974), Cleave (1974), and Gelfand (1975). The list is presented in Table 12.1. It appears that any disease state that is prevalent in developed societies and missing in Africa could be attributed to the lack of dietary fibre.

A report covering medical aspects of dietary fibre, and the areas where fibre might play a role, was written by The Royal College of Physicians (1980), and 20 years later remains one of the most useful books in the field. The report discussed the actions of dietary fibre along the intestinal tract, these having been summarised by Heaton (1983) (Table 12.2). The effects of fibre in the hind gut (faecal weight, transit time and composition) were among the factors which brought effects of dietary fibre to the attention of the early investigators.

Table 12.1 'Western' diseases.

Colonic and abdominal diseases Appendicitis; cancer of the large bowel; constipation; Crohn's disease; diverticular disease; haemorrhoids; hiatal hernia; irritable bowel syndrome; ulcerative colitis
Metabolic diseases Angina; cerebrovascular disease; cholesterol gallstones; deep-vein thrombosis; diabetes mellitus; essential hypertension; ischaemic heart disease; osteoporosis; Paget's disease; phlebitis; pulmonary embolism; renal calculi; toxaemia of pregnancy; varicose veins
Endocrine diseases Addison's disease; Hashimoto's thyroiditis; myxoedema; thyrotoxicosis
Autoimmune diseases Multiple sclerosis; pernicious anaemia; rheumatoid arthritis

Source: Trowell (1979)

Table 12.2 Actions of dietary fibre.

Site	Actions
Food	Solidifier, hardener, water trapper
Mouth	Saliva stimulant, cleaner, work demander
Stomach	Diluter, distender, storage-prolonger
Small intestine	Diluter, distender, absorption-delayer
Large intestine	Diluter, distender, ion-binder, bacterial substrate/inhibitor, water trapper
Stool	Softener, enlarger, trauma/strain preventer

Source: Heaton (1983)

12.2 Fibre and the gastrointestinal tract

Fibre appears to exert the greatest influence on faecal output (Williams & Olmstead 1936; Cummings *et al* 1979). Stool weight is a function of the amount of fibre in the diet. When Dutch students were fed a diet rich in fruit, vegetables and wholemeal bread, their average stool weight increased by 167% over output on a diet containing no fibre-rich foods (Stasse-Wolthuis *et al.* 1979). Different types of fibre have different effects; thus, Cummings *et al.* (1978) found that faecal weight rose by 127% in young men fed 20 g/day of bran fibre, but increases on 20 g/day of cabbage, carrot or apple fibre or guar gum were 69%, 59%, 40% and 20%, respectively. Increasing levels of dietary fibre may relieve constipation, but effects on irritable bowel syndrome are inconclusive (Soltoft *et al.* 1976; Manning *et al.* 1977).

Prevalence of diverticular disease of the colon is related to ageing and it has been attributed to a low-residue diet (Painter & Burkitt 1971, 1975). This condition had actually been treated with a low-residue diet until Painter *et al.* (1972) showed that a high-fibre diet relieved most of the symptoms in a group of 62 patients. Their finding have been confirmed in other trials (Plumley & Francis 1973; Brodribb 1977).

The foregoing have been conditions which can be relieved by the mechanical action of dietary fibre. Effects of fibre on other conditions such as obesity, gallstones, diabetes, coronary disease and colon cancer involve effects of fibre on metabolic functions.

12.3 Obesity

There is little evidence that an increase in dietary fibre can, in itself, affect obesity. Bonfield (1995) has recently reviewed effects of dietary fibre on weight management. Some 50 studies have been published relating to the effects of fibre on weight loss. In 17 studies of soluble fibre effects, 11 (65%) reported appreciable weight loss, while six did not. In addition, when insoluble fibre was fed (10 studies), 60% of studies reported no appreciable weight loss, but in 78% of studies (18/23) in which mixed fibres were fed, the subjects showed appreciable weight loss. Most of the effective studies used barley, and several possible mechanisms of fibre action have been proposed, including longer time spent chewing, stomach distension, delayed stomach emptying, reduced nutrient absorption, effects on gastrointestinal hormones (such as cholecystokinin, gastrin or glucagon), the ileal 'brake' (slowing of transit time), and increased caloric excretion. The effect of fibre may be more useful in weight

maintenance than in weight loss, although long-term effects are yet to be determined. Weight control is still largely a matter of caloric book-keeping.

12.4 Gallbladder disease

Most gallstones are composed primarily of cholesterol. In the bile, cholesterol is solubilised in micelles, which also contain bile acids and phospholipids. The optimal concentrations of these three components lie within a narrow range such that, when the range is exceeded the bile becomes saturated with cholesterol, which may precipitate in the form of gallstones. The addition of large quantities of wheat bran to the diet will reduce cholesterol saturation significantly (Pomare *et al.* 1976; McDougall *et al.* 1978). The mechanism of action of bran may also involve effects on the bile acid pool. Interest in dietary treatment of gallstones has waned with the development of effective drugs and less complicated surgery.

12.5 Diabetes

The ameliorating influence of dietary fibre on plasma glucose and insulin levels in diabetics has been demonstrated through the work of Jenkins and Anderson (Anderson 1981; Anderson *et al.* 1990; Jenkins *et al.* 1995). There is little evidence that diabetes occurs as a result of a low-fibre diet, however. This suggests that fibre affects the symptomatic evidence of diabetes rather than the source. The diabetic patient is grateful for the relief of symptoms and is willing to let the academic world worry about mechanisms and aetiology.

Jenkins and his colleagues demonstrated that diets rich in complex carbohydrates or specific soluble fibres such as guar or pectin, normalised glucose tolerance and plasma insulin levels. Anderson's group pioneered the use of high-carbohydrate, high-fibre diets in reducing glycaemia and insulinaemia in diabetic patients. Jenkins has suggested that gelling fibres such as guar or pectin form a matrix in which glucose and other dietary components are rendered unabsorbable. There is little evidence that diets low in dietary fibre play a role in the development of non-insulin-dependent diabetes mellitus.

12.6 Lifestyle diseases

The major causes of deaths in the developed world are heart disease and cancer. These are often referred to as 'lifestyle' diseases, suggesting that diet is only a part of the disease complex. There being no simple test for either disease, we depend instead on identification of metabolic behavioural aberrations as portents of eventual illness – these are called 'risk factors'. Insofar as heart disease is concerned, some of the major risk factors are high blood cholesterol levels, cigarette smoking, high blood pressure and male gender. Hopkins and Williams (1981) have identified over 200 risk factors (both positive and negative) for the development of heart disease and, while most of them are minor, each represents a possible clue. New risk factors such as lipoprotein(a) (Rhoades *et al.* 1986), homocysteinaemia (Ueland *et al.* 1992) and *Chlamydia* infection (Grayston 1999) continue to arise. We look to diet for clues to the aetiology of both diseases.

12.6.1 *Heart disease*

Insofar as atherosclerosis is concerned, one universal measurement serves as a diagnostic indicator, and that is serum or plasma total cholesterol. One can also measure lipoprotein levels, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). There are data attributing predictive powers to total cholesterol, as well as to either major lipoprotein fraction. The earliest interest in dietary fibre centred on wheat bran, but it has no effects on cholesterol levels (Eastwood 1969). It is now established that, whereas insoluble fibres (wheat bran, cellulose) do not influence serum lipids, soluble fibres (guar, pectin, psyllium) exert a hypocholesterolaemic effect (Schneeman & Lefevre 1986; Anderson (1995) (Table 12.3). The mechanism(s) by which soluble fibre exerts its hypolipidaemic effect involve increased viscosity of contents of the stomach and small intestine, and they may also influence bile acid metabolism (Lairon 1997).

A study of dietary fibre and serum lipid levels in Seventh Day Adventists showed that only the true vegans had significantly lower serum cholesterol levels (149 ± 8 mg/dl; 3.85 ± 0.21 mmol/l), when compared with lacto-ovo vegetarians (serum cholesterol 192 ± 7 mg/dl; 4.97 ± 0.18 mmol/l), non-vegetarian Seventh Day Adventists (serum cholesterol 207 ± 7 mg/dl; 5.35 ± 0.18 mmol/l), or the general public (serum cholesterol 214 ± 7 mg/dl; 5.53 ± 0.19 mmol/l). The only difference in fibre intake among the four groups was a higher intake of pectin (7.5 g/kcal/day versus 4.0–4.5 g/kcal/day) (Kritchevsky *et al.* 1984).

There is developing epidemiological evidence, however, that a high-fibre diet may lower heart disease-related mortality. Humble (1997) summarised the available data from several prospective studies (Table 12.4). Morris *et al.* (1979) reviewed their study involving 337 healthy middle-aged men and found an inverse correlation between cases of coronary heart disease and fibre intake, the best correlation being with cereal fibre. Kromhout *et al.* (1982) and Fehily *et al.* (1993) found a significant protective effect of fibre which disappeared upon adjustment. Khaw and Barrett-Connor (1987) found fibre intake to be protective in both men and women. Humble *et al.* (1993) studied a cohort of 1801 men aged 45–59 years for nine years, and found fibre intake to be protective. Rimm *et al.* (1996) studied 43 757 men for six years and reported that dietary fibre intake was significantly protective against coronary events (Table 12.5). Table 12.6 summarises other differences between health professionals

Table 12.3 Influence of dietary fibres on human serum total and low-density lipoprotein (LDL)-cholesterol (% of control).

Fibre	Total cholesterol	LDL-cholesterol
Wheat bran	102	97
Corn bran	97	98
Cellulose	104	108*
Soy hulls	107	104
Oat bran	87*	86*
Guar gum	87*	84*
Locust bean gum	86*	86*
Gum karaya	90*	90*
Legumes	87*	95

Source: Schneeman and Lefevre (1986).
* Significant effect.

Table 12.4 Fibre effects in coronary heart disease.

Study	No. of patients/sex	Age (years)	Years	Findings	
				Unadjusted	Adjusted
Morris *et al*. (1977)	337/M	30–67	10–20	<0.05	<0.05
Kromhout *et al*. (1982)	871/M	40–59	10	<0.05	–
Kushi *et al*. (1985)	1001/M	30–69	19	<0.05	–
Khaw & Barrett-Connor (1987)	356/M 503/F	50–79 –	12 <0.05	<0.05	<0.05
Burr *et al*. (1989)	1879/M	56.6	2	–	*
Fehily *et al*. (1993)	2423/M	45–59	5	<0.05	–
Humble *et al*. (1993)	1801/M	45–59	9	<0.05	–
Rimm *et al*. (1996)	43 757/M	40–75	6	<0.05	<0.05

Source: Humble (1997).
*Deleterious effect.

Table 12.5 Relative risk of myocardial infarction across quintiles of food sources of fibre in 43 757 US male health professionals.

Fibre	*P*-value for trend (RR)
Fruit	0.11
Fruit plus vegetable and cereal fibre	0.10
Vegetable	0.06
Vegetable plus fruit and cereal fibre	0.05
Cereal	0.02
Cereal plus vegetable and fruit fibre	0.007

Source: Rimm *et al*. (1996).

Table 12.6 Baseline characteristics across quintiles of dietary fibre intake among 43 757 US male health professionals aged 40–75 years.

	% Difference
Characteristic	Quintile 1 versus Quintile 5
Fibre (g/day)	+133
User of vitamin E (%)	+92
Smokers (%)	–239
Physical activity	+42
Total fat (g/day)	–21
Saturated fat (g/day)	–31
Dietary cholesterol (g/day)	–26

Source: Rimm *et al*. (1996).

in the first and fifth quintiles of fibre intake. It is evident that increased fibre intake is but one aspect of total lifestyle changes. A 15-year study of 75 521 nurses suggests that increased intake of whole grains may protect against coronary heart disease (Liu *et al.* 1999). Another recent study (Ludwig *et al.* 1999) reports on data from a 10-year study of 3609 probands. Fibre consumption predicted insulin levels, weight gain and other risk factors (blood pressure, lipids, lipoproteins) more strongly than did total or saturated fat consumption. The investigators concluded that high-fibre diets may protect against obesity and cardiovascular disease by lowering insulin levels.

The data offer suggestions that soluble fibre lowers serum lipid levels, but a high-fibre diet may actually afford protection against cardiovascular heart disease.

12.6.2 *Cancer*

Research on the relationship of fibre to risk of cancer has been focused primarily on large bowel diseases, since that is the immediate site of fibre action. Higginson and Oettle (1960), when surveying cancer incidence in South Africa, suggested that increased dietary bulk might play a protective role. Burkitt (1971 1980) suggested that variations in dietary fibre could explain international differences in incidence of colon cancer. Other reviews of the literature (summarised by Kritchevsky 1995) have not echoed Burkitt's certainty. Ecological studies suggest a more protective effect than do case-control studies. A relatively recent review (Byers 1995) found that fruit and vegetable fibre were more protective than cereal grain, a view which is disputed by Hill (1997), Slavin *et al.* (1997) and Jacobs *et al.* (1998). A meta-analysis of 13 studies involving 5225 cases and 10 349 controls showed a highly significant protective effect for dietary fibre (Howe *et al.* 1992). Thun *et al.* (1992) also adduced a protective effect of dietary fibre in both men and women. Jacobs *et al.* (1998) reviewed 40 case-control studies of 20 cancers and colon polyps. High whole grain intake was protective.

There are several ways by which fibre might affect colon cancer (Table 12.7). Cummings *et al.* (1992) have shown a significant relationship between low stool output, low fibre intake, and increased risk. Dietary fibres bind the bile acids and bile salts which may promote carcinogenesis (Story & Kritchevsky 1976). The concentration of bile acids in faeces is much higher in colon cancer-prone populations, suggesting that there has been a lower fibre intake and less dilution of colonic contents. It may be noted parenthetically that, while the binding of bile acids and bile salts by fibre has been a focus of studies relating to colon cancer, there is recent evidence that binding of oestrogen by fibre may affect the course of breast cancer (Rose *et al.* 1991; Rose 1996). Fibre may increase faecal energy loss (Southgate & Durnin 1970), and caloric intake has been suggested to be an important variable *vis-à-vis* colon cancer risk

Table 12.7 Effects of dietary fibre in the human colon.

- Increases faecal weight
- Increases frequency of defaecation
- Decreases transit time
- Dilutes colonic contents
- Increases microbial growth
- Alters energy metabolism
- Adsorbs organic and inorganic substances
- Decreases bile acid hydroxylation
- Produces H_2, CH_4, CO_2 and short-chain fatty acids

(Kritchevsky 1999a). Lower caloric intake is associated with reduced risk of colon cancer (Jain *et al.* 1980; Lyon *et al.* 1987).

Fermentation of fibre by the anaerobic microflora of the gut produces short-chain fatty acids, principally acetic, propionic and butyric. Butyrate slows growth of human colorectal cell lines *in vitro* (Kim *et al.* 1980), and induces differentiation (Augeron & Laboisse 1984) and apoptosis (Hague *et al.* 1993). Faecal butyrate concentration is correlated negatively with tumour mass. In rats, wheat bran provides the most favourable ratio of butyrate to propionate and acetate (McIntyre *et al.* 1993). Walker *et al.* (1986) found that the risk of colon cancer for ethnic populations in South Africa was correlated with faecal pH, but not with fibre intake.

Most of the data relating fibre intake to colon cancer risk suggest that whole grains or wheat bran may be the most protective fibre sources. Several large clinical trials of effects of wheat bran on colon cancer are under way in the United States, but no data are available at the time of this writing. In rats given chemical carcinogens, wheat bran is almost uniformly protective (Kritchevsky 1999b).

Southgate and Penson (1983) presented the following statements regarding the Dietary Fibre Hypothesis:

(1) A diet rich in foods containing plant cell walls (dietary fibre) is protective against a range of diseases prevalent in Western communities (coronary heart disease, diabetes, stroke, large bowel cancer, etc.).
(2) In some instances, a diet that is poor in foods containing plant cell walls is a causative factor in the development of the disease (e.g. diverticular disease), while in others it provides the conditions where other aetiological factors are active.

A reason for the dichotomies between population and case-control studies may be that the epidemiologists are studying a diet rich in plant foods, while others (in the reductionist mode) try to extract one item from that diet (such as fibre) on which to base their hypotheses, analyses or treatments.

A diet rich in plant foods contains a plethora of possibly anti-carcinogenic phytochemicals which may augment fibre action, exert a specific action of their own, or even antagonise the action of other food components. These have been discussed by Potter and Steinmetz (1995), and their metabolic actions have been discussed and well referenced. A formidable list of anti-carcinogenic phytochemicals is shown in Table 12.8, but interactions among these substances remain to be elucidated.

The interaction of nutrients is a subject that is rarely broached in discussions of diet and disease, but eventually it must be addressed. As Byers (1995) has pointed out, fibre co-varies

Table 12.8 Anti-carcinogenic phytochemicals.

Allium compounds	Inositol hexaphosphate
Carotenoids	Isoflavones
Coumarins	Isothiocyanates
Dithiolthiones	Saponins
Ellagic acid	Selenium
Flavonoids	Sterols
Folic acid	Thiocyanates
Glucosinolates	Vitamin C
Indoles	Vitamin E

with both micro- and macronutrients. Observational epidemiology is limited by the multiple co-linearity between nutrients (Smith *et al.* 1991), but the problem must be addressed. For example, in a study of diet and colon cancer (Slattery *et al.* 1988), it was found that the third quartile of vegetable and grain intake was more protective than the fourth quartile. However, in the case of fruit, the third quartile of intake was less protective than the second quartile (Table 12.9). A simplistic way to look at these findings is to say that in going from the third quartile to the fourth quartile of vegetable intake, the increase in vegetables reflected a reduction of intake of some other protective nutrient. Identification of what change in intake led to the observed effects might teach us much about devising effective diets.

12.7 Summary

In summary, the general observation that an increase in dietary fibre may help in the prevention of prevalent Western diseases has been a boon to public health. The investigators who pioneered these views – Cleave, Burkitt, Trowell and Walker – deserve our gratitude, as their observations and hypotheses set the stage for current advice. We now need to study the influences of individual phytochemicals and their interactions with the remainder of the dietary components. With regard to individual dietary behaviour and/or advice, there is no need for dietary extremism. A balanced diet taken in moderation is sufficient. Much dietary advice can be summarised as variety, balance and moderation. Or, put another way, moderation – not martyrdom.

Table 12.9 Colon cancer risk and dietary factors.

	Quartile (OR)			
	Lowest	2	3	4
Starch				
Male	1.0	1.5	0.9	1.7
Female	1.0	0.4	0.7	1.2
Vegetables				
Male	1.0	0.4	0.6	0.4
Female	1.0	1.0	0.8	0.3
Fruit				
Male	1.0	0.4	0.5	0.3
Female	1.0	0.8	1.6	0.6
Grains				
Male	1.0	1.1	1.6	1.0
Female	1.0	0.7	0.6	1.1

Source: Slattery *et al*. (1988).

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13 Dietary Fibre, Carbohydrate Metabolism and Chronic Disease

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13.1 Introduction

Historically, although this field owes much to Surgeon Captain Cleave and others before him, it was Denis Burkitt and Hugh Trowell who brought the idea of fibre and health together and into the public eye. Burkitt's main interest was in the connection between fibre and colonic disease (Burkitt & Spiller 1993), while Trowell (1993) carried the torch for the metabolic effects of fibre on carbohydrate and lipid metabolism and their related disorders. Many of the concepts they proposed found support in the work of Walker in South Africa, on whose findings they drew extensively.

As the debate progressed in the 1970s, many of the health benefits originally observed for a high vegetable fibre diet in Uganda became transferred to wheat bran for the Western world, possibly because of the visible immediacy of the colonic effect. Over the subsequent years, interest has shifted to viscous fibres, resistant starch and fructo-oligosaccharides – all of which fulfil the definition of fibre, as carbohydrates which enter the colon, but which have been excluded from the definition of fibre by those who consider that only cell-wall materials can be classed as dietary fibre. Nevertheless, they share many of the attributes of cell-wall polysaccharides, both in the small intestine and in the colon.

13.2 Effect of viscous and non-viscous fibre on glucose, insulin and gut hormones

Early on, it was shown that insoluble fibre sources such as wheat bran had little effect on glucose tolerance and the postprandial insulin response. However, the effects of viscous fibres on glucose tolerance and insulin responses was marked, and dependent on their viscosity (Jenkins *et al.* 1978). Studies using xylose excretion as a marker of the rate of absorption confirmed the slower rate of absorption with the more viscous fibres. These studies have been confirmed with other fibre sources, notably oat β-glucan (Metz *et al.* 1994). The mechanism appears to relate to impedance of solute movement through the unstirred water layer (Blackburn *et al.* 1984) and impedance to bulk phase diffusion of the products of digestion from lumen to the enterocyte (Flourie *et al.* 1984). Studies have also shown that the benefits of slowing the rate of absorption can be mimicked by sipping glucose over 3 hours rather than taking it in a bolus in 5 minutes (Jenkins *et al.* 1990). The concept of the advantage of spreading the nutrient load over time (e.g. nibbling versus gorging) has been shown to be of benefit in diabetes. Diabetics provided with small, frequent meals showed lower mean glucose and insulin levels over the day, than when the same amount of food was taken in larger discrete meals (Jenkins *et al.* 1992; Bertelsen *et al.* 1993).

Studies after gastric surgery have suggested that the so-called 'dumping syndrome' can be improved metabolically by viscous fibres which blunt not only the glucose rise but also the endocrine response, including insulin and the incretin or insulinotrophic gut hormone, gastric inhibitory polypeptide (GIP) (Jenkins *et al.* 1980; Leeds *et al.* 1981).

13.2.1 *Effect of other fibre-like carbohydrates*

True chemically resistant starches, RS_2 and RS_3 have no effect on the glycaemic response of the available starch (Jenkins *et al.* 1982a) when the resistant starch is accounted for by subtraction from the total starch in determining the test dose, i.e. the remaining starch gives a predictable high blood glucose rise. These starches are chemically resistant in that they require solubilisation with sodium hydroxide, etc. prior to analysis, and owe their resistance to hydrogen bonding. Entrapped starch (RS_1), which may be trapped by fibre reacts as slow-release carbohydrate (see below). This starch simply requires to be freed from its retaining physical matrix to be made 'available', e.g. by fine grinding.

Fructo-oligosaccharides have no major effects on the glycaemic response of accompanying carbohydrate, and have no glycaemic effect *per se*.

13.3 Fibre in whole food: food form and glycaemic index

Fibre may be one of the factors reducing the availability, or limiting enzymatic access to the starch. A portion of the starch may be digested so slowly that some enters the colon and is called resistant starch. It is not truly resistant starch, but is slowly absorbed, and thus unlike chemically resistant starches (RS_2 and RS_3) it results in a flattened blood glucose profile. Foods containing these fibre and starch matrixes are low glycaemic index foods and are more slowly digested *in vitro* (Jenkins *et al.* 1982a; Englyst *et al.* 1999). They have also been described as Slowly Available Glucose (SAG) sources (Englyst *et al.* 1999). Associated with prolonged free fatty acids (FFA) suppression, these foods also appear to improve the second meal glucose tolerance (Axelsen 1999; Jenkins *et al.* 1982b; Wolever *et al.* 1988). Examples of these foods are dried legumes, peas, beans and lentils, pumpernickel rye bread and bulgur or cracked wheat. In these cases fibre and food form may reduce the rate at which these foods are digested.

13.3.1 *Fibre and glycaemic index: diabetes, cardiovascular disease and cancer*

Fibre and its influence on glycaemic index, as a determinant of food form, may influence a number of chronic diseases. Viscous fibres may reduce serum lipid levels, secondary to inducing increased bile acid loss (Anderson *et al.* 1984; Lia *et al.* 1995; Jenkins *et al.* 1997). They also blunt the glucose and insulin responses. There are further means by which low-glycaemic index and viscous fibre-rich foods may confer benefit. Postprandially, they may reduce the level of oxidative stress and the production of free radicals generated after consuming refined carbohydrate meals (Geriello *et al.*. 1998). Low serum cholesterol, postprandial glucose, insulin, insulin-like growth factors and reactive oxygen species may all therefore influence the risk for cardiovascular disease, diabetes and cancer.

Diabetes

Ironically, it is the insoluble cereal fibre which appears to offer protection from diabetes in the Nurses Health Study, the Health Professionals Study and the Iowa Women's Health Study (Salmeron *et al.* 1997a, b; Jacobs *et al.* 1998). In addition, low-glycaemic index diets or diets with a low glycaemic load (dietary glycaemic index × dietary carbohydrate) were also negatively related to the development of Type 2 diabetes over a six-year period (Salmeron *et al.* 1997a, b). The glycaemic effect is understandable, but it is not as easy to see why the cereal fibre has an effect in reducing the incidence of Type 2 diabetes, unless it acts through associated nutrients such as magnesium or antioxidants. The effects of wheat bran on glycaemia are not marked. It is however possible that bran phenolics may be useful antioxidants of importance in diabetes prevention, or that other systems related to diabetes are favourably improved by cereal fibre.

In terms of disease treatment, low-glycaemic index diets have been shown to reduce glycated proteins in the majority of Type 1 or 2 diabetic subjects studied in over 13 trials of low-glycaemic index foods. One of the most recent studies also demonstrated a reduction in plasminogen activator inhibitor (PAI; Jarvi *et al.* 1999). This reduction of a haemostatic risk factor associated with thrombosis may have a definite advantage in reducing some of the risks of the complications of diabetes.

The effect of viscous fibres showed early promise in the treatment of diabetes; however, the lack of readily available palatable formulations has made further work in this area difficult. In addition, although some early insoluble fibre studies looked hopeful in terms of Type 2 diabetes treatment (Bosello *et al.* 1980), there have been no recent reports on the successful use of wheat bran in diabetes. There is also the question of whether fibre has a role in weight loss. In this area, recent data in obese boys indicate that favourable metabolic change and increased satiety accompany meals of reduced glycaemic load (Ludwig *et al.* 1999a, b).

Cardiovascular disease

Epidemiologically, wheat bran in a wide range of studies appears to be protective against the development of cardiovascular disease (Jacobs *et al.* 1998; Lui *et al.* 1999). The reasons for this are not apparent, since cereal fibre is relatively lipid neutral. Alternative explanations presented to explain diabetes protection may apply here also. Unfortunately, viscous soluble fibres are not eaten in sufficient quantities to allow an assessment of these types of fibre to be made in Western population studies.

Low-glycaemic index diets have also been implicated in reducing the risk of cardiovascular disease. Both the Nurses Health and the Health Professionals studies showed a reduction in the risk of cardiovascular risk when low-glycaemic index diets or low-glycaemic load diets were consumed (Lui *et al*. 1998). These benefits are therefore in addition and probably even stronger than the effects seen with low-glycaemic index diets in reducing diabetes incidence.

Clinically, viscous rather than insoluble fibres have long been known to reduce serum lipids in both normal and hyperlipidaemic subjects (Jenkins *et al.* 1975; Braaten *et al.* 1994). Viscous fibres may therefore play a key role in dietary cholesterol reduction and be part of the dietary portfolio of food and lifestyle factors, which if additive could reduce serum lipids to the same extent as currently used drug therapies (Table 13.1) (Jenkins and Kendall 1999). Diets containing low-glycaemic index foods have also been associated with reduced lipids in

Table 13.1 A portfolio of dietary factors for cholesterol reduction.

Dietary component	Dietary changes	Approximate LDL reduction (%)
Saturated fat*	<7% of calories	10
Dietary cholesterol	<200 mg/day	5
Body weight	Lose 5 kg (10 lb.)	5
Viscous fibre	5–10 g/day	5
Soy protein	25 g/day	5
Plant sterols†	1–3 g/day	5
Total	Full portfolio‡	35

* Reduce *trans* fatty acid as close to zero as possible.
† Depending on the sterol or stanol.
‡ Assuming that the effects are additive.
LDL, low-density lipoprotein.

hyperlipidaemic subjects. Reductions occurred especially in serum triglycerides, and there was either no change, or a tendency to higher HDL cholesterol levels (Jenkins *et al*. 1987). It is therefore relevant that assessment of British adults and the NHANES III data have shown that the lower the diet glycaemic index, the higher the HDL cholesterol (Ford and Liu 1999; Frost *et al.* 1999), suggesting a further reason for coronary heart disease (CHD) risk reduction on a low-glycaemic index diet. It may be that the strongest case for fibre in CHD reduction lies in the ability of fibre to reduce the glycaemic index of foods.

Cancer

It is has been suggested that insulin and insulin-like growth factors may be important in the promotion of cancers (McKeown-Eyssen 1994; Giovannucci 1995).

In this respect, it is worth noting that in a large Italian case-control study, glycaemic index was negatively related to colon cancer (Augustin *et al.* 2000). This opens the door to assessment of the possible role of glycaemic index in other malignancies. Again, the association of higher fibre with low glycaemic index may be the reason for the fibre effect. It may also be failure to assess this association which has led to negative findings in the past, and the lack of glycaemic effect may be the reason for the poor showing of wheat bran in recent trials in relation to polyp recurrence prevention (Alberts *et al.* 2000; Schatzkin *et al.* 2000).

13.4 Conclusion

An important feature by which fibre may act is by reducing the rate of absorption. One effect which fibre and fibre-like substances may have on the diet is to convert the carbohydrate component of foods into a slow release form that requires less insulin for tissue uptake, and also increasing the elimination of bile acids in the faeces. These factors, in addition to altered colonic short-chain fatty acid profiles, may be key to the benefits of high-fibre diets on carbohydrate metabolism, with potential benefits in diabetes, cardiovascular disease and cancer reduction.

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14 Dietary Fibre and Gastrointestinal Function

Barbara O. Schneeman

14.1 Introduction

Dietary fibre is a macronutrient whose target of action, as a nutrient, is the gastrointestinal tract. One of its primary roles is to serve as a substrate for the microflora normally present in the large bowel, but it also functions to regulate the rate of nutrient digestion and absorption, as well as to promote normal laxation. Fibre has these effects in the gut because, unlike other dietary components, it is not broken down or digested in the mammalian small intestine. Research over the past 25 years has revealed that the effects of fibre on gastrointestinal function have important metabolic consequences that can result in reduction of risk for non-communicable diseases, such as cardiovascular disease, certain types of cancer and Type 2 diabetes mellitus. The interest in dietary fibre as a food component that can alter disease risk grew from the association of diets high in fruits, vegetables, legumes and whole grains with lower incidence of non-communicable disease. Although dietary fibre may not be the only component of these foods that reduces the risk of non-communicable disease, the clinical and experimental data accumulated in the past 25 years clearly indicate a unique and distinct role for fibre in the modification of disease risk. This chapter will review the characteristics of dietary fibre that affect gastrointestinal function, and how the gastrointestinal response to dietary fibre is related to modification of disease risk. While modification of risk for non-communicable disease attracts considerable attention for evaluation of the benefits of a diet high in fibre, it is equally important to keep in mind the unique function of fibre in maintaining normal gastrointestinal function and health. Such an understanding allows definition of essential functions and requirements for fibre in the diets of normal, healthy individuals, not just as an adjunct to modifying risk factors for chronic disease (Cummings & Englyst 1995; Schneeman & Li 1997).

The characteristics of fibre that affect gastrointestinal function include viscosity, water-holding capacity (or dispersibility in water), bulk, binding of bile acids, and fermentability. These characteristics vary, based on the chemistry of the polysaccharides. In most cases, an understanding of these physical characteristics is useful in predicting the physiological response to new sources of dietary fibre (Gallaher & Schneeman 1996; Schneeman 1999a). Perhaps the greatest limitation in using these characteristics is that standardised *in-vitro* methods, which correlate with *in-vivo* parameters, have not been developed and tested adequately. Several laboratories have relied upon measures of soluble and insoluble fibre; however, this approach has not been useful in understanding the relationship between gastrointestinal effects of fibre and metabolic consequences (Food and Agricultural Organisation 1998; Schneeman 1999b).

14.2 Characteristics of fibre that affect gastrointestinal function

14.2.1 *Viscosity*

Viscosity refers to the ability of certain polysaccharides to thicken when mixed with liquid. Gums, pectins and β-glucans can form viscous solutions; however, the degree of thickening will depend on the chemical composition of the polysaccharide (Gallaher & Schneeman 1996). Hydrolysing these polysaccharides is known to reduce their viscosity (Tietyen *et al*. 1995). Within the gut, the shear rate and fluid added from gastrointestinal secretions will affect the actual viscosity within the gut contents. Marciani *et al.* (2000) demonstrated that viscosity of a locust bean gum solution declines immediately after swallowing due to mixing with saliva and gastric secretions. However, the *in-vivo* measurement of viscosity by echo-planar magnetic resonance imaging was well correlated with the *in-vitro* measurement of viscosity.

A theoretical concept referred to as the unstirred layer has been used to understand the rate of water-soluble nutrient absorption from the small intestine. Water-soluble nutrients must penetrate this unstirred layer at the cell surface to reach the transport mechanisms on the brush border of enterocytes. Thickening the unstirred layer presents a greater barrier to absorption. In *in-vitro* systems, the thickness of the unstirred layer can be altered by stirring; a more rapid rate decreases the barrier, while a slower rate increases the barrier. Viscous polysaccharides reduce mixing and cause an apparent thickening of the unstirred layer within the small intestine (Johnson & Gee 1981). This change in the physical characteristics of the intestinal contents slows the rate of absorption from the small intestine. However, the small intestine has excess capacity for absorption, so slowing the rate of absorption involves more distal areas of the intestine in the absorptive process. Histological studies on long-term feeding of fibre suggest more nutrient exposure in the distal small intestine (Schneeman & Richter 1993). Likewise, adding a viscous polysaccharide to an enteral infusion increases the feedback from the distal gut due to nutrient exposure and slows intestinal transit (Lin *et al*. 1997).

Viscosity is associated with prolonged gastric emptying and slower transit through the small intestine (Schwartz *et al*. 1982; Lin *et al*. 1992, 1997; Marciani *et al*. 2000). The slower gastric emptying is likely due to several factors, such as the dilution of viscous polysaccharides in the stomach which expands the volume to be emptied, feedback from more distal areas of the small intestine which slows intestinal transit, and enhanced cholecystokinin (CCK) release which slows gastric emptying. The enhanced CCK release may be related to slower fat digestion and absorption when viscous polysaccharides are consumed (Burton-Freeman & Schneeman 1996; Bourdon *et al*. 1999; Burton-Freeman 2000).

14.2.2 *Water-holding capacity (WHC)*

WHC refers to the ability of a fibre source to swell when mixed with water and to hold water within its matrix. Viscous polysaccharides typically will have a high WHC. The value of WHC can be estimated *in vitro* (Robertson & Eastwood 1981). Within the small intestine, polysaccharides with high WHC can be shown to increase the total volume of the intestinal contents, and specifically the volume of the aqueous phase of intestinal contents (Schneeman 1999a). Nutrients are absorbed from the aqueous phase, and expanding this volume will dilute the concentration of nutrients; such dilution will then slow absorption. Expansion of the aqueous phase in the small intestine may also have implications for the absorption of

lipids. Lipids are not soluble in the aqueous phase, but the formation of micelles allows their transport through this phase to the cell surface. Expansion of this phase can contribute to slowing the rate of lipid absorption (Gallaher & Schneeman 1986; Lairon 1996).

A high WHC allows penetration of water-soluble or hydrophilic substances into the fibre matrix, and reduces their diffusion to the cell surface. In some cases this may partition nutrients into the fibre matrix, which will contribute to reduced availability for absorption. In the large bowel, it allows microorganisms to penetrate the fibre matrix and provides greater access for digestion of the polysaccharides by microbes. Polysaccharides with very low WHC, such as certain forms of cellulose, are not very susceptible to microbial degradation.

14.2.3 *Bulk*

Bulk refers to the non-digestible nature of dietary fibre. Because fibre is not degraded in the small intestine, it increases the dry weight of the contents (Schneeman 1982). The small intestine moves contents by peristaltic muscle contractions. Very little research has been conducted on the relationship between greater bulk of intestinal contents from fibre and the efficiency of peristalsis for mixing contents and moving them along the small intestine. However, it is likely that the greater bulk associated with fibre is likely to slow this process to allow for sufficient mixing of the material with digestive secretions.

The bulk associated with dietary fibre is primarily polysaccharides. These polysaccharides are the primary energy substrate for the microflora in the large intestine. The degree to which the microflora are able to gain access and degrade the polysaccharide structure depends on the WHC of the fibre matrix. Undegraded polysaccharides remain as bulk from fibre in the stool.

14.2.4 *Binding bile acids*

The earliest work on dietary fibre demonstrated the ability of certain fibres to bind bile acids *in vitro* (Vahouny *et al*. 1980; Story & Kritchevsky 1982). In human and animal studies, an increase in bile acid excretion in the stool is evidence that bile acid binding also occurs *in vivo* (Story & Furumoto 1990). Likewise, *in-vivo* data that certain fibres increase activity of the enzymes involved with the conversion of cholesterol to bile acids, demonstrate that these fibres increase the turnover of bile acids and thus of cholesterol, as has been shown for psyllium, pectin and sugarbeet fibre (Nishina *et al*. 1991; Buhman *et al*. 1998; Hara *et al*. 1999). Originally investigators hypothesised that the nature of the interaction between fibre and bile acids was hydrophobic, especially for those fibre sources high in lignin. However, evidence also exists for a hydrophilic interaction (Selvendran *et al*. 1987). A hydrophilic interaction may be more consistent with the fact that fibre sources with a high WHC are more likely to enhance bile acid excretion (Story & Furumoto 1990; Lairon 1996).

14.2.5 *Fermentation*

The polysaccharides in dietary fibre, as well as undigested starch are the primary substrates for the microflora in the large gut. Digestion of this substrate leads directly to an increase in microbial mass, which is a significant proportion of the stool. The exact contribution of microbial mass depends on the relative fermentability of the polysaccharides (Stephen & Cummings 1979; Chen *et al*. 1998). Chen *et al*. (1998) demonstrated that either wheat bran or

oat bran fed to young male subjects would increase stool weight. For wheat bran, most of the increase in weight was from fibre residue, while for oat bran most of the increase was in microbial mass. The metabolism of polysaccharides in the large bowel has many consequences other than simply growth of the microflora. Because of the complex nature of the microflora in the large intestine we do not fully understand all of the metabolic implications of their growth and metabolism. Of particular interest in recent years has been the production of short-chain fatty acids (SCFA), including acetate, propionate and butyrate. The colonocytes utilise all of the butyrate produced, and also some of the propionate and acetate. Only acetate appears in the peripheral blood, as the liver clears any propionate that appears in the portal system. Based on the amount of SCFA produced, fibre sources contribute up to 2.5 kcal/g to the diet (Livesey *et al*. 1995). The SCFA may be essential for maintaining the health of the gastrointestinal mucosa (Velázquez *et al*. 1997).

14.3 Relationship between gastrointestinal function and physiological response

Examining three physiological responses to fibre sources illustrates how the characteristics of fibre that affect gastrointestinal function are important for understanding the effects of fibre on metabolism and reducing risk factors for non-communicable disease.

14.3.1 *Cholesterol lowering*

The ability of certain fibres to lower plasma and low-density lipoprotein (LDL)-cholesterol is well documented, and several meta-analyses have been published (Brown *et al*. 1999; Anderson *et al*. 2000) to this effect. These data are consistent with epidemiological associations between diets high in fruits, vegetable, legumes and whole grains and reduced risk of cardiovascular disease (Rimm *et al*. 1996; Stamler *et al.* 1997; Harsha *et al*. 1999; Jacobs *et al*. 1999; Ludwig *et al*. 1999). Many of these reports suggest that so-called soluble fibre is the active component that lowers cholesterol; however, not all soluble fibres will lower plasma cholesterol. For example, inulin and oligofructose – which are both classified as soluble fibre – do not lower plasma cholesterol concentration, probably because of their non-viscous properties (Schneeman 1999a). When hydrolysed, viscous polysaccharides such as β-glucan are still detectable as soluble fibre, but no longer have as significant a cholesterol-lowering effect (Tietyen *et al*. 1995).

Viscosity is a primary characteristic of fibres that lower plasma and LDL-cholesterol. Because of their effect on small intestinal absorption, viscous polysaccharides can affect lipid metabolism through several routes, including enhanced bile acid excretion and slower lipid absorption. Enhanced bile acid excretion will increase the conversion of cholesterol to bile acids and enhance cholesterol excretion from the body via bile acids, as demonstrated by increased activity of enzymes at the rate-limiting steps for this conversion (Nishina *et al.* 1991; Buhman *et al.* 1998). Slower lipid absorption may be important in enhancing reverse cholesterol transport (Bourdon *et al.* 1999). An increase in reverse cholesterol transport is consistent with the observation that most viscous fibres decrease LDL-cholesterol levels, while maintaining high-density lipoprotein (HDL)-cholesterol concentration.

The bulk and WHC associated with certain viscous polysaccharides undoubtedly contribute to their cholesterol-lowering ability. This is due in part to the fact that these two parameters

contribute to the ability of viscous polysaccharides to increase bile acid excretion and modify lipid absorption (Lairon 1996). However, either property by itself does not result in lowering plasma cholesterol levels. Likewise, fermentability of polysaccharides is not sufficient by itself to lower cholesterol levels (Gallaher & Hassel 1995; Gallaher & Schneeman 1996). Carr *et al*. (1996) demonstrated in hamsters that viscous polysaccharides, but not fermentable polysaccharides, lower cholesterol levels. In rats, viscous hydrocolloids reduce cholesterol levels by inhibiting cholesterol absorption (Levrat-Verny *et al*. 2000).

14.3.2 *Glycaemic and insulinaemic response*

In Type 2 diabetics, an important route to blunt glucose and insulin response is to slow gastric emptying, a response that is associated with viscosity (Johnson & Gee 1981; Schwartz *et al*. 1982). Non-insulin-dependent diabetes mellitus (NIDDM) subjects have been reported to have a more rapid gastric emptying of meals, and this effect was associated with lower CCK levels (Rushakoff *et al*. 1993; Schwartz *et al*. 1995, 1996). Viscosity is important in the small intestine as well as the stomach, since increasing the apparent thickness of the unstirred layer will slow glucose absorption (Johnson & Gee 1981; Leclére *et al*. 1994). By slowing fat absorption, certain sources of viscous polysaccharides appear to prolong or enhance the CCK response to a meal (I. Bourdon *et al*., unpublished results; Burton-Freeman 2000). Enhancing CCK response has been associated with improved glycaemic control in NIDDM patients (Rushakoff *et al*. 1993).

As with the cholesterol-lowering effects of fibre, both WHC and bulk contribute to the effect of viscous polysaccharides on glycaemic control, but are probably not sufficient in themselves to modify glycaemic response.

14.3.3 *Improving large bowel function*

Fibre improves large bowel function by reducing transit time, increasing stool weight and frequency, diluting large intestinal contents, and providing fermentable substrate for the microflora normally present in the large intestine. Consequently, the characteristics fermentability, bulk and WHC all contribute to the ability of fibre to improve large bowel function. Cummings *et al*. (1992) reported that stool weight is correlated with non-starch polysaccharide intake. From population data we know that low fibre intakes are generally associated with delayed transit times (Spiller 1986). The bulk provided, either from fibre or increased microbial mass, is needed to provide normal laxation (Nyman *et al*. 1986; Chen *et al*. 1998). Dietary fibre is the primary dietary component that can increase stool weight in healthy individuals. Stool weights less than 100 g/day have been associated with constipation, and a stool weight of <150 g/day has been associated with increased risk of large bowel cancer, diverticulitis, and high deoxycholate concentrations in bile (Sonnenberg & Koch 1989; Cummings & Englyst 1995).

Our understanding of the relationship between stool weight and fibre intake provides a good understanding of how the various characteristics of fibre can promote normal function. We are still developing our understanding of the role that SCFA production plays in the health of the large intestine. Production of SCFA is determined by the fermentability of the fibre, which in turn, is influenced by the WHC of the fibre source. Early research demonstrated that highly fermentable fibres promote growth of the large intestinal mucosa (Jacobs & Lupton 1984; Sakata 1987). The hypertrophy of the large intestine mucosa is associated with the abil-

ity of colonocytes to use SCFAs for energy. More recent research has focused on the ability of butyrate to protect against the development of colon cancer by inhibiting cancer cell growth (Archer *et al*. 1998; Emenaker & Basson 1998; Wang & Friedman 1998).

14.4 Conclusion and summary

The ability of certain sources of fibre to lower plasma and LDL-cholesterol or to blunt glycaemic response to a meal is mostly dependent on their altering the characteristics of the stomach and small intestinal contents. As summarised in Table 14.1, viscosity is a key factor in understanding these responses, and WHC and bulk of non-digestible material contribute to the response. The ability to bind bile acids is primarily of importance for lowering cholesterol levels.

The ability of dietary fibre to maintain large bowel function are summarised in Table 14.2. The most important characteristic of fibre is probably provision of polysaccharides that can contribute to stool bulk directly, or act as a substrate for the microflora.

The characteristics of fibre sources, such as viscosity, water-holding capacity, bulk, bile acid binding and fermentability are important for gastrointestinal functions. These physical–chemical properties regulate the rate and site of digestion and absorption, which is important for understanding the role of dietary fibre in disease prevention and health promotion. More effort is needed to standardise the measurement of these properties and their ability to predict physiological and metabolic response to consumption of fibre.

Table 14.1 The characteristics of fibre that contribute to lowering plasma and LDL-cholesterol and blunting glycaemic response.

Viscosity	• Slows digestion and absorption of lipid and carbohydrate • Delays gastric emptying • Enhances release of cholecystokinin
WHC	• Expands the aqueous phase of the small intestinal contents
Bulk	• Expands bulk phase of intestinal contents
Bind bile acids	• Increases bile acid excretion – primarily important for cholesterol response

Table 14.2 Characteristics of fibre that contribute to improved large bowel function.

WHC	• Creates an aqueous phase in the fibre matrix for penetration of microorganisms to break down the polysaccharide structure
Bulk	• Provides more material entering the large intestine which serves as substrate for microflora or undigested residue for stool
Fermentability	• Provides polysaccharides that can be used by the large intestine microflora and results in increased microbial mass and production of CO_2, H_2, CH_4 and short-chain fatty acids

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15 Dietary Fibres and Dietary Lipids

Denis Lairon

15.1 Introduction

A series of epidemiological studies has stressed that a high dietary fibre intake is associated with a lower incidence of coronary heart disease (Kromhout *et al.* 1982; Rimm *et al.* 1996; Ludwig *et al.* 1999; Wolk *et al.* 1999). This is the case in men as well as in women, and cereal fibres seem to play a particularly important role in risk-lowering in the cohorts studied in North America.

Among the acknowledged risk factors for cardiovascular diseases, several parameters including plasma and low-density lipoprotein (LDL) cholesterol (Andersson & Tietyen-Clark 1986; Ripsin *et al.* 1992; Glore *et al.* 1994; Mekki *et al.* 1997; Brown *et al.* 1999; Andersson *et al.* 2000), plasma triglycerides, insulin status and thrombotic status, have all been shown to be improved by the intake of dietary fibres.

Dietary fibres – and especially soluble fibres – can alter several physiological and metabolic processes involving dietary lipids. These include energy and nutrient intake, stomach emptying, carbohydrate and fat digestion and absorption, ileal/faecal excretion, postprandial metabolic response, peripheral tissues sensitivity, fermentation in the colon and liver metabolism (Schneeman 1990; Jenkins *et al.* 1993; Lairon 1996, 1997).

These different aspects will be briefly presented and discussed.

15.2 Fibres can reduce dietary lipid intake

Intake of fibre-rich food items can reduce, to some extent, the energy density of the diet, and thus the energy intake (by about 100–200 kcal/day). More specifically, the amount of ingested saturated fat and cholesterol can be reduced, thanks to the predominance of vegetable foodstuffs. It is noteworthy that both an increased satiety and a reduced food intake have been reported, following a fibre-rich meal (Burley & Blundell 1995).

For these reasons, improvement in body weight losses, or control, have been reported when subjects rely on a high-fibre diet (Ludwig *et al.* 1999).

15.3 Fibres alter fat digestion

Reducing the rate of gastric emptying, and thus the subsequent postprandial rise in blood glucose, is an acknowledged mechanism of action of viscous fibres. Nevertheless, this effect remains to be fully documented in the case of dietary lipids (Lairon 1997).

Digestion of dietary fat begins within the stomach, where dietary fat is coarsely emulsified and hydrolysed (up to about 10–15%; Armand *et al.* 1996). In the duodenum, no marked

further emulsification occurs, but colipase-pancreatic lipase hydrolyses triglycerides very efficiently (Armand *et al.* 1996). Subsequently, phospholipids and cholesterol esters are enzymatically cleaved.

15.3.1 *Effect of fibres on pancreatic lipase activity*

Limited data are available regarding the *in-vivo* inhibitory effects in humans. In healthy subjects, adding 5–15 g of pectin to a meal has been reported to lower pancreatic lipase output by 49% (Sommer & Kasper 1980). However, in other studies, this lowering effect was not observed (Flourié *et al.* 1985). In pancreatectomised patients receiving enzyme supplement therapy, 5 g of pectin reduced the lipase activity by 63% (Isaksson *et al.* 1984). Conversely, several sources of soluble dietary fibres such as pectins, oat bran, psyllium and sugarbeet fibre can reduce the hydrolytic activity of pancreatic lipase *in vitro* (Schneeman 1978; Isaksson *et al.* 1982; Lairon *et al.* 1985; Hendrick *et al.* 1992). Part of this loss in enzyme activity could result from some binding of the enzyme to fibres (Schneeman 1978; Isaksson *et al.* 1982; Lairon *et al.* 1985). The implication of inhibitory proteins in lipase inhibition by wheat bran and wheat germ has also been reported (Lairon *et al.* 1985; Borel *et al.* 1989a).

15.3.2 *Effects of fibres on lipid emulsification and lipolysis*

Because the mechanisms whereby soluble fibres might alter the lipolysis process were only poorly understood, we decided to investigate some of the physicochemical aspects involved. It is well known that emulsification is a key step in fat digestion, because gastric and pancreatic lipases act at the fat globule–water interface, and that the size of the fat globule *per se* is a parameter governing the activity of gastric and pancreatic lipases both *in vitro* and *in vivo* (Armand *et al.* 1999). We tested the hypothesis that dietary fibre could interfere with the normal process of dietary fat emulsification (Pasquier *et al.* 1996a, b). *In-vitro* experimental conditions were used to mimic closely the physiological conditions.

The two key physicochemical parameters tested were viscosity and electric charge. Thus, five sources of dietary fibres with different properties were used, namely gum arabic (not viscous), two differently charged pectins (low viscosity), and three uncharged guar gums (low, medium and high viscosity). In the gastric conditions (pH 5.4), the amount of emulsified lipid was not affected by any of the fibres, but viscous fibres did increase the size of the emulsified droplets, and the effect was more pronounced as the concentration of these fibres increased. Only viscous fibres significantly increased droplet size and reduced droplet surface area. Overall, the droplet size was positively correlated, and the droplet surface area was negatively correlated with the concentration of the medium viscosity guar gum in the range 0 to 20 mPa.s. The high-viscosity guar gum significantly reduced (by 32%) the extent of triglyceride lipolysis of such emulsions by human gastric lipase, as compared with control and low- or medium viscosity fibres (Pasquier *et al.* 1996a).

In the duodenal conditions (pH 7.5 and addition of bile), the amount of emulsified lipids was reduced and the size of emulsified droplets was increased only by raising the concentration of viscous fibres. The extent of emulsification ($r = -0.79$), the droplet size ($r = 0.88$) and consequently, the droplet surface area ($r = -0.59$) were strongly correlated to the concentration of the medium viscosity guar gum in the range 0 to 4 mPa.s (and also 0 to 20 mPa.s). The high- and medium-viscosity guar gums and one type of pectin significantly reduced the extent of triglyceride lipolysis catalysed by colipase-pancreatic lipase only. Overall, the extent of

triglyceride lipolysis was negatively correlated ($r = -0.97$) to the viscosity of the reconstituted duodenal medium (Pasquier *et al.* 1996b).

These new findings thus indicate that a mechanism by which soluble, highly viscous fibres can alter lipid assimilation is by a lowering of the emulsification of dietary lipids, and subsequently of lipolysis, in both the stomach and the duodenum.

In order to evaluate the existence of such a mechanism *in vivo*, we subsequently performed an experiment in rats. A coarsely emulsified lipid mixture with or without 0.3% high-viscosity guar gum was intragastrically intubated (Lairon 1997). After 30 min of digestion in the stomach, the median droplet diameter of the emulsion in the presence of guar gum was about two-fold larger. Consequently, the specific surface area displayed by the emulsion in the gastric content, was about half that of the control. At the same time, in the presence of guar gum, the extent of triglyceride lipolysis was about three-fold lower in the stomach content and about two-fold lower in the duodenum content. Thus, those dietary fibres that noticeably raise the median viscosity in the stomach or the duodenum content, reduce the extent of fat emulsification and subsequent triglyceride lipolysis. A proposed model which summarises the observed effects of fibres in the upper digestive tract is shown in Fig. 15.1.

15.3.3 *Dietary fibres and lipid absorption*

The lipolytic products of the digestion process (i.e. mainly monoglycerides, free fatty acids, lysophospholipids and free cholesterol) are absorbed by the small intestinal mucosa, where they are quickly used as substrates for *de novo* triglyceride, phospholipid and cholesterol ester synthesis. After the intracellular packaging of lipid moieties and apoproteins, they are

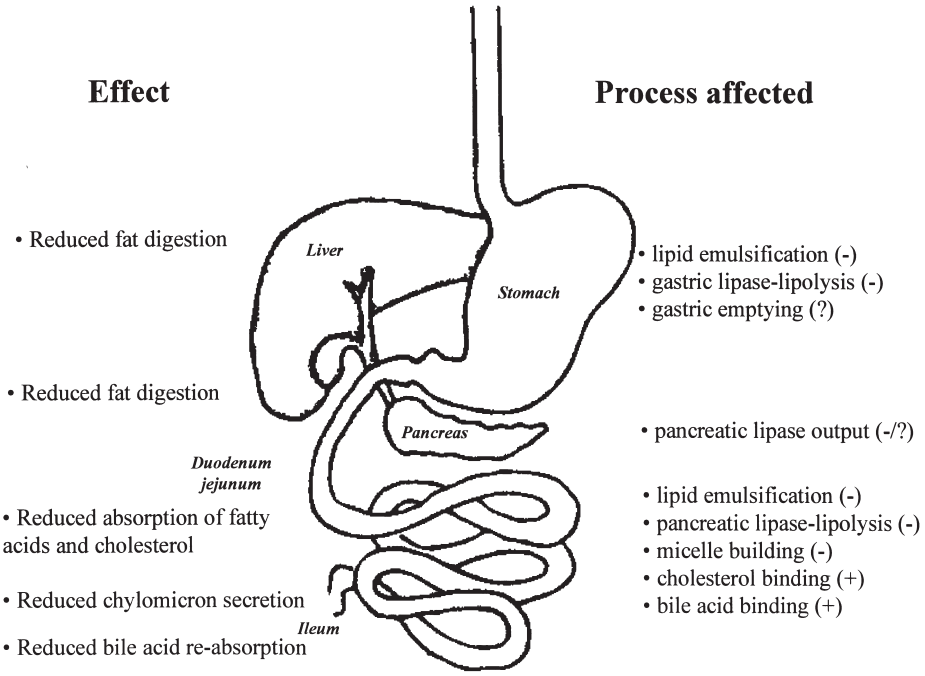

Fig. 15.1 Sites of action of dietary fibres on lipid digestion and assimilation in the upper digestive tract.

secreted in the form of chylomicrons into the lymph and subsequently into blood circulation.

In addition to the effects reported above, other mechanisms can contribute to alterations in lipid absorption. The partial binding or entrapment of lipolytic products as well as bile salts in the presence of viscous fibres such as oat bran, guar gum and pectins (but not wheat bran or cellulose) can counteract the building of vesicles and mixed micelles in the aqueous phase of the small intestine content (Vahouny *et al.* 1981; Lairon *et al.* 1985; Ebihara & Schneeman 1989; Lia *et al.* 1997). Finally, the limiting step in the process of lipid absorption *per se* is the transport across the unstirred water layer associated with the enterocyte brush-border membrane. Increasing the thickness of the unstirred water-layer (Flourié *et al.* 1984), and reducing the rates of diffusion and absorption of cholesterol and free fatty acids, have been observed during acute experiments in the presence of highly viscous fibres (Gee *et al.* 1983). These effects have not been observed in the presence of slightly viscous soluble fibres (hydrolysed guar gum or chitosan) or insoluble fibres such as cellulose or some hemicelluloses (Borel *et al.* 1989b). Alterations induced by viscous soluble fibres in the intestinal lumen (Gallaher *et al.* 1993) have been shown to reduce significantly the recovery of dietary fatty acids and cholesterol in the lymph, after infusion of a test meal in the rat (Vahouny *et al.* 1988; Ikeda *et al.* 1990).

Some alterations of the mucosal morphology have also been reported after chronic fibre feeding.

15.4 Lipid and sterol ileal excretion

As a result of the processes described above, an increased excretion of lipid moieties from the small intestine should be observed. Indeed, augmented ileal excretions of lipids have been repeatedly shown in ileostomised human subjects after addition to diets and test meals of either citrus pectin, alginate, oat bran or β-glucans and barley fibre (Sandberg *et al.* 1983; Bosaeus *et al.* 1986; Zhang *et al.* 1992; Lia *et al.* 1995, 1997). Some studies also reported increased faecal or ileal lipid excretions after enrichment of the diet with wheat bran or wheat germ (Borel *et al.* 1989b).

Cholesterol present in the small intestine, essentially in the form of free cholesterol, is derived either from bile secretion (1–2 g/day) or the diet (0–1 g/day). Early studies in this area have shown that different kinds of dietary fibres can bind cholesterol moieties *in vitro* as well as their bile acid derivatives (Vahouny *et al.* 1981; Lairon *et al.* 1985). The existence of such a mechanism *in vivo* has been fully demonstrated in animal experiments (Ebihara & Schneeman 1989) and in numerous human studies (Sandberg *et al.* 1983; Bosaeus *et al.* 1986; Zhang *et al.* 1992; Lia *et al.* 1995, 1997). The ileal (or faecal) excretion of cholesterol (or neutral sterols) and/or bile acids is markedly increased after addition of various kinds of dietary fibres to diets or test meals. Soluble and viscous fibres such as β-glucans, pectins or psyllium are particularly efficient in increasing cholesterol excretion. Nevertheless, some sources of wheat fibres can also increase excretion of cholesterol from the small intestine.

The interaction between dietary lipids and fibres has been studied in detail in short-term experiments in which diets with reduced fat content were employed to which mixed fibres from cereals, fruits and vegetables were employed (Ellegard & Bosaeus 1991). The data obtained showed that reducing fat alone was not enough to change cholesterol excretion; however, adding mixed fibres resulted in a higher excretion of both fat (+50%) and choles-

terol (+56%). Phytosterols present in some fibre-rich foods may have an additional effect in preventing cholesterol absorption.

15.5 Postprandial lipid metabolism and dietary fibres

The postprandial state is the first metabolic response to the process of digestion-assimilation of lipid nutrients. Given the usual dietary pattern found in Western countries (three to six intakes each day) and the duration of this phenomenon after regular meals, i.e. about 5–6 h, subjects are in a postprandial state for most of the day-time. The principal feature of the postprandial state is an increase in glycaemia and insulinaemia, and a marked increase in triglyceridaemia due to secretion of chylomicrons (that transport absorbed dietary lipids and cholesterol) by the small intestine into the circulation. A series of studies with test meals, either supplemented or not supplemented, has shown that different kinds of dietary fibres (wheat fibres, legume fibres, guar gums, pectins, oat bran, β-glucans, psyllium and mixed fibres), and especially those rich in soluble fibres, can reduce the postprandial rise in insulinaemia and triglyceridaemia (Lairon *et al.* 1996). In some studies, some fibre sources were shown to reduce the rise in chylomicron triglycerides or cholesterol induced by oat bran or guar gum (Cara *et al.* 1992; Morgan *et al.* 1993; Lia *et al.* 1997). Other studies, where soluble fibres have been incorporated in foodstuffs such pasta, have provided overall comparable data (Gatti *et al.* 1984; Bourdon *et al.* 1999).

In our studies, the aim was to evaluate directly the expected relationship between absorption of dietary lipids from the small intestine and the postprandial response. This was studied in ileostomised human subjects who ingested, on separate days, test meals providing fat and cholesterol, together with either wheat bran fibre (3 g) or oat bran fibre (12 g) (Lia *et al.* 1997). After the oat bran meal, the ileal excretion of triglycerides and diglycerides increased markedly (+610%), as also did the levels of monoglycerides and free fatty acids (+400%), as compared with results obtained with the low-fibre wheat meal. At the same time, the 7-h postprandial rise in chylomicron lipid moieties, i.e. triglycerides, phospholipids and cholesterol, was lowered by about 40% after the oat bran meal. As previously shown, blunting the postprandial chylomicron rise alters the postprandial variation in LDL and HDL lipid composition (Dubois *et al.* 1993). Chronic intake of oat bran might induce even more complex metabolic changes (Dubois *et al.* 1995).

15.6 A comprehensive view of the effects and mode of action of dietary fibres

Epidemiological data have established an association between a high intake of dietary fibres and a reduced risk for coronary heart disease. This relationship can be understood as a combination of several complementary mechanisms, as illustrated in Fig. 15.2.

Replacement of foods which are rich in saturated fat, by low-fat, fibre-rich foods, leads to maximum cholesterol-lowering effects, due to the combined beneficial influence of the two measures in various dietary contexts. In addition, these fibre- and carbohydrate-rich diets do not lead to an elevation of fasting triglyceridaemia – an independent risk factor for coronary heart disease – as generally observed when shifting to diets rich in refined carbohydrates. These two beneficial effects are obtained through various complementary effects of dietary

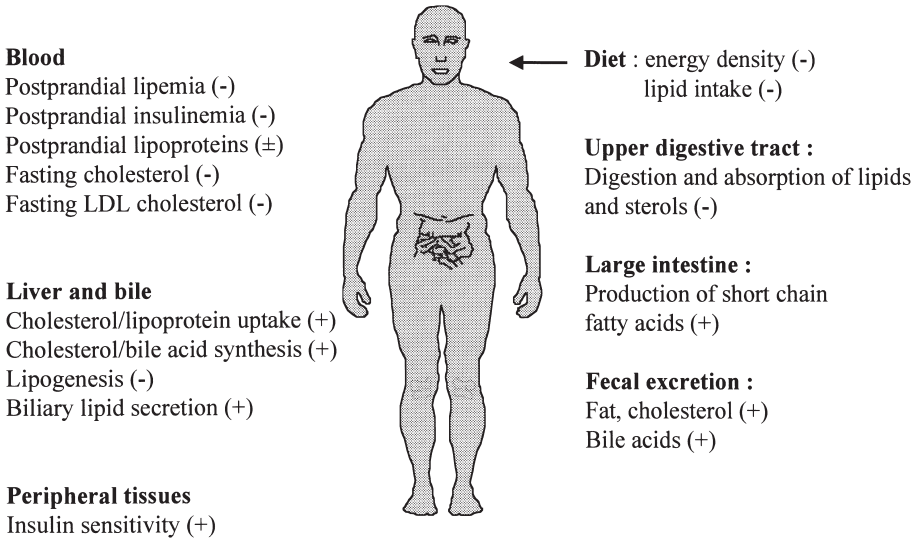

Fig. 15.2 Documented effects of dietary fibres on lipid assimilation and overall matabolism in humans.

fibres. A high-fibre diet reduces fat and energy intake and increases satiety. Subsequently, emulsification and enzymatic digestion of fat is impaired and/or delayed in the stomach and the small intestine, resulting in somewhat increased losses of fat and cholesterol. Overall, the energy input into the body is lowered, thus helping the maintenance of desirable body weight. Conditions of overweight and obesity are other acknowledged risks factors for cardiovascular disease due to resulting metabolic disorders such as hyperinsulinaemia and hyperlipidaemia.

Increased ileal loss of cholesterol and/or bile salts (produced in the liver from cholesterol moieties) is a key mechanism leading to increased cholesterol demand by the liver. This results in slightly increased neosynthesis of cholesterol by the liver, and over-expression of lipoprotein hepatic receptors. HDL uptake is expected to be stimulated as well as LDL, generating the cholesterol and LDL cholesterol-lowering effect observed in plasma under chronic feeding.

The reduced bioavailability of fatty acids and cholesterol induced by fibres (especially viscous fibres) makes the small intestine mucosa less efficient in lipid synthesis and assembly and thus, in secreting chylomicrons into the lymph and then to circulation, as observed postprandially. In turn, the rate of clearance of triglyceride-rich lipoproteins from the plasma could be stimulated. This leads to a lowered atherogenic profile of postprandial lipoproteins. The lowered insulin response elicited postprandially by the presence of viscous soluble fibres in the small intestine content generates less stimulation of the various biochemical processes activated by insulin (such as lipogenesis, cholesterol synthesis, etc.) and thus improves peripheral insulin sensitivity.

The hypothesis that some end-products of fermentation of fibres in the colon (e.g. propionate) could play a noticeable role in the cholesterol-lowering effect of dietary fibre, has not received firm support from results of *in-vivo* studies. The importance of this effect has essentially been ruled out by the key observation that oat bran has a comparable cholesterol-

lowering effect in ileostomised subjects as it does in healthy subjects with an intact colon (Zhang *et al.* 1992).

Finally, some factors that are implicated in the thrombotic process (such as factor VII) and which could play a role in the reduced risk for coronary heart disease, are decreased after chronic intake of fibre-rich diets (Marckmann *et al.* 1994). Because most of the effects reported are directly linked to the physicochemical properties exhibited by dietary fibres in the digestive tract, the most effective fibres seem to be those that are soluble and viscous.

Although there have been impressive advances in our scientific knowledge of the effect of dietary fibre on lipid metabolism during the past two decades, several aspects still need further investigation. This is particularly true for our understanding of the relationships between the acute effects elicited by dietary fibres in the digestive tract and the various metabolic changes related to health and diseases, as observed after chronic intake.

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16 Food Polysaccharides, Glucose Absorption and Insulin Sensitivity

John C. Mathers and Mark E. Daly

16.1 Syndrome X: central role of insulin resistance and hyperinsulinaemia

Reaven (1988) designated as Syndrome X, a constellation of cardiovascular disease (CVD) risk markers consisting of abdominal obesity, hypertension, raised fasting concentration of very low-density lipoprotein (VLDL) triglycerides, reduced concentration of high-density lipoprotein (HDL) cholesterol, hyperinsulinaemia, glucose intolerance and non-insulin-dependent diabetes mellitus (NIDDM). This increased CVD risk may be compounded by increased circulating concentrations of plasminogen activator inhibitor type 1 (PAI-1) (a potent inhibitor of fibrinolysis), which is also strongly associated with peripheral insulin resistance (Potter van Loon *et al.* 1993). The aetiology of the syndrome remains uncertain, not least because of the difficulties inherent in disentangling cause and effect when several potential aetiological determinants coexist in the same individual. Recent attempts using factor analysis in several cohort studies with a range of racial mixes, subject ages and pathology (Meigs *et al.* 1997; Edwards *et al.* 1998; Gray *et al.* 1998; Chen *et al.* 1999; Snehaltha *et al.* 2000) have produced slightly different clustering of the key components. However, insulin resistance leading to increased pancreatic secretion of insulin and hyperinsulinaemia may be an underlying cause in most, if not all (Meigs *et al.* 1997), cases (Reaven 1993).

While genetic inheritance contributes to the degree of susceptibility to Syndrome X (Beatty *et al.* 1993), it seems probable that most cases are due to a 'double hit' of environmental insults imposed on susceptibility genes. Martyn *et al.* (1998) reported that plasma insulin concentrations in adults (men and women, mean age 52 years) were negatively correlated with abdominal circumference at birth. Given that the latter is an indicator of growth of the liver during foetal life (Campbell & Thoms 1977), factors which affect liver development adversely may contribute to reduced hepatic insulin sensitivity in adulthood (Martyn *et al.* 1998). However, adult lifestyle factors appear to be at least as important. Low habitual energy expenditure was strongly associated with the metabolic cardiovascular syndrome in a cross-sectional study of young (30- to 40-year-old) adults (Wareham *et al.* 1998). The secular rise in incidence of obesity (a feature of the metabolic syndrome) in the UK is coincident with a reduction in physical activity (Prentice & Jebb 1995), which suggests a possible target for intervention studies. Food choice may also contribute to the risk of Syndrome X. In the San Luis Valley Diabetes Study, fasting insulin concentrations in persons with normal glucose tolerance were positively associated with total saturated fat intake and inversely associated with dietary fibre and starch intake (Marshall *et al.* 1997). Alteration in habitual macronutrient consumption is an attractive strategy for primary prevention of hyperinsulinaemia and its adverse consequences.

This review focuses on the effects of food polysaccharides on the kinetics of carbohydrate digestion and absorption and on the subsequent endocrine responses.

16.2 Food polysaccharides and glucose absorption rates

It is well established that many factors other than amount of α-linked glucose polymer in foods determine the rate of glucose delivery by the gut to the portal vein, and hence to peripheral tissues. Most of the supporting data are derived from studies of the glycaemic (and insulinaemic) response to test meals. Pioneering studies by Crapo and colleagues demonstrated that the botanical source of starchy foods strongly influenced postprandial glycaemia (Crapo *et al.* 1976, 1977, 1981). In part, such effects could be explained by the amylose:amylopectin ratio in the starches (Behall *et al.* 1988, 1989; Behall & Howe 1995), since amylopectin has a much faster rate of hydrolysis by pancreatic α-amylase (Kabir *et al.* 1998). Unchewed foods result in much flatter glycaemic responses than do the same foods chewed well before swallowing (Read *et al.* 1986). Analogous responses can be produced through alteration in the milling of cereals (Heaton *et al.* 1988; Holt & Brand Miller 1994), demonstrating the importance of food structure and particle size which probably exert their influence via modulation of α-amylase access to the starch substrate and via surface area effects. Other food constituents, most notably non-starch polysaccharides (NSP) which form viscous solutions, may slow the rate of starch digestion and glucose absorption (Jenkins *et al.* 1978; Wood *et al.* 1994). The effects of viscous NSP may arise through:

- delayed gastric emptying (Rainbird & Low 1986; Cherbut 1995);
- reduced activity of hydrolytic enzymes (Dunaif & Schneeman 1981);
- poorer mixing of intestinal contents with digestive secretions (FAO/WHO Expert Consultation (1998); and/or
- increases in the thickness of the unstirred water layer, slowing diffusion of glucose to transporters on the apical membrane of enterocytes (Johnson & Gee 1981).

Jenkins *et al.* (1981) introduced the concept of glycaemic index (GI) as a means of comparing the physiological effects (at least in terms of postprandial glycaemia) of food carbohydrates. Low-GI foods are slowly, or incompletely, digested in the small bowel, or contain fructose which is only partially converted to glucose (Mayes 1993). On this basis, foods such as unripe banana [rich in amylase-resistant starch (RS)] and over-ripe banana (rich in fructose) have a lower GI than fresh white bread (Hermansen *et al.* 1992), the standard against which glycaemic responses of other foods are measured.

The starch in freshly cooked potatoes is readily hydrolysed by α-amylase (Englyst & Cummings 1987), and consumption results in very high glycaemic and insulinaemic responses (Jenkins *et al.* 1984). Allowing cooked potatoes to cool (Mathers & Dawson 1991), or subjecting them to repeated cycles of cooking and cooling increases the proportion of starch resistant to α-amylase (RS), so that a greater proportion of the starch flows from the ileum into the large bowel (Englyst & Cummings 1987). To investigate the effects of simple processing of potatoes on rate of glucose uptake from the gut and disposal by oxidation, potatoes were labelled with ^{13}C by growing in an environment enriched with $^{13}CO_2$. Eight (one female) healthy volunteers consumed the potatoes in test meals according to a randomised cross-over design (Daly *et al.* 1998a). On one occasion, the volunteers consumed freshly boiled and mashed potatoes, while on the other occasion the potatoes had been cooked and chilled overnight before serving. For both plasma glucose and serum insulin, peak postprandial concentrations and areas under the concentration–time curve (AUC) were higher with the freshly cooked potatoes (Figs. 16.1 and 16.2). The rate of exogenous glucose oxidation rose more

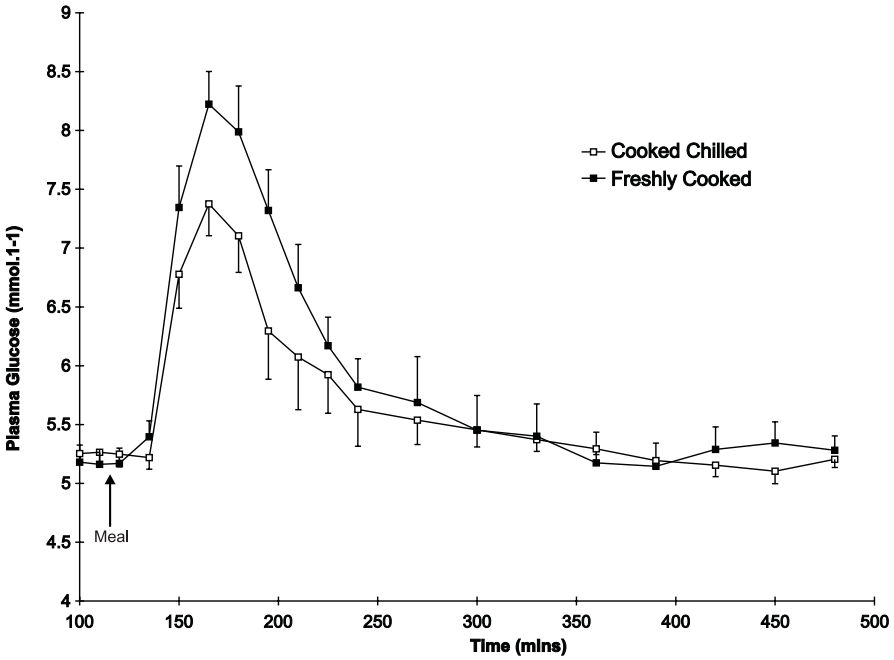

Fig. 16.1 Postprandial changes in blood glucose concentration following test meals containing freshly cooked (solid squares) or cooked and cooled (open squares) potato.

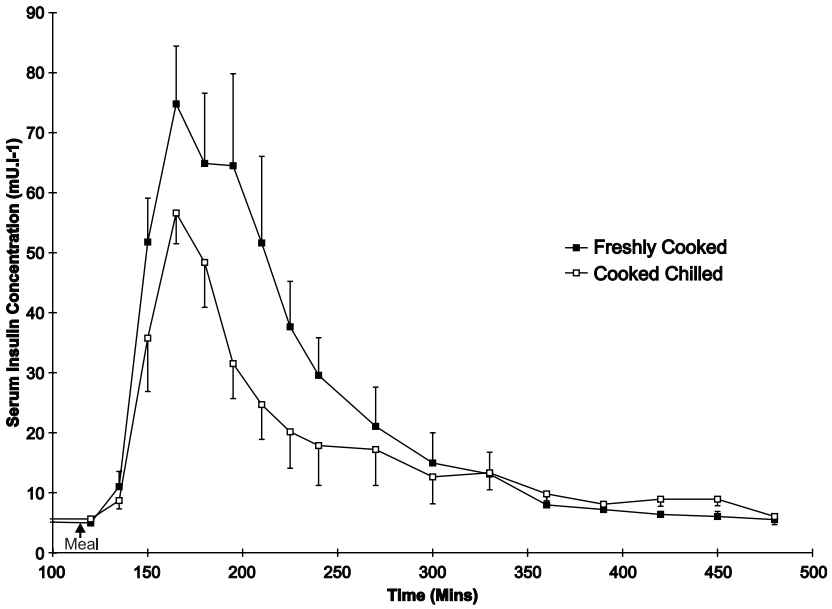

Fig. 16.2 Postprandial changes in serum insulin concentration following test meals containing freshly cooked (solid squares) or cooked and cooled (open squares) potato.

slowly and peaked later with the cooked, chilled potatoes (Fig. 16.3). These data suggest that simple changes to the method of preparation of potatoes (and, by analogy, of other starchy foods) may have profound effects on the rate of intestinal hydrolysis of starch and on the rate of delivery of glucose for tissue oxidation, which could be exploited to the benefit of insulin-resistant patients.

16.2.1 *Fuel selection following high-carbohydrate meals*

Most of the characteristic metabolic effects of high sucrose intakes, i.e. those not shared by high starch intakes, are thought to be due to the fructose component of sucrose (Hollenbeck 1993; Frayn & Kingman 1995; Abraha *et al.* 1998). Despite this, there has been a paucity of experimental work focused on tissue fuel selection and, specifically, on the relative rates of oxidation of the two constituent monomers, glucose and fructose. To address this question we fed high-carbohydrate test meals to seven healthy men in a randomised cross-over design (Daly *et al.* 1997a). Two of the meals were high-sucrose (one supplemented with 200 mg uniformly labelled ^{13}C-fructose, one with 200 mg labelled ^{13}C-glucose) and the other high-starch (supplemented with 200 mg labelled ^{13}C-glucose). The high-sucrose test meal provided 50% of energy as sucrose and the high-starch meal, 50% as starch.

From concurrent measurements of whole body O_2 and CO_2 production and of breath $^{13}CO_2$ enrichment, it was apparent that exogenous carbohydrate oxidation rate was greater for the high-sucrose test meal (Fig. 16.4), and that the patterns of oxidation of the constituent monomers were quite different. When glucose and fructose were both available to the tissues (following the high-sucrose meal), there was preferential oxidation of the fructose, and glucose oxidation rate was considerably suppressed relative to that with the high-starch meal. Peak

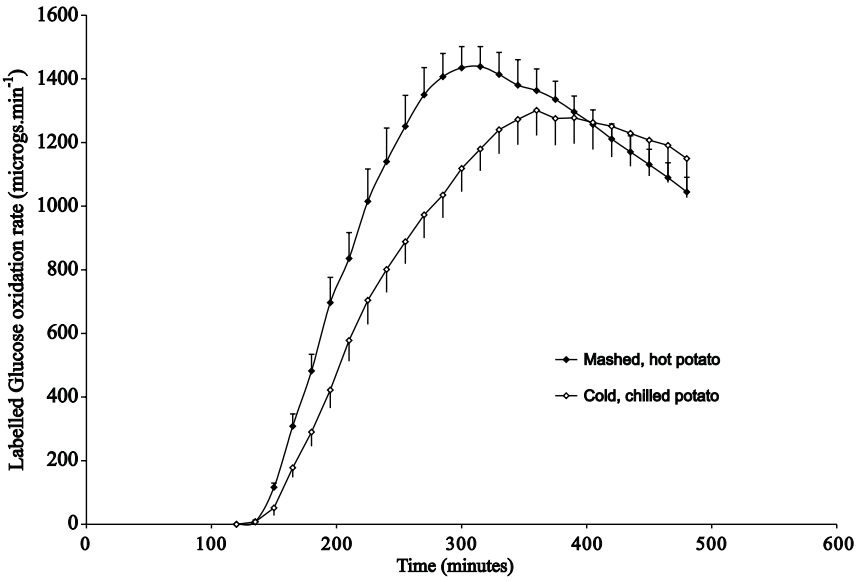

Fig. 16.3 Postprandial changes in rate of exogenous glucose oxidation following test meals containing freshly cooked (solid diamonds) or cooked and cooled (open diamonds) ^{13}C-labelled potato.

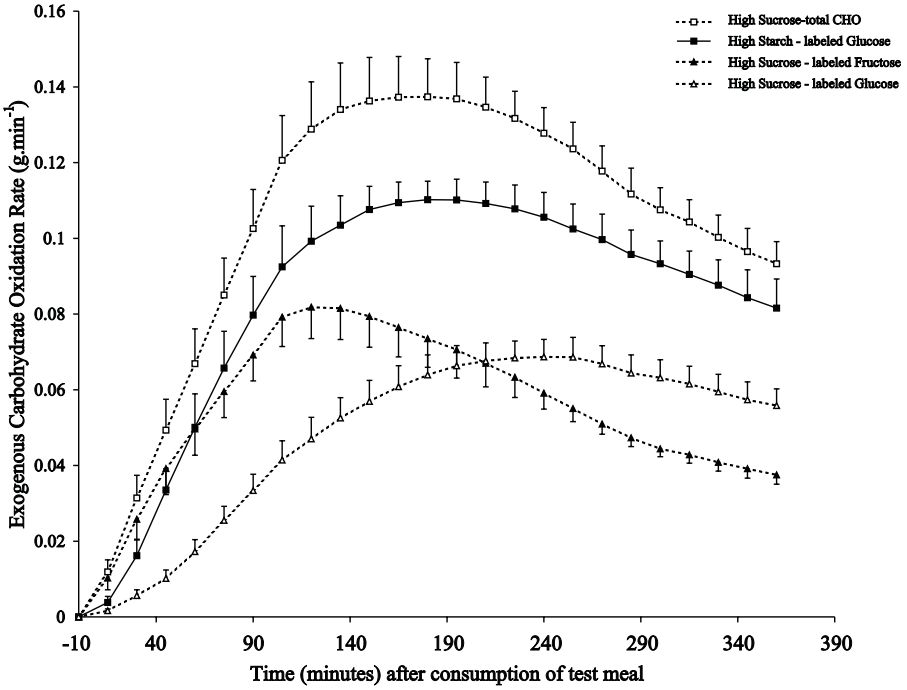

Fig. 16.4 Oxidation of glucose and fructose following test meals containing starch or sucrose labelled with ^{13}C-glucose (starch meal) and both ^{13}C-glucose and ^{13}C-fructose (sucrose; two separate test meals).

blood glucose concentration was slightly, but not significantly ($P = 0.18$) higher with the high-sucrose test meal. However, from 75 min after consumption of the test meals, blood glucose concentrations were consistently higher with the high-starch meal. Both test meals provoked a similar rapid suppression of plasma non-esterified fatty acids (NEFA) concentrations (Fig. 16.5). Whereas these returned to fasting levels by 6 h after the test meal with the high-sucrose challenge, plasma NEFA remained below 50% of fasting concentrations with the high-starch meal throughout the period of observation. To our knowledge, this is the first *in-vivo* study in humans demonstrating the preferential oxidation of fructose, which would be expected on the basis of knowledge of this sugar's metabolism (Mayes 1993).

16.2.2 *Glucose-sparing and insulin resistance*

This glucose-sparing effect of fructose consumption raises some interesting questions about its evolutionary significance. Some clues may come from the 'carnivore connection' hypothesis for the aetiology of insulin resistance and hyperinsulinaemia. According to this hypothesis, the selective force for insulin resistance was the high-meat, low-carbohydrate diet that was common across much of the World throughout the latter 2 million years of human evolution during the Ice Ages (Brand-Miller & Colagiuri 1999). During earlier primate evolution, dietary carbohydrate intake was plentiful and glucose became the chief metabolic fuel of the brain, foetus and mammary gland (Frienkel 1980). This specific demand for glucose could

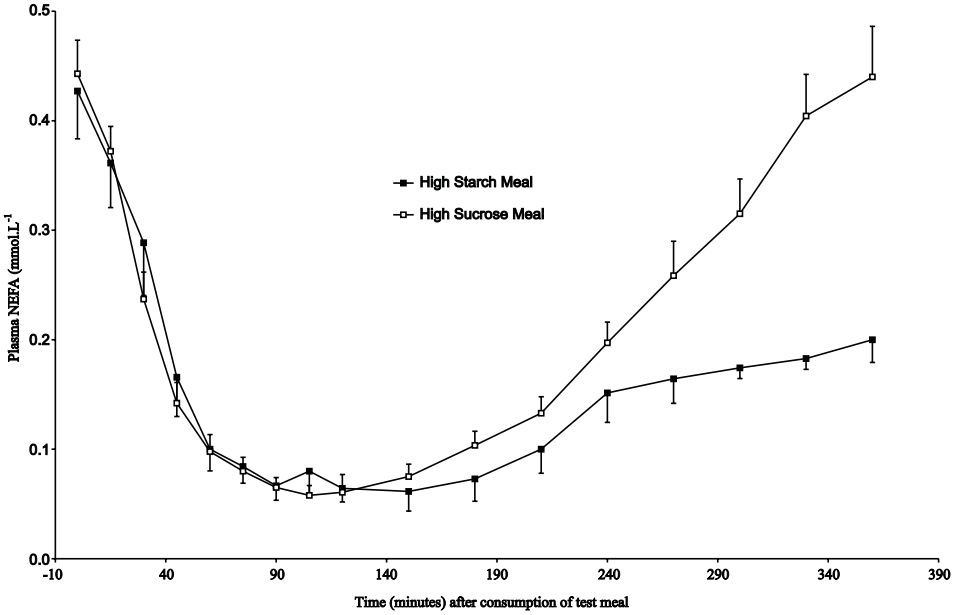

Fig. 16.5 Postprandial changes in plasma non-esterified fatty acids (NEFA) concentration following high-starch (solid squares) or high-sucrose (open squares) test meals.

be met, at least in part, by hepatic gluconeogenesis from amino acids, but this route is limited (DeFronzo 1988). Fruits would have provided fructose – a source of carbohydrate readily oxidisable by muscle and other tissues – and thus allowed available glucose to be used for those tissues having an obligate requirement.

16.3 Experimental studies of effects of food carbohydrates on insulin sensitivity in humans

The balance of evidence from experimental studies in animals indicates that very high intakes of sucrose or fructose have detrimental effects on whole body insulin sensitivity, particularly in association with induction of hypertriglyceridaemia (reviewed by Daly *et al.* 1997b). It is more difficult to draw a convincing conclusion from the more limited studies in human subjects, not least because of the heterogeneity of study designs and the greater difficulty with control of potential confounders (Daly *et al.* 1997b). In a study in patients with advanced CVD, those randomised to a low-GI diet for 4 weeks showed improved whole body glucose tolerance and greater insulin sensitivity in adipocytes from a presternal fat biopsy. No such improvements were detected in those patients randomised to a high-GI diet (Frost *et al.* 1996). A low-GI diet also improved peripheral insulin sensitivity, and normalised PAI-1 in Type 2 diabetics (Jarvi *et al.* 1999). In contrast, 4 weeks of exposure to a low-GI diet in fit young men decreased insulin sensitivity at high plasma insulin concentrations (euglycaemic clamp assay), but not at low insulin concentrations (Kiens & Richter 1996).

Short-chain fructo-oligosaccharides (FOS) are not hydrolysed by human pancreatic or enterocytic enzymes, and pass to the large bowel where they are fermented to short-chain fatty acids (SCFA) and gases (Luo *et al.* 1996). Dietary supplementation of healthy volunteers with 20 g FOS/day for 4 weeks had no significant effect on whole body insulin sensitivity when compared with a similar dose of sucrose (Luo *et al.* 1996). The colon is a major source of circulating glucagon-like peptide 1 (GLP-1) (Robertson *et al.* 1999), which is the most potent insulinotrophic hormone known (Holst *et al.* 1987). Stimulation of colonic fermentation is associated with a prolonged GLP-1 response (Qualmann *et al.* 1995), but it remains to be determined whether SCFA are the luminal-derived signals for GLP-1 release.

UK national policy to reduce the burden from CVD includes efforts to reduce energy intake from total fat and from saturated fatty acids (Department of Health 1994), with this energy being replaced by greater intakes of starchy foods. However, there is considerable evidence that those who choose a lower fat intake often have a higher sugar (sucrose) intake (the so-called sugar–fat seesaw) (Macdiarmid *et al.* 1998). Consequently, it is of interest to know whether, in the context of lower fat intakes (35% of dietary energy from fat), there is a difference in insulin sensitivity when volunteers consume diets rich in sucrose versus those rich in starch. In each of the studies described below, the whole diet was provided for the volunteers to minimise confounding and to maximise compliance.

16.3.1 *Acute feeding study*

After 24 h, during which 50% of the dietary energy was provided by sucrose or starch, there was no difference in whole body insulin sensitivity, as assessed by the short insulin tolerance test (ITT). There were, however, changes in metabolic profiles throughout the experimental day, with higher peaks but lower troughs in blood glucose and insulin concentrations with the high-sucrose diet (Daly *et al.* 1998c).

16.3.2 *Short-term study 1: dose–response effects in younger adults*

Four diets, three lower fat (35% of energy) with sucrose contents ranging from 4.4% to 22.8% of dietary energy, and one higher fat diet (similar to the current UK diet) were administered to 16 healthy younger adults according to a randomised partial cross-over design, such that each volunteer consumed two of the four experimental diets, each for 7 days. None of the treatments altered whole body insulin sensitivity for glucose disposal as assessed by ITT (M. Daly, C. Vale, A. Littlefield, M. Walker, K. Alberti & J. Mathers, unpublished results).

16.3.3 *Short-term study 2: older, sedentary adults*

Older, fatter and more sedentary adults are more likely to have reduced insulin sensitivity and therefore might show greater responses to dietary modifications than younger, fitter adults. In a further study, eight overweight volunteers (aged 45–65 years) consumed each of three experimental diets for 7 days in a cross-over design. No differences in insulin sensitivity between the treatments which were: (1) average UK diet; (2) lower fat, high sucrose; and (3) lower fat, high starch, were detected (M. Daly, C. Vale, A. Littlefield, M. Walker, K. Alberti & J. Mathers, unpublished results).

16.3.4 *Month-long feeding study*

In the final study, a high starch (38% of dietary energy) and a high sucrose diet (33% of dietary energy) were each consumed by seven healthy volunteers for 4 weeks, according to a cross-over design, with whole body insulin sensitivity assessed by the euglycaemic clamp at baseline and at the end of each experimental period. There was no difference between treatments in whole body insulin sensitivity at either baseline or after 4 weeks of dietary intervention, but insulin sensitivity was improved by a mean of 18% with both treatments (Daly *et al.* 1998b). Part of this improvement may have resulted from the modest reduction in body mass (1.1 kg/4 weeks) observed with both treatments, but some may have been a consequence of altered pattern of food intake.

16.4 Clinical implications

In theory, regimes for matching glucose uptake from the gut with the availability of insulin from residual pancreatic activity plus exogenous supply, which are simple to use, would be of considerable benefit in the management of diabetics. Despite being heavily promoted internationally for two decades, the approach based on GI has not been adopted widely in the UK. One disadvantage of the GI approach is that it considers the postprandial period as a whole, and is unable to address the pattern of glucose uptake (and, therefore, insulin need) over time. In the short term, consumption of more slowly digested starches with flatter (and perhaps more prolonged) postprandial glycaemia would better parallel current insulin dosing methods. Future strategies for exogenous insulin supply which had the flexibility to match rate of release to the circulation to rate of appearance of glucose in the posthepatic circulation, would allow diabetics greater freedom in food choice without the penalties associated with hyper- or hypoglycaemia.

16.5 Role of food polysaccharides in healthy eating advice for the public

By comparison with that for fats, the evidence base for recommendations on optimal ranges for food polysaccharides to maximise health benefits is poor. It is probable that diets rich in starches are to be preferred over those rich in sucrose because of reduced risk of dental caries and obesity and because they are associated with higher intakes of a range of micronutrients (Cummings & Englyst 1992). Those containing more slowly digested (lower GI) starches, which exert a smaller demand on the pancreatic β-cell, may be preferable for long-term maintenance of health (FAO/WHO Expert Consultation 1998). Such diets are likely to be richer in NSP, which may have protective effects against colorectal cancer (Department of Health 1998), although recent epidemiological evidence provides no support for this hypothesis (Fuchs *et al.* 1999).

16.6 Conclusions

Our recent studies with healthy volunteers aged 20–60 years [body mass index (BMI)

20–30 kg/m^2] have found little evidence that varying the proportions of starch and sucrose over the ranges likely to be consumed in normal UK diets had a marked effect on whole body insulin sensitivity. However, it should be noted that these test diets were reduced fat (35% of food energy), and it is not known whether alterations in the source of carbohydrate would have greater effects with higher fat diets. Secondly, in design of the test diets, commonly eaten starchy foods, without regard to the rate or extent of digestion of the starch fraction, were used. It remains to be established whether substitution of slowly digested starches (with lower GI) in such lower fat diets would improve insulin sensitivity in healthy subjects, but the available evidence is encouraging (Frost *et al.* 1998). Individuals with raised blood triglyceride concentrations (perhaps because of their genetic inheritance) may be more sensitive to high intakes of sucrose (and fructose) (reviewed by Daly *et al.* 1997b), and future studies should focus on this aspect of diet–gene interactions. In our longest term study (4 weeks per treatment), there was strong evidence that imposition of a regular eating pattern consisting of either a high-starch or a high-sucrose diet, in combination with lower fat improved insulin sensitivity. This area of eating pattern and health risk is likely to repay investment in further research.

Acknowledgements

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17 Fermentation of Oligosaccharides and Influences of Fermentation Products

Akiko Inagaki and Takashi Sakata

17.1 Bacterial breakdown of oligosaccharides in the large intestine

Indigestible oligosaccharides are subjected to bacterial attack in the large intestine, the process producing short-chain fatty acids (SCFA) such as acetic, propionic and *n*-butyric acids as well as gases (Macfarlane & Macfarlane 1997). Lactic or succinic acids may be produced under certain conditions. Bacteria in the large intestine utilise the energy released during this process for their proliferation (Macfarlane & Macfarlane 1997), which is accompanied by the synthesis of protein from peptide, amino acids and ammonia (Macfarlane & Macfarlane 1997). Thus, the energy supply to bacteria in the large bowel stimulates bacterial proliferation and consumption of these materials, at the same time producing a range of metabolites. Therefore, a considerable part of the effects of indigestible oligosaccharides should be to provide energy to the large intestinal bacteria, thereby increasing the production of organic acids and reducing that of peptides, amino acids, ammonia and branched-chain fatty acids.

In this chapter, we summarise the effects of bacterial metabolites on the energy metabolism and digestive functions, and discuss aspects of the regulation of large bowel fermentation.

17.2 Absorption of bacterial metabolites from the large intestine

More than 95% of SCFA produced in the large bowel are absorbed via passive diffusion and anion-exchange (von Engelhardt *et al*. 1998). Absorption of SCFA, but not of lactic or succinic acids, accompanies bicarbonate secretion (Umesaki *et al*. 1978). The mechanism of lactic acid absorption is unknown, but succinic acid is absorbed via a mechanism common for di- and tricarboxylic acids (Wolffram *et al*. 1995). The absorption of lactic or succinic acids occurs far more slowly than that of SCFA (Umesaki *et al*. 1978), and this often leads to an accumulation of these acids in the lumen (Ichikawa & Sakata 1997).

17.3 Influence of bacterial metabolites

17.3.1 *Influence on lumen pH*

Lactic and succinic acids, but not SCFA, are the major determinants of lumen pH in the large bowel (Hoshi *et al*. 1994).

17.3.2 *Energetic contribution of SCFA*

A part of the SCFA absorbed into colonocytes is used by these cells, while the remainder

enters the systemic energy pool (Livesey & Elia 1995). The absorption of lactic or succinic acids is too slow (Umesaki *et al*. 1978) to contribute to the energy balance of the host animal; thus, the metabolisable energy content of an oligosaccharide depends on the proportion of its gross energy that was converted to SCFA.

n-Butyric acid is the premier fuel for colonic epithelial cells (Livesey & Elia 1995; Roediger 1995), providing approximately 70% of energy to colonocytes *in vivo* (Roediger 1995). Virtually all butyric and propionic acids that enter the portal vein are metabolised by the liver. Only part of the acetic acid enters the systemic blood flow to be metabolised in the kidney, mammary gland and adipose tissue as a substrate for energy production and fat synthesis (Livesey & Elia 1995).

17.3.3 *Influence on absorption of water and solutes*

SCFA, but not lactic or succinic acids, stimulate the absorption of water and sodium from the large intestine (von Engelhardt *et al*. 1998). Therefore, SCFA are not likely to induce diarrhoea when the production rate of SCFA is within the absorptive capacity of the large intestine (Wolever *et al*. 1995; von Engelhardt *et al*. 1998). Succinic acid stimulates water secretion from the ileum *in vivo* (Shimazaki 1992). The absence of symport of water or sodium with lactic or succinic acids, together with their slow absorption (Umesaki *et al*. 1978; von Engelhardt *et al*. 1998), may be the reason why these acids induce diarrhoea.

The large intestine absorbs a considerable amount of calcium and magnesium, this being stimulated by the presence of fructo-oligosaccharides (Ohta *et al*. 1995) and by SCFA (Trinidad *et al*. 1996).

Ammonia increases the absorption of SCFA and vice versa (Boedeker 1993); this may be due to the provision of protons from the ammonium cation to the SCFA anion, making both more lipophilic.

17.3.4 *Effect of SCFA on mucosal blood flow*

SCFA increase blood flow and oxygen supply of the colonic mucosa (Kvietys & Granger 1980; Mortensen *et al*. 1991; Mortensen & Nielsen 1995). The effect is local and directly on smooth muscle cells of the arteriole, and involves a fall in intracellular calcium levels (Mortensen & Nielsen 1995).

17.3.5 *Influence on epithelial cell kinetics and tissue mass*

SCFA in the large bowel stimulate epithelial cell proliferation in the small and large intestine (Sakata & von Engelhardt 1983; Rollandelli *et al*. 1986; Sakata 1986, 1987, 1995). L-Lactic acid (Ichikawa & Sakata 1997) or ammonia (Ichikawa & Sakata 1998) continuously infused into the isolated caecum or colon also stimulate the crypt cell production rate in the large bowel. Tropic effects of SCFA and lactic acid are pH-dependent (Ichikawa & Sakata 1997), the effect of SCFA being stronger at pH 7.0 than at pH 5.0, while that of lactic acid is significant at pH 5.0 but not at pH 7.0. The effects of ammonia and SCFA are not additive (Ichikawa & Sakata 1998); rather, the coexistence of ammonia and SCFA seems to reduce the tropic effect of these factors alone.

It is interesting that lowering lumen pH with hydrochloric acid inhibits the caecal epithelial cell proliferation both *in vivo* (Ichikawa & Sakata 1997) and *in vitro* (A. Inagaki & T.

Sakata, unpublished results). Therefore, the tropic effect of SCFA or lactic acid in the large intestine is not due to the low pH, but is mediated via both neural and non-neural mechanisms, even to a distant segment (jejunum) (Sakata 1989; Frankel *et al*. 1994).

Although SCFA increases the mucosal and submucosal tissue mass and crypt cell numbers, lactic acid or ammonia do not (Ichikawa & Sakata 1997, 1998); thus, SCFA might provide important physiological stimuli in maintaining the normal tissue mass, while lactic acid or ammonia may not have such an effect.

Succinic acid has been shown to inhibit crypt cell proliferation of the caecum or colon both *in vivo* and *in vitro* (A. Inagaki, H. Ichikawa & T. Sakata, unpublished results).

17.3.6 *Effect on mucus release from the large intestine*

A mixture of SCFA instilled into the caecal or colonic lumen stimulates the release of mucus from these segments *in vivo* (Sakata & von Engelhardt 1981; Sakata & Setoyama 1995). The effect is local and partly requires cholinergic transmission.

17.3.7 *Influence on gut motility*

SCFA acutely stimulate the propulsive motility of the washed distal colon *in vivo* (Yajima & Sakata 1987), although prolonged exposure to SCFA leads to the distension of the segment. Low pH, lack of SCFA or presence of succinic acid in chronic infusion into the rat caecum for 2 weeks reduced the motility ratio of the caecum (Hoshi 1994). Thus, both succinic acid and low pH reduce the motility, while SCFA are necessary to maintain normal motility.

Both colonic (Cherbut *et al*. 1997) or ileal (Cuche & Malbert 1999) SCFA inhibit gastric emptying via a vago-vagal reflex.

17.3.8 *Effects on the pancreas*

Administration of SCFA into the large intestine increases pancreatic juice flow and amylase secretion, mainly via a vago-vagal reflex (Katoh 1995; Ohbo *et al*. 1996).

Rectal infusion of SCFA acutely increases the blood level of glucagon in humans (Wolever *et al*. 1991), although it is unclear whether the glucagon was of pancreatic or enteric origin.

17.4 Characteristics of SCFA effects

Most of the *in-vivo* effect of SCFA is the sum of direct, local indirect and systemic effects (Sakata 1994) with, in some cases, their systemic effect superseding their local effect. Accordingly, *in-vitro* results may not be extrapolated directly to *in-vivo* conditions. As most effects of SCFA are dose-dependent and vary between different acids (Sakata 1994), it is important to know both the nature of the acid and the extent of its production from oligosaccharides by the large intestine bacteria population.

17.4.1 *The need to control colonic fermentation*

As production of lactic or succinic acids and the resultant low lumen pH may be hazardous in humans, it is important to regulate gut fermentation processes so that SCFA are produced

rather than lactic or succinic acids. It is also important to regulate the proportion of each SCFA, considering their different efficacies and ultimate destinations as energy substrates. Thus, it is important to clarify the mechanism that regulates fermentation in the large intestine.

17.4.2 *Estimation of fermentation rates*

It is important to measure the rate of production of each metabolite in order to clarify the mechanism that regulates bacterial fermentation in the large intestine. The different approaches are compared briefly in the following sections.

Lumen or faecal concentration

As most SCFA produced in the large bowel are absorbed, it is dangerous to estimate their production rates from their lumen or faecal concentrations. It is also important to consider the time-course of the lumen concentration of metabolites. This is especially important in investigations using nocturnal rodents (e.g. rats), which often show a high luminal lactic acid concentration during the night, i.e. just after feeding (A. Inagaki & T. Sakata, unpublished results.)

Lumen pool size

Some researchers have adopted lumen 'pool size' (lumen concentration × contents volume) as a parameter for the production rate (Hara *et al*. 1996; Campbell *et al*. 1997; Topping *et al*. 1997). However, in addition to the danger of using lumen concentration as part of this calculation, the pool size may be strongly influenced by the volume of the large intestine. Accordingly, 'pool size' is a poor parameter for production rate. Lumen concentration is a better parameter because this value at least represents the environment to which the mucosal surface is exposed.

Portal arteriovenous (A-V) difference

It is possible to estimate the net gain of bacterial metabolites from portal-drained organs by multiplying the concentration difference between arterial and portal blood by the portal flow rate. However, as most of the *n*-butyric acid and a considerable part of the propionic acid are consumed by epithelial cells (Roediger 1982; Livesey & Elia 1995; Livesey *et al*. 1995; Roediger 1995), this may result in an underestimation of either (or both) of these compounds.

Care must be taken to maintain the normal blood supply to the mucosa when the portal concentration of organic acids is measured, because some SCFA absorption depends on active sodium transport, which in turn requires oxidative energy production in epithelial cells (von Engelhardt *et al*. 1998). Oxygen supply is also necessary for the oxidation of propionic acid and *n*-butyric acid in epithelial cells, and in this regard chronically catheterised animals/subjects, without anaesthesia, should be used (Uhing & Kimura 1995).

Isotope dilution

In theory, isotope dilution methods (Pouteau *et al*. 1998) can be used to measure the rates of consumption and production of organic acids by bacteria, their metabolism in epithelial cells, and their penetration into the organs. This method is expensive and tedious, and not yet sufficiently sensitive, and requires extensive mixing of the isotope in the lumen – a process that is not always easy to perform, or to guarantee. Moreover, it is not always easy to establish 'steady state' in the lumen of the large bowel of humans who are leading their normal life in dietary terms.

Batch culture

Batch culture using either large intestinal contents (Kikuchi & Sakata 1992; Monsma & Marlett 1995; Kihara & Sakata 1997; Sakata *et al*. 1999), faeces (Barry *et al*. 1995; Monsma & Marlett 1995) or bacteria isolated from such materials (Kiriyama, Hariu *et al*. 1992) is both easy to perform and inexpensive. It is possible to conduct many concurrent cultures, even when using the same source of inoculum. This enables a mathematical description of the time-course and kinetic analysis of metabolite production (Kikuchi & Sakata 1992). A batch culture method has been developed which uses as little as 1 ml or even 100 µl (Kikuchi & Sakata 1992; Kihara & Sakata 1997), and this in turn permits the amount of test oligosaccharide to be reduced. However, in batch culture the initial culture conditions cannot be maintained as the substrate is consumed and metabolic products accumulate during incubation (Kiriyama *et al*. 1992).

It is important to use mixed bacterial species to evaluate the fermentation of oligosaccharide(s). Otherwise, it is impossible to simulate the complex and interactive metabolism of the bacterial ecosystem in the large intestine. It is possible to use autoclaved caecal contents or ileal effluents as the basal vehicle to which the oligosaccharide(s) will be added. It is also reasonable to ask ileostomates to provide their ileal effluent after consuming the oligosaccharide(s) of interest, for use as the test substrate to be evaluated in batch cultures.

Continuous culture

This method can employ various patterns of substrate supply. It is possible to alter the dilution rate, to regulate the pH, or to adapt the culture to certain substrate(s). It is also possible to remove the fermentation products by dilution with buffer or through a dialysis membrane (Czerkawski 1986). However, continuous culture is more tedious, expensive and space-consuming than batch culture. Continuous culture methods require greater quantities of test oligosaccharides than batch cultures. The choice of different methods depends on the objective of the study, though oversimulation is not always productive (Czerkawski 1986).

17.5 Regulating factors of bacterial metabolism in the large intestine

17.5.1 *Substrates*

The monosaccharide composition of an oligosaccharide seems to affect the fermentation.

For example, feeding of a pentose (xylose), a sugar alcohol of pentose (xylitol) or a pentose-containing oligosaccharide (xylosylfructoside) results in less lumen butyric acid than their hexose counterparts (Kiriyama *et al*. 1992; Hoshi 1994).

The degree of polymerization of the oligosaccharide or polysaccharide is another important factor. Feeding partially hydrolysed guar gum, or its ileal infusion, resulted in the accumulation of lactic acid in the rat caecum (Hoshi 1994). Other studies using batch cultures of washed pig caecal bacteria showed that, the higher the degree of polymerisation of guar gum, the higher the proportion of acetic acid and the lower the proportion of propionic acid (Hoshi 1994).

A rapid entrance of substrate(s) into the proximal large intestine often leads to the accumulation of lactic and succinic acids (Hoshi 1994); this may be due to the effect of dilution rate (Wallace 1995) or to a low pH (Ushida & Sakata 1998).

17.5.2 *Dilution rate*

Dilution rate is defined as the rate of digesta entry relative to the volume of the (fermentation) chamber. Monocultures of *Streptococcus ruminantium* produced propionic and acetic acids from glucose at low dilution rate and lactic acid at high dilution rates (Wallace 1995). Thus, the mode of digesta flow and the size of fermentation chamber are important determinants of fermentation.

17.5.3 *Lumen pH*

The lumen pH affected the fermentation of oligosaccharides by pig caecal bacteria in a batch culture (Ushida & Sakata 1998). Although SCFA are the main products when the pH is higher than 6.0, lactic acid is produced when the pH is lower than 6.0. Succinic acid is also produced when the initial pH is lower than 5.0. Such a shift in fermentation products is mainly due to the increase in the production of lactic and succinic acids at pH values above 4.0, and to the decrease in the utilisation of these acids at pH values below 4.0 (A. Inagaki, M. Sato & T. Sakata, unpublished results). Such an effect of pH is common for the fermentation of different types of oligosaccharides, namely fructo-oligosaccharides, isomalto-oligosaccharides and xylosylfructoside (Ushida & Sakata 1998).

17.5.4 *Interaction among substrate entry rate, pH and fermentation pattern*

The effects of pH on the fermentation pattern suggest that the flux rate of fermentable carbohydrates into the large bowel is important. If the flux rate exceeds the absorption rate for organic acids, then the excess acids remains in the lumen to reduce lumen pH, favouring the production of lactic and succinic acids. The latter acids have smaller pK_a values than SCFA, and thus their absorption occurs much more slowly than that of SCFA. Accordingly, once the lumen pH is lowered, lactic or succinic acid may lower it further. The lack of bicarbonate secretion to counteract the effect of the acids left in the lumen (Umesaki *et al*. 1978), will further amplify the effect of low pH.

In this regard, factors affecting the flux rate of carbohydrates into the large bowel should have a significant effect on gut fermentation. Oligosaccharides or sugar alcohols ingested as a drink may reach the large bowel rapidly as a bolus, while the flux of such carbohydrates contained in a structured solid food may be slower and follow a smoother pattern. Accordingly,

the form, structure and surface area of the food should be considered when the effect of an oligosaccharide component of that food is investigated.

17.5.5 *Coexisting material*

A high concentration of succinic acid is observed in rats fed a diet containing resistant starch prepared from amylomaize. However, this effect can be abolished by the simultaneous feeding of protein that is resistant to pancreatic digestion (Morita & Kiriyama 1996).

17.6 Perspectives and conclusions

Studies on the effects of fermentation products should employ *in-vivo* experiments, because *in-vivo* and *in-vitro* effects can be contradictory. Dose–response studies and time-course studies are essential. When *in-vitro* methods are used, the actual concentration of the metabolite(s) in the region of the effector cells should be reproduced, and these may be quite similar to arterial concentrations in many cases.

Either experimental or mathematical approaches should be developed to analyse the complex bacterial ecosystem in the large intestine to help clarify the effects of oligosaccharides. It is important to study interactive effects between different carbohydrates and other substrates such as resistant protein under various dilution rates, pH values or mixture conditions. It is also important to compare the fermentation profile before and after adaptation of the bacterial population to the oligosaccharide of interest.

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18 Cholesterol-lowering Properties of Cereal Fibres and Fractions

Talwinder S. Kahlon

18.1 Introduction

Healthful effects of cereal fibres and fractions were recognised by observing the lower incidence of degenerative diseases in the ancient cultures and developing world. A growing body of evidence suggests a protective effect of dietary fibre and fractions from cereal grains against atherosclerosis and other diseases common to the Western world. Various fractions of dietary fibre from cereals have been shown to moderate or prevent hypercholesterolaemia, one of the primary factors contributing to atherosclerosis and heart disease. There are considerable differences in the composition of dietary fibre from oat bran, rice bran and barley (Table 18.1), and hypocholesterolaemic effects have been demonstrated with each of these cereal fibres. De Groot *et al.* (1963) first reported cholesterol-lowering effects with rolled oats. Rice fibre and fractions were demonstrated to have hypocholesterolaemic activity in hamsters at the USDA Western Regional Research Center, where a rice bran stabilisation process (Randall *et al*. 1985) was developed, deactivating lipase that produced high free fatty acid content. The stabilisation process changed rice bran from a low-value animal feed to a high-value food product. Montana State University researchers have contributed the major share of the scientific data on hypocholesterolaemic effects of barley. This review encompasses research on the cholesterol-lowering influence of oat, rice and barley dietary fibre and fractions in animal and human studies, including current knowledge of mechanisms that may be responsible for this activity.

18.2 Oat fibre and fraction

Studies on the effects of oat bran and fractions on cholesterol metabolism in animals are summarised in Tables 18.2, and human studies in Table 18.3.

Table 18.1 Composition of oat bran, rice bran and dehulled barley (% dry matter).

Cereal	TDF	IDF	SDF	β-Glucan	Fat	Protein
Oat bran	18.6	10.6	8.0	8.3	7.7	0.5
Rice bran	22.9	21.5	1.4	1.8	20.6	13.7
Barley	17.2	11.2	6.0	5.7	3.1	12.5

TDF, Total Dietary Fibre; IDF, Insoluble Dietary Fibre; SDF, Soluble Dietary Fibre.
Source: data from Kahlon *et al*. (1993).

Table 18.2 Total plasma cholesterol (TC) reductions with oat bran: animal studies.

Species	Oat fraction	%	TC reduction %*	Reference
Rat	Rolled oats	25.0	73	De Groot *et al*. 1963
	Defatted oats	23.2	61	
	Oat fat	1.8	54	
Rat	Oat bran	37.0	26	Chen *et al*. 1981
	Oat gum	10.0	41	
Rat	Oat fibre	6.0	38	Ney *et al*. 1988
Rat	Oat flour	41.0	26	Shinnick *et al*. 1990
Rat	Oat bran	23.0	22	Mongeau *et al*. 1991
Rat	Oat bran	40.0	13	Illman *et al*. 1991(expt. 1)
Rat	Oat bran	36.0	24	Lupton & Yung 1991
Rat	Oat bran	49.0	15	Roach *et al*. 1992
Rat	Oat bran	30.0	33	De Schrijver *et al*. 1992
Rat	Oat bran conc.	8–24	45	Malkki *et al*. 1993
Chick	Oat bran	30.0	42	Welch *et al*. 1988
Chick	Oat bran	68.0	48	Pettersson & Aman 1992
Chick	Oatrim	26.4	18	Inglett & Newman 1994
Hamster	Oat bran	46.0	27	Kahlon *et al*. 1990
Hamster	Oat bran	53.7	14	Kahlon *et al*. 1993
Hamster	Enriched oat flour	14.9	21	Yokoyama *et al*. 1998
	Oatrim-10	39.6	36	
	Oatrim-5	67.3	32	
	Oat hydrolysate	40.1	17	

*All reductions in these studies were significantly lower than the corresponding control or comparison treatments. Other studies (Nishina *et al*. 1991; Illman *et al*. 1991, expt. 2; Arjmandi *et al*. 1992; Tietyen *et al*. 1995; Rieckhoff *et al*. 1999) found no significant reductions with oat bran or oat fibre.

Table 18.3 Total plasma cholesterol (TC) reductions with oat bran: human studies.

Pre-treatment TC (mg/dl)	Oat bran (g/day)	Days reduction (%)	TC	LDL-cholesterol	Reference
251	140 (rolled oats)	21	11*	–	De Groot *et al*. 1963
260	100	10	13*	14*	Kirby *et al*. 1981
260	100	21	19*	23*	Anderson *et al*. 1984
208	60 (bran/meal)	42	3*	–	Van Horn *et al*. 1986
268	25	14	5*	9*	Anderson *et al*. 1990
186	87	42	0	0	Swain *et al*. 1990
266	106	21	13*	12*	Anderson *et al*. 1991
247	38	20	3	7*	Blumenschein *et al*. 1991
>185	38	28	2	5	Gold *et al*. 1991
242	55	42	0	0	Mackay & Ball1992
266	110	21	13*	12*	Bridges *et al*. 1992
227	100	28	8*	10*	Kelley *et al*. 1994
164	9 (gum)	14	0	0	Beer *et al*. 1995
211	63 (1% β-glucans)	35	10*	15*	Behall *et al*. 1997
	63 (10% β-glucans)	35	15*	21*	
278	84	42	13*	17*	Gerhardt & Gallo 1998

*Reductions were significantly lower than corresponding controls or comparison treatments.

18.2.1 *Studies in rats*

De Groot *et al.* (1963) reported a 73% reduction in serum cholesterol with a 25% rolled oat diet compared with wheat starch in six male and six female rats fed 1% cholesterol and 0.2% cholic acid diets for 4 weeks. Supplementing the diets with defatted rolled oats, or its extracted fat, showed that the cholesterol-lowering effect was equally divided between the oat fat and non-fat material. Other investigators in later studies focused on the hypocholesterolaemic effects of the fibre component of oats. Studies in which either cholesterol or cholic acid were added to rat diets (Chen *et al.* 1981; Ney *et al.* 1988; Shinnick *et al.* 1990; Mongeau *et al.* 1991; Lupton & Yung 1991) or not (Roach *et al.* 1992; De Schrijver *et al.* 1992) resulted in significant total plasma cholesterol (TC) reductions with oat bran. Adding fish oil to the oat bran diet resulted in further significant reductions in plasma cholesterol values over the unsupplemented oat bran diet (Roach *et al.* 1992). Plasma high-density lipoprotein cholesterol (HDL-C) levels were raised with oat bran diets in studies by Chen *et al.* (1981) and Ney *et al.* (1988), but were significantly lowered in the study by Mongeau *et al.* (1991). Nishina *et al.* (1991), Arjmandi *et al.* (1992) and Tietyen *et al.* (1995) did not observe significant plasma cholesterol reductions in rats fed oat bran diets containing cholesterol. Liver cholesterol was significantly lowered by oat bran in cholesterol-fed rats in several studies (Chen *et al.* 1981; Shinnick *et al.* 1990; Lupton & Yung 1991; Tietyen *et al.* 1995).

Illman *et al.* (1991) found that the cholesterol-lowering ability of oat bran was lost by extracting oat bran with *n*-pentane, and it was not restored by adding back the lipid extract. The authors concluded that the cholesterol-lowering effects were related to a pentane-soluble component of oat bran, but not to tocotrienols or tocopherols, since their content in the diets was similar in treatments which lowered cholesterol and in those which had no effect. Oat bran β-glucan (1.5%) diets significantly lowered plasma cholesterol in cholesterol-fed rats compared with those fed a cellulose control diet (Malkki *et al.* 1993). Treating oat bran with *endo*-D-glucanases significantly elevated liver cholesterol compared with those fed untreated oat bran (Tietyen *et al.* 1995), showing that glucans are involved in liver cholesterol metabolism.

Significant increase in faecal cholesterol excretion (Jonnalagadda *et al.* 1993) and bile acid excretion (De Schrijver *et al.* 1992) with oat bran diets have been reported. An inverse linear relationship ($r = -0.80$) was observed between plasma cholesterol concentration and bile acid excretion (De Schrijver *et al.* 1992).

18.2.2 *Studies in chicks*

Welch *et al.* (1986, 1988) and Pettersson and Aman (1992) reported significant reductions in TC with oat bran diets in chickens. Significant reductions in liver cholesterol and increased small intestinal length with oat bran diets (Welch *et al.* 1986, 1988) in chickens were also reported. The authors suggested that increased small intestinal length might be associated with alterations in hormonal regulation of cholesterol metabolism. Pettersson and Aman (1992) determined that tocotrienol-extracted oat bran diets resulted in significant TC reductions, similar to intact oat bran diet, compared with β-glucanase-treated oat bran. They concluded that the cholesterol-lowering effect of oat bran was due to its soluble fibre rather than to the presence of tocotrienols. Inglett and Newman (1994) reported significant reductions in TC and low-density lipoprotein cholesterol (LDL-C) in chicks fed 2.2% soluble β-glucan (oatrim) diet containing cholesterol.

18.2.3 *Studies in hamsters*

Kahlon *et al.* (1990, 1993) reported that oat bran diet showed significantly lower elevations of plasma and liver cholesterol compared with a cellulose diet containing added cholesterol. Yokoyama *et al.* (1998) observed significant TC and LDL-C reductions with diets containing oat hydrolysate which contained no β-glucans, as well as with oat or oatrim with 4.2–4.3% β-glucans compared with cellulose control diet. The authors speculated that in addition to β-glucans, other components such as phytosterols of oats may also be responsible for the cholesterol lowering. Oat bran diet lowered TC, LDL-C and liver cholesterol compared with wheat bran diet, and extrusion cooking (two energy levels) did not significantly alter the cholesterol-lowering potential of oat bran (Kahlon *et al.* 1998). However, Rieckoff *et al.* (1999) did not observe significant reductions in TC, very low-density lipoprotein cholesterol (VLDL-C), LDL-C and liver cholesterol with oat [10–13% total dietary fibre (TDF)] diets containing 20% fat and 0.12% cholesterol in 5 weeks compared with wheat bran control. In a 6-week study, Kahlon *et al.* (1999) reported significant reduction (59–63%) in the plaque formation in the inner bend of the aortic arch with oat bran diets containing 20% fat and 0.5% cholesterol compared with cellulose control diet. Relative plaque area in the aortic arch was 0.82–0.90 mm^2 with oat diets compared with 2.19 mm^2 with cellulose control diet.

18.2.4 *Studies in humans*

De Groot *et al.* (1963) reported a significant cholesterol-lowering effect of rolled oats in 21 male hypercholesterolaemic volunteers (average age 40 years) after 3 weeks of consuming 140 g/day of rolled oats incorporated in bread; cholesterol levels decreased significantly from 251 to 223 mg/dl (–11%). Replacing rolled oat bread with their usual bread resulted in a rise in serum cholesterol to 246 mg/dl in 2 weeks. Nearly 20 years later, renewed interest in the cholesterol-lowering effects with oats resulted in a number of studies (Kirby *et al.* 1981; Anderson *et al.* 1984, 1990, 1991; Kashtan *et al.* 1992; Whyte *et al.* 1992; Poulter *et al.* 1993; Braaten *et al.* 1994) in which significant reductions in TC and LDL-C were observed in hypercholesterolaemic men who consumed oat bran or oat gum. In several reports, oat bran diet resulted in a significant increase in the HDL-C:LDL-C ratio (Kirby *et al.* 1981; Anderson *et al.* 1984; Mackay & Ball 1992) and in faecal bile acid excretion (Kirby *et al.* 1981; Anderson *et al.* 1984).

Van Horn *et al.* (1986) reported that feeding 60 g/day of oat bran [10.8 g TDF/day; 5.2 g insoluble dietary fibre (IDF) and 5.6 g soluble dietary fibre (SDF) or oat meal (4.9 g TDF/day; 2.2 g SDF and 2.7 g IDF)] for 6 weeks to subjects consuming the American Heart Association's National Cholesterol Education Program (NCEP) step 1 diet (30% fat calories, sat:mono:poly 1:1:1, and cholesterol <250 mg/day), significantly lowered TC in free-living moderately hypercholesterolaemic (208 mg/dl) adults. NCEP step 1 diet lowered TC 5%, oat bran further lowered TC by 5% and oatmeal by 3%, compared with the unsupplemented control group.

Swain *et al.* (1990) fed healthy normocholesterolaemic (186 mg/dl) hospital workers high-fibre oat bran (87 g/day) or a low-fibre refined wheat product supplement (93 g/day) for 6 weeks, along with the subjects' usual free-living regular dietary pattern in a cross-over design. At the conclusion of the study, no differences were observed in effects on TC, LDL-C

and HDL-C values between the two supplements. TC was 7–8% lower with both supplements compared with the baseline value, which was attributed to 33% lower cholesterol intake, as well as reduced saturated fat and higher polyunsaturated fat intake during the treatment period. These lower saturated fat and cholesterol intakes most likely contributed to the serum cholesterol reductions during the low-fibre supplementation period. However, total dietary fat intake during the oat bran period was significantly higher than during the wheat and baseline periods, which may account in part for the lack of difference in cholesterol reductions by the two cereals.

Evidence for cholesterol reductions in children eating oat bran, was reported by Blumenschein *et al.* (1991). They found that consumption of oat bran (1 g/kg/day for 3 weeks) by hypercholesterolaemic (247 mg/dl) 5- to 12-year-olds resulted in significant reductions in LDL-C and LDL-C/HDL-C ratios, and significant elevations in HDL-C. In addition, Gold *et al.* (1991) fed a daily supplement of 38 g of oat bran for 4 weeks to school-age children (mean age 10 years) following the NCEP step 1 diet. Initial TC levels (>185 mg/dl) were lowered by 9%, LDL-C by 8%, and HDL-C by 1%, but results were not significant compared with the NCEP Step 1 control. A decrease in apolipoprotein B in the oat bran group was significant compared with the low-fat control group, suggesting that oat bran may reduce risk factors for atherosclerosis in hypercholesterolaemic children. Bridges *et al.* (1992) observed that with 3 weeks of oat bran supplementation (106 g/day) in hypercholesterolaemic men, TC was significantly lowered by 12.8% and LDL-C by 12.1%, compared with pre-treatment control values. Plasma acetate, one of the short-chain fatty acids which are claimed to inhibit cholesterol synthesis, was significantly increased (45%) with oat bran supplementation compared with control values, which may have contributed to oat bran's cholesterol-lowering effects. Kelley *et al.* (1994) reported that consumption of 100 g/day of oat bran for 4 weeks resulted in a significant reduction in TC and LDL-C in subjects (initial TC, 227 mg/dl) who were following low-fat (24.2% of the calories) and low-cholesterol (166 mg/day) diets. Beer *et al.* (1995) observed that daily consumption of 9 g of oat gum for 14 days resulted in no significant effect on TC and LDL-C, but HDL-C was significantly increased in normocholesterolaemic young men. The authors attributed the lack of effect on LDL-C to the low viscosity of the oat gum used in the study, and possibly to low initial cholesterol levels in the subjects. Behall *et al.* (1997) reported significant reductions in TC and LDL-C in mildly hypercholesterolaemic subjects with oat fibre extract (1 or 10% β-glucans) diets in 5 weeks. Gerhardt and Gallo (1998) observed significant reductions in TC and LDL-C (13 and 17%, respectively) with consumption of 84 g/day of an oat product for 6 weeks in moderately hypercholesterolaemic, non-smoking, non-obese adults.

These studies indicate that adding oat products to the diet in moderate and realistic levels can produce significant reductions in plasma cholesterol and/or improve beneficial lipoprotein ratios to reduce the risk of heart disease in hypercholesterolaemic individuals.

Animal and human studies reported to date suggest that the cholesterol-lowering components of oat bran include β-glucans, soluble fibre, phytosterols and soluble fibre-lipid interactions. Possible mechanisms of cholesterol reduction in hypercholesterolaemic subjects included increased faecal cholesterol and bile acid excretion, increased gastrointestinal tract length, and increased production of short-chain fatty acids in the gastrointestinal tract. Tocotrienols in oat bran do not appear to play a significant role.

18.3 Rice fibre and fractions

Evaluation of the cholesterol-lowering properties of rice bran in animal and human studies is summarised in Table 18.4.

18.3.1 *Studies in animals*

TC and LDL-C were significantly lowered in hypercholesterolaemic animals by rice bran oil in rats (Sharma and Rukmini 1986) and monkeys (Nicolosi *et al.* 1991). Significant TC reductions with diets containing stabilised or par-boiled rice brans were reported by Topping *et al.* (1990) in rats, Hundemer *et al.* (1991) in mice, and by Kahlon *et al.* (1990, 1992a, b) in hamsters. Topping *et al.* (1990) observed a further significant reduction with the supplementation of 5% fish oil to the rice bran diet. No significant TC reductions were observed with stabilised rice bran in chickens (Newman *et al.* 1992a) or hamsters (Kahlon *et al.* 1993, 1996), when diets were less hypercholesterolaemic. The cholesterol-lowering effect of a test substance appears to be related to the degree of hypercholesterolemia in the test animals (Mongeau *et al.* 1991; Kahlon *et al.* 1992a, b; Newman *et al.* 1992a).

Table 18.4 Total plasma cholesterol (TC) reductions with rice bran: animal and human studies.

	Rice bran (RB)							
		Diet		Pre-treatment		Reduction (%)		
Species	Fraction	%	g/day	Days	TC (mg/dl)	TC	LDL-C	Reference
Rat	RB oil	10	–	56	–	37*	52*	Sharma & Rukmini 1986
Rat	RB	32	–	10	–	10*	–	Topping *et al*. 1990
Rat	RB oil	10	–	56	–	23*	27*	Sharma & Rukmini 1987
	RB oil + U	10 + 0.4	–	56	–	35*	41*	
Rat	Rice protein	25	–	14	–	20*	–	Morita *et al*. 1997
Rat	RB oil	7	–	28	–	14	–	Sunitha *et al*. 1997
Chick	RB	60	–	10	–	0	9	Newman *et al*. 1992a
Hamster	RB	45	–	21	–	32*	–	Kahlon *et al*. 1990
Hamster	RB	50	–	21	–	27*	–	Kahlon *et al*. 1992a
Hamster	RB	44	–	21	–	64*	–	Kahlon *et al*. 1992b
Hamster	RB	44	–	21	–	9	10	Kahlon *et al*. 1993
	RB, defatted	35	–	21	–	15	–	
Hamster	RB	48	–	21	–	5	12	Kahlon *et al*. 1996
	RB + U	48	–	21	–	14*	20	
Monkey	RB oil	17	–	56	–	37*	46*	Nicolosi *et al*. 1991
Human	RB oil	–	39	15	247	17*	–	Raghuram *et al*. 1989
	RB oil	–	39	30	247	26*	–	
Human	RB	–	60	28	245	0	0	Kestin *et al*. 1990
Human	RB	–	15	21	176	2	–	Sanders & Reddy 1992
	RB	–	30	21	176	4	–	
Human	RB	–	100	21	235	9	–	Hegsted *et al*. 1993
	RB	–	100	21	217	4	–	
Human	RB	–	84	42	268	8*	14*	Gerhardt & Gallo 1998

* Reductions were significantly lower than the corresponding controls or comparison treatments in the respective studies.
LDL-C, low-density lipoprotein-cholesterol; U, unsaponifiable matter from rice bran oil.

Kahlon *et al.* (1990, 1992a, b) conducted a series of studies demonstrating the effects of processing on the potential of rice bran to moderate hypercholesterolaemia in hamsters (Table 18.5). Full-fat stabilised rice bran consistently lowered TC compared with cellulose in all the studies, whereas defatted rice bran was ineffective. Liver cholesterol was significantly lowered by both stabilised rice bran and the combination of defatted stabilised rice bran plus either rice bran oil or degummed-dewaxed rice bran oil. Par-boiled rice bran resulted in TC reductions, but not in liver cholesterol; defatted, par-boiled rice bran did not influence plasma or liver cholesterol levels. Both the plasma and liver data suggest that when recombined, defatted rice bran and rice bran oil are not as effective as full-fat rice bran in lowering cholesterol in hamsters.

The dose effect of full-fat, stabilised rice bran was evaluated (Table 18.6) by Kahlon *et al.* (1992b). Significant TC reductions were observed in animals fed 43.7% full-fat stabilised rice bran with or without added cholesterol, compared with those fed cellulose control diets. TC reductions were significantly correlated with the level of rice bran (10.9, 21.8, 32.8 and 43.7%) in the diet, resulting in 8, 11, 15 and 21% reductions, respectively.

A significant TC reduction was observed by feeding hamsters a combination of stabilised rice bran and oat bran (5% TDF from each) diet (Kahlon *et al.* 1993). Oat bran provided 2.3% β-glucans, with 0.3% β-glucans from rice bran, suggesting that cholesterol-lowering activity with rice bran was due to components other than β-glucans. The above suggestion is supported by lack of significant TC reductions with the combination of oat bran and enriched barley fraction diet with 4.5% β-glucans (2.3% from oat bran and 2.2% from barley fraction). Viscosity measurements of diet slurries revealed that diets containing rice bran had a viscos-

Table 18.5 Plasma and liver cholesterol of hamsters consuming rice bran diets for 3 weeks.

Treatment	% of diet	Plasma cholesterol (mg/dl)	Liver cholesterol (mg/g)
Cholesterol (0.5%)			
Control (cellulose)	10.0	401.8 ± 15.9^{a}	56.6 ± 1.5^{a}
Rice bran	47.8	274.2 ± 13.7^{e}	31.2 ± 1.5^{e}
Defatted rice bran	37.1	$353.5 \pm 16.2^{a,b,c}$	$44.6 \pm 1.2^{b,c}$
Par-boiled rice bran	31.8	$302.3 \pm 18.6^{c,d,e}$	$53.2 \pm 2.3^{a,b}$
Defatted par-boiled rice bran	19.6	$383.4 \pm 21.3^{a,b}$	56.9 ± 1.5^{a}
Cholesterol (0.3%)			
Control (cellulose)	10.0	322.4 ± 19.2^{a}	36.2 ± 1.4^{a}
Rice bran	50.2	236.9 ± 11.9^{b}	22.8 ± 2.2^{b}
Defatted rice bran + degummed-dewaxed rice bran oil	41.5, 7.9	$282.5 \pm 17.3^{a,b}$	25.7 ± 2.3^{b}
Cholesterol (0.3%)			
Control (cellulose)	10.0	326.6 ± 21.7^{a}	37.1 ± 1.4^{a}
Rice bran	43.7	254.7 ± 17.1^{b}	28.1 ± 2.3^{c}
Defatted rice bran + rice bran oil	35.0, 9.0	$287.3 \pm 13.4^{a,b}$	$31.4 \pm 1.8^{b,c}$

Values are mean ± SEM; $n = 10$. Means within a column with different superscript letters differ significantly ($P < 0.05$); all diets contained 10% total dietary fibre.
Source: data from Kahlon *et al*. (1990, 1992a, b).

Table 18.6 Plasma and liver cholesterol in hamsters fed various levels of stabilised rice bran diets for 3 weeks.

Diet	Cellulose (%)	Rice bran (%)	Cholesterol	
			Plasma (mg/dl)	Liver (mg/g)
No added cholesterol				
Cellulose	10.0	–	145 ± 7^{c}	4.2 ± 0.1^{d}
Rice bran	–	43.7	115 ± 6^{d}	3.8 ± 0.1^{d}
0.3% added cholesterol				
Cellulose	10.0	–	327 ± 22^{a}	37.1 ± 1.4^{a}
Rice bran-44	–	43.7	255 ± 17^{b}	28.1 ± 2.3^{c}
Rice bran-33	2.5	32.8	$275 \pm 20^{a,b}$	$34.1 \pm 2.3^{a,b}$
Rice bran-22	5.0	21.8	$290 \pm 20^{a,b}$	$32.8 \pm 1.9^{a,b,c}$
Rice bran-11	7.5	10.9	$299 \pm 19^{a,b}$	$35.8 \pm 1.8^{a,b}$

$n = 10$; values within a column with different superscript letters differ significantly ($P < 0.05$).
Source: data from Kahlon *et al*. (1992b).

ity (<10 cp over a 3-h period) similar to that of the cellulose control diet, while oat bran diet viscosity was 10-fold greater (104 cp). This suggests that the cholesterol-lowering effect of rice bran is related to a mechanism other than sequestering or entrapping lipids, bile acids or their metabolites. With barley diets, viscosities were very high initially, but continued to decrease with time, suggesting the presence of endogenous glucanase in barley fractions.

Unsaponifiable matter (UM) from rice bran oil was shown to lower plasma cholesterol in cholesterol-fed rats (Sharma & Rukmini 1987) and plasma and liver cholesterol in hamsters (Kahlon *et al.* 1996). In the hamster study, liver cholesterol concentrations were significantly lowered, and to an equal extent, by both a rice bran diet containing 0.4% UM, and by a cellulose diet with 0.8% added UM. Increasing the UM level to 0.8% in the rice bran diet resulted in further significant reductions in liver cholesterol. These reductions were significantly correlated with faecal fat and neutral sterol excretion, suggesting that a possible mechanism for liver cholesterol reductions by rice bran UM may be through diminished absorption/reabsorption of cholesterol, fat and bile acids from the intestinal tract.

Morita *et al.* (1997) reported a significant serum cholesterol reduction in rats fed a rice protein diet as compared with a casein diet. Sunitha *et al.* (1997) observed significant reduction in TC, LDL-C and liver cholesterol in rats fed rice bran oil plus safflower/sunflower oil (in a 70:30 ratio) for 4 weeks. Faecal neutral sterols and bile acids increased with rice bran oil. Rong *et al.* (1997) reported significant reduction in TC, VLDL-C, and LDL-C in hamsters fed 1% oryzanol (non-saponifiable lipid of rice bran oil) in diets containing 5% coconut oil and 0.1% cholesterol for 7 weeks. Aortic foam cell accumulation was significantly reduced (67%) with the oryzanol diet. Kahlon *et al.* (1999) observed significant reduction (49–65%) in foam cell formation in the aortic arch of hamsters with rice bran diets containing 20% fat and 0.5% cholesterol in 6 weeks.

Kahlon *et al.* (1998) reported significant TC, VLDL-C and liver cholesterol reductions with rice bran diets compared with corn bran or wheat bran diets, and extrusion cooking (two energy levels) did not influence the hypocholesterolaemic potential of rice bran. In the same study, extruding wheat bran at low energy levels resulted in significantly lower plasma and liver cholesterol levels compared with those of non-extruded wheat bran, suggesting that

extrusion of wheat bran under the appropriate conditions may enhance its potential to lower cholesterol.

18.3.2 *Studies in humans*

Raghuram *et al.* (1989) reported a significant reduction in TC in 15 and 30 days when 12 hypercholesterolaemic subjects replaced their customary cooking oil with rice bran oil. Kestin *et al.* (1990) observed a significant increase in HDL-C/TC after 4 weeks of feeding 11.8 g dietary fibre from rice bran or oat bran (total non-starch-polysaccharides 21 g/day) to mildly hypercholesterolaemic free-living men. In normocholesterolaemic men, feeding rice bran (15 or 30 g/day) for 3 weeks resulted in non-significant TC reductions (Sanders & Reddy 1992). Hegsted *et al.* (1993) reported that consumption of stabilised rice bran (100 g/day) for two 3-week periods resulted in a 4–10% reduction in TC in moderately hypercholesterolaemic subjects fed diets in which 37% of calories were provided as fat. Qureshi *et al.* (1997) observed significant reduction in TC and LDL-C in hypercholesterolaemic subjects given a tocotrienol-rich extract of rice bran oil in addition to consuming NCEP step 1 diet for 4 weeks. Gerhardt and Gallo (1998) reported significant reductions in TC and LDL-C in moderately hypercholesterolaemic subjects consuming 84 g/day of stabilised rice bran product for 6 weeks.

Rice bran has been shown to have cholesterol-lowering activity in hypercholesterolaemic animals and human subjects. This activity is apparently in the non-saponifiable matter and oil as well as other components of rice bran. Proposed mechanisms of cholesterol-lowering by rice bran include an increased faecal excretion of fat, cholesterol and bile acids. Soluble fibre and viscosity do not appear to play a significant role in the hypocholesterolaemic activity of rice bran.

18.4 Barley fibre and fractions

The results of animal and human studies evaluating barley fibre and fractions are summarised in Table 18.7.

18.4.1 *Studies in animals*

Studies on the hypocholesterolaemic effects of barley are influenced by the type of barley (hull-less or covered with an attached hull), starch (normal or waxy), and cultivar tested, the solubility and content of the β-glucans present, and environmental conditions during growth (Newman *et al.* 1989). Processing and formulating food products with barley may alter the functionality of both the product and the barley – aspects that also must be taken into consideration when evaluating the hypocholesterolaemic effects of barley.

Significant reductions in TC in chickens were reported with rolled Hiproly barley diet (Prentice *et al.* 1982). These effects were possibly related to TC reductions with barley β-glucans as well as inhibition of hepatic cholesterol biosynthesis by barley oil tocotrienols (suppression of HMG-CoA reductase activity). Later work by Qureshi *et al.* (1986) determined that d-α-tocotrienol isolated from high-protein barley flour suppressed cholesterol synthesis and induced TC reductions in chicks. Other studies showed hypocholesterolaemic effects with hull-less, waxy barley types in chicks (Fadel *et al.* 1987; Martinez *et al.* 1991;

Table 18.7 Total plasma cholesterol (TC) reductions with barley and fractions: animal and human studies.

	Barley fraction		Pre-treatment		Reduction (%)		
Species	(diet %)	(g/day)	(days)	TC (mg/dl)	TC	LDL-C	Reference
Chick	73	-	28	-	32*	-	Prentice *et al.* 1982
Chick	65	-	21	-	14*	45*	Fadel *et al.* 1987
Chick	60	-	17	-	45*	-	Martinez *et al.* 1991
Chick	58	-	21	-	38*	60*	Newman *et al.* 1991
Chick	35	-	42	-	31*	59*	Newman *et al.* 1992b
Chick	70	-	8	-	27*	67*	Wang *et al.* 1992
Rat	61	-	10	-	32*	41*	Newman *et al.* 1992b
Rat	53	-	42	-	12*	-	Wang & Klopfenstein 1993
Hamster	14 tdf (6 β-g)	-	21	-	15*	46*	Kahlon *et al.* 1993
Hamster	25	-	28	-	16*	9	Ranhotra *et al.* 1998
	50	-	28	-	20*	24	
Human	-	42 tdf	28	179	3	6	Newman *et al.* 1989b
Human	-	62 bsg, 30 tdf	7	217	10	15	Zhang *et al.* 1990
Human	-	16 tdf	28	240	6*	7*	McIntosh *et al.* 1991
Human	-	100 wbf	28	217	+1	Narain *et al.* 1992	
Human	-	NCEP1+30 bf	30	280	8*	6*	Lupton *et al.* 1994
	-	NCEP1+ 3 bo	30	280	7*	9*	
Human	-	50/50 boiled barley/rice	28	251	10*	12*	Ikegami *et al.* 1996

*Reductions were significantly lower than the corresponding controls or comparison treatments in the respective studies.
tdf, β-g, bsg, wbf, NCEP1, bf and bo = total dietary fibre from barley, β-glucans, brewer's spent grain, whole barley flour, National Cholesterol Education Program-Step 1, barley flour and barley oil, respectively.

Ranhotra *et al.* 1991, 1998; Newman *et al.* 1991, 1992b; Wang *et al.* 1992). In several reports (Fadel *et al.* 1987; Newman *et al.* 1991, 1992b; Wang *et al.* 1992), the barley diets resulted in significant reductions in LDL-C, while in most (Fadel *et al.* 1987; Martinez *et al.* 1991; Ranhotra *et al.* 1991; Newman *et al.* 1991, 1992b; Wang *et al.* 1992) significant elevations in HDL-C were reported. The addition of β-glucanase to a barley diet eliminated the significant TC reduction of the diet (Fadel *et al.* 1987). However, Newman *et al.* (1991) found that even in the presence of supplemental β-glucanase, barley diet was effective in lowering TC, suggesting that β-glucans contribute to only part of the plasma cholesterol-lowering activity of barley.

Liver cholesterol was also significantly reduced in chicks by some barley diets (Martinez *et al.* 1991; Newman *et al.* 1992b). Ranhotra *et al.* (1991) reported significantly lower serum and liver cholesterol in rats with diets containing bran or flour from hull-less waxy barley, compared with meal from the same barley. The reductions were related to the soluble fibre/total dietary fibre ratio in each fraction, the greater reductions occurring with a higher percentage of soluble fibre. Newman *et al.* (1992b) found significant reductions in serum but not in liver cholesterol with hull-less waxy barley diet fed to rats, while Wang and Klopfenstein (1993) observed significantly lower serum and liver cholesterol in rats with raw or extruded barley (with hulls), the extruded barley producing the greatest effects. Total and soluble β-glucan content, along with diet viscosity was suggested as the principal contributing factors. Significant TC reductions have also been reported in hamsters (Kahlon *et al.* 1993)

fed β-glucan-enriched barley diet (14% TDF, 6% β-glucans, 0.25% cholesterol). Liver cholesterol reductions were also significant in hamsters fed β-glucan-enriched barley diets containing 3.3% β-glucans in that study (Kahlon *et al.* 1993). Significant TC reductions were reported (Wang *et al.* 1997) in hamsters fed whole barley and defatted barley diets compared with those fed cellulose control, cellulose with barley oil, barley with soluble fibre removed and barley with fat and soluble fibre removed diets (containing 11% TDF, 9–10% fat and 0.3% cholesterol), while none of these diets significantly affected liver cholesterol values. Ranhotra *et al.* (1998) observed 16.4 and 20.5% reductions in total serum cholesterol in hamsters in 4 weeks with 25% and 50% barley diets, while a 75% barley diet did not result in any further cholesterol reductions. Rieckhoff *et al.* (1999) reported no effect on TC in hamsters fed 13% TDF barley diet for 5 weeks.

18.4.2 *Studies in humans*

Newman *et al.* (1989a) found no significant effect on TC with barley food supplements compared with wheat products in normocholesterolaemic, free-living volunteers; however, there was a marked reduction in TC with barley diet in individuals with higher pre-treatment cholesterol levels. The authors presented a regression model for predicting the effects of barley diets based on pre-treatment cholesterol levels. In a subsequent study (Newman *et al.* 1989b), hypercholesterolaemic subjects consumed products (170 g/day) formulated with weight (not fibre) equivalent amounts of oat or barley (waxy hull-less) flour for 6 weeks; similar cholesterol reductions were observed in both the oat and barley groups, compared with the mean initial TC and LDL-C levels of the individuals. Zhang *et al.* (1990) reported that brewer's spent-barley grain, which is nearly free of β-glucans, significantly lowered LDL-C in ileostomy patients with a low daily excretion of bile acids. McIntosh *et al.* (1991) observed that a daily consumption of 170 g of a barley food (38.4 g total TDF, 25.3 g from barley, of which 8 g was β-glucan) by mildly hypercholesterolaemic men, resulted in a significant reduction in TC and LDL-C and no change in HDL-C in 4 weeks, compared with the control subjects. Narain *et al.* (1992) reported significant elevation in HDL-C in six subjects consuming 100 g whole barley flour daily for 4 weeks. Consumption of 30 g barley bran flour or 3 g of barley oil daily by hypercholesterolaemic subjects following NCEP step 1 diet for 30 days resulted in significant reductions in TC and LDL-C compared with the cellulose control (Lupton *et al.* 1994). Ikegami *et al.* (1996) observed significant reductions in TC and LDL-C over a 4-week period in hypercholesterolaemic subjects who had consumed a 50/50 mixture of boiled barley-rice compared with consumption of boiled rice alone.

The hypocholesterolaemic effects of barley appear to be due to its soluble β-glucans, tocotrienols and other components. Possible mechanisms for cholesterol-lowering by barley are increased faecal fat and bile acid excretion, and inhibition of HMG-CoA reductase and 7α-hydroxylase – the rate-limiting enzymes for cholesterol and bile acid synthesis.

18.5 Conclusions and recommendations

Cereal fibres and fractions (oat, rice and barley) contribute significantly to reducing TC in a variety of animal species and in hypercholesterolaemic subjects. Long-term population studies (Framingham, LRC and Helsinki) have demonstrated that for each 1% reduction in TC, there is a two- to four-fold decrease in atherosclerosis in otherwise healthy individuals. In a

6-year prospective study of US male health professionals diagnosed free of cardiovascular disease, cereal fibre was found to be most strongly associated with a reduced risk of total myocardial infarction (Rimm *et al.* 1996). Increasing the soluble fibre of diets by including oat and barley, in addition to other high-soluble fibre foods, proved efficacious in lowering cholesterol even further in subjects on the NCEP Step 2 diet (Jenkins *et al.* 1993). In normocholesterolaemic subjects, although the effect of cereal dietary fibre on plasma lipids and lipoproteins is small, these changes would have a significant potential for reducing cardiovascular risk for the whole population.

Future research needs include the conducting of more studies to evaluate the ability of rice and barley fractions to prevent hypercholesterolemia in humans; testing combinations of oat, rice and barley fractions for possible synergistic effects on cholesterol metabolism; the development of cultivars which contain enhanced levels of the cholesterol-lowering components of these cereals; investigating the effects of antioxidant activity in these cereal fibres on ameliorating or preventing the development of atherosclerosis; and development of palatable food products incorporating these cereal fibres to increase the availability of healthful cereal-based foods to the consumer. The USDA Food Guide Pyramid recommends 6 to 11 servings of cereal products daily, underscoring the importance of cereals as a necessary, healthful food.

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19 Effects of Psyllium on Serum Cholesterol Levels

Victor L. Fulgoni, III

19.1 Introduction

Psyllium seed or psyllium seed husk has been used for centuries as a digestive aid and laxative. Psyllium has been mentioned in various Ayurvedic texts from India, and was cited in Shen Nung's Pen T'sao from China over 3000 years ago. Commercially available psyllium is from blonde psyllium (*Plantago ovata* [Forsk]), which comes primarily from India. However, in the past, psyllium has been grown in France, Spain and more recently in Australia and the American Southwest. The French psyllium is called black psyllium and comes from *Plantago indica* (L.). Spanish psyllium comes from *Plantago psyllium* (L.). Two other species have also been reported: *Plantago asiatica* (L.) from Japan and *Plantago major* (L.) from Norway (BeMiller 1973).

Differences in polysaccharide content across the Plantago species have only very recently been studied. Psyllium polysaccharides are obtained from the seed husk, and are composed primarily of heteroxylans with 1:4 and 1:3 linkages as the backbone. Short side chains containing arabinose, galactose and rhamnose are also present. Samuleson *et al.* (1999) reported that *Plantago major* does not have rhamnose in the side chain. Whether these structural changes have an impact in physiological function is not known.

Traditional food uses of psyllium have been widely reported, including bread, marmalade, chocolates, confectionery base, ice cream and sherbet (Kellogg 1996). Most of these uses were associated with the ability of psyllium to bind water. More recent uses of psyllium have been in various grain-based foods such as pasta and ready-to-eat cereals.

The polysaccharide structure of commercially available psyllium leads to the compound's unique physiological function, namely a soluble fibre that also has faecal bulking properties. Given the high soluble fibre content of psyllium (approaching 80%), many research teams have examined the effects of psyllium on cholesterol metabolism.

19.2 Cholesterol-lowering effects

Over 60 published studies have examined the effects of psyllium on serum cholesterol levels.

Studies reported before 1989 (Table 19.1) were designed primarily to examine the faecal bulking effects of psyllium, but concomitantly measured blood lipids. These studies were of short duration and utilised small numbers of subjects. The amount of psyllium used in these studies also varied widely. However, there was a strong indication that psyllium might be able to reduce elevated cholesterol levels, with total cholesterol (TC) levels being significantly decreased from between 2.6% and 20%, while reported low-density lipoprotein (LDL)-cholesterol decreases were even greater (data not shown).

Table 19.1 Studies examining effects of psyllium on cholesterol levels: 1965–1988.

Study	Subjects	Amount of psyllium (g/day)	Duration of trial	Cholesterol reduction (%)
Garvin *et al.* 1965	15	9.6	5 weeks	–8
Forman *et al.* 1968	NA	9.6	6 weeks	–17
Lieberthal & Martens 1975	10 HC 9 normal	11.5	5 weeks	–14.4 (HC) –9.8 (normal)
Danielsson *et al.* 1979	13 HC	7.2–10.8	2–29 months (average 8)	–16.9
Capani *et al.* 1980	9 NIDD	21	9 days	–12
Enzi *et al.* 1980	22 obese	6	30 days	–14
Fagerberg 1982	40 NIDD 10.8 for 2 months	3.6–7.2 for 2 months	4 months	–5
Nakamura *et al.* 1982	9	6–12	5 weeks	–14.4
Frati-Munari *et al.* 1983	19 obese 8 NIDD	15	10 days	–12.6 (all) –14.1 (obese) –10.5 (NIDD)
Borgia *et al.* 1983	65	10.5	4 weeks	–2.6
Kies 1983	85	14–20	NA	–8
Abraham & Mehta 1988	7	21	3 weeks	–16
Anderson *et al.* 1988	26	10.2	8 weeks	–14.8

HC, hypercholesterolaemia; NA, not applicable; NIDD, non-insulin-dependent diabetes mellitus.

Studies between 1989 and 1996 (Table 19.2) were specifically designed to evaluate the effects of psyllium on cholesterol levels. The amount of psyllium used ranged from 2 to 30 g/day, but the majority of studies used around 10 g/day of psyllium. The duration of these trials was more consistent, with most studies being 4 weeks or longer. Also, significantly larger numbers of subjects were used in most of the studies. Of particular interest is that several special subgroups were also evaluated, namely the elderly and children. In these better-designed studies, psyllium was found significantly to decrease TC by about 5–8% (again, LDL-cholesterol was decreased slightly more than total cholesterol, by about 9–11%).

More recent publications supported the cholesterol-lowering effects of psyllium. In a meta-analysis of 12 studies (eight published and four unpublished) using psyllium-containing cereals, Olson *et al.* (1997) reported lower TC and LDL-cholesterol of 0.31 mmol/l (5%) and 0.35 mmol/l (9%), respectively. It was noted that the cholesterol-lowering effects of psyllium were independent of gender and age. The studies included in this analysis were randomised controlled experiments that had been conducted in human subjects where a control group consumed cereal with <3 g of soluble fibre each day. All of the studies in this analysis added psyllium cereals to a low-fat diet, and thus the cholesterol-lowering effect of psyllium was over and above that expected with a low-fat diet alone.

Davidson *et al.* (1998) assessed the effects of 0, 3.4, 6.8 and 10.2 g psyllium per day in a 24-week randomised, double-blind controlled study with 286 men and women with moderately elevated cholesterol. Randomisation to treatment groups occurred after following a low-fat diet for 8 weeks. Psyllium at 10.2 g/day significantly lowered LDL-cholesterol (5.3%) as compared with the control group. There were no effects on high-density lipoprotein (HDL)-cholesterol or triglycerides. There was an indication of a psyllium dose–response relationship on LDL-cholesterol levels, but the effect did not reach statistical significance.

Table 19.2 Studies examining effects of psyllium on cholesterol levels: 1988–1996.

Study	Subjects	Amount of psyllium (g/day)	Duration of trial	Cholesterol reduction (%)
Bell *et al.* 1989	75 HC	10.2	8 weeks	–4.8
Taneja *et al.* 1989	11 adolescents	25	3 weeks	–7
Miettinen & Tarpilla 1989	9 HC	30	11 days	–15.7
Lerman-Garber *et al.* 1990	14 HC	10.2	6 weeks	–5.9
Bell *et al.* 1990	58 HC	6	9 days	–12
Neal & Balm 1990	54 HC	20.4	13 weeks	–7.1
Levin *et al.* 1990	58 HC	10	16 weeks	–5.6
Glassman *et al.* 1990	36 HC children	2.5–10	8 months	–14
Anderson *et al.* 1991	105 HC	10.2	8 weeks	–4.3
Stewart *et al.* 1991	175 elderly	9–19	1 month to 1 year	–4.1 (taking 15–17 g/day)
Everson *et al.* 1992	20 HC	15.3	40 days	–3.5
Haskell *et al.* 1992	55 HC	2.1–6.3	4–12 weeks	–3 to –12
Anderson *et al.* 1992	44 HC	11.4	6 weeks	–8.4
Jenkins *et al.* 1993	43 HC	NA	4 months	–4.9
Jensen *et al.* 1993	29 HC	15 (mixture)	4 weeks	–10
Dennison & Levine 1993	20 HC children	6	4–5 weeks	0
Schechtman *et al.* 1993	297 HC	10.4	46 months	–2.8
Sprecher *et al.* 1993	118 HC	10.2	8 weeks	–4.5
Stoy *et al.* 1993	23 HC	15	5–8 weeks	–4.4
Summerbell *et al.* (1994)	37 HC	9.6	6 weeks	–7.3
Gelissen *et al.* (1994)	10 (4 with ileostomies)	9.9	3 weeks	–6.4
Gupta *et al.* (1994)	24 NIDD	7	90 days	–19.7
Roberts *et al.* (1994)	81 HC	12	6 weeks	–3.2
Wolever *et al.* (1994a)	42 HC	6.7	2 weeks	–6.4
Wolever *et al.* (1994b)	18 HC	7.3	2 weeks	–8.4
Williams *et al.* (1995)	50 children	3.2–6.4	12 weeks	–9.6
Davidson *et al.* (1996)	25 HC children	6.4	6 weeks	–5.2

HC, hypercholesterolaemia; NA, not applicable; NIDD, non-insulin-dependent diabetes mellitus.

Thus, in this large, relatively long-term study psyllium was found significantly to reduce LDL-cholesterol levels.

The data provided above convinced the US Food and Drugs Administration to approve a health claim for foods with psyllium, which allows foods and supplements with at least 1.7 g of soluble fibre from psyllium per serving to claim, 'soluble fibre from psyllium, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease'. This was only the second new health claim approved by FDA. While the agency did not find 'reliable data to establish a dose response', it 'did find that in placebo-controlled studies that tested an intake of 10.2 grams (g) of psyllium seed husk per day as part of a diet low in saturated fat and cholesterol, there was consistently significant effects of psyllium husk on blood total- and LDL-cholesterol levels'. The FDA easily found the requisite 'significant scientific agreement' regarding the ability of psyllium to significantly lower elevated cholesterol levels.

Now that food manufacturers can communicate the benefits of psyllium, more foods with psyllium may become available in the near future.

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20 Non-digestible Carbohydrates and Gut Function: Implications for Carcinogenesis

Ian Rowland

20.1 Introduction

There is evidence that the type of carbohydrate in the diet can have marked effects on gut function, particularly on events in the colonic mucosa, possibly influencing colon cancer.

Dietary carbohydrates likely to have the greatest effect on the colon are those that are poorly digested in the small intestine and hence pass intact into the large bowel. There are three main types of such non-digestible carbohydrates:

(1) *Non-starch polysaccharides* (dietary fibre). These encompass a wide range of polysaccharides including soluble and insoluble fibres (e.g. cellulose, hemicellulose, gums) and pectins, and are largely derived from plant cell walls or algae.
(2) *Resistant starch* (RS). Most dietary starch is broken down by amylase in the small intestine, but some starches resist digestion as a consequence of their inaccessibility to amylase (e.g. starch in intact seeds and grains), chemical resistance to the enzyme (e.g. raw potato starch and amylomaize starches such as Hylon VII), or because of retrogradation of the amylose polymer caused by successive heating and cooling (e.g. heated and cooled potatoes, and the processed starch CrystaLean) (Englyst *et al.* 1992).
(3) *Non-digestible oligosaccharides* (NDO). Some low-molecular weight carbohydrates comprising 3–10 sugar moieties, such as stachyose raffinose, fructo-oligosaccharides (FOS) and xylo-oligosaccharides, possess particular glycosidic linkages that are not susceptible to the hydrolytic enzymes in the small bowel, and so pass into the colon (Rumney & Rowland 1995). Inulin, a carbohydrate extracted from chicory, is comprised of a range of NDO with short and longer chain lengths [up to a degree of polymerisation (DP) of 50].

Non-digestible carbohydrates have a wide range of effects on gut physiology and function. This chapter will be confined to effects in the colon rather than the upper gastrointestinal tract, and will consider the implications for colorectal cancer. The effects can be divided into direct effects of the intact carbohydrates in the gut and indirect effects that are a consequence of the fermentation of the carbohydrate by the intestinal microflora, or indeed carbohydrate-induced changes to the composition and activity of the microflora itself.

20.2 Direct effects of non-digestible carbohydrates on gut function

Insoluble fibre such as that in cereal brans has direct effects in the colon by increasing faecal bulk, decreasing transit time, and binding substances such as bile acids and carcinogens. Potentially, these events can have a preventive action on colorectal cancer by, respectively,

decreasing the concentration of carcinogen in the colon, decreasing the time of contact between carcinogens in the faecal stream and the gut mucosa, and decreasing the concentration of water-soluble toxicants and carcinogens in the faeces. Cummings *et al.* (1992) has reported an inverse relationship between stool weight and colon cancer incidence. Direct effects are largely confined to non-starch polysaccharides (NSP), although NDO and RS can have modest effects on faecal bulk and transit time. For example, in human volunteers, FOS increased stool weight by about 20% (Birkbeck 1999). A number of studies in humans with either chemically resistant or retrograded forms of RS have shown an increase in stool wet weight of up to 30% (Phillips *et al.* 1995; Cummings *et al.* 1996; Heijnen *et al.* 1998). The most likely reason for the increase in stool weight associated with NDO and RS was increased bacterial biomass.

20.3 Non-digestible carbohydrates and gut microflora composition

All non-digestible carbohydrates reach the colon relatively intact and are fermented to greater or lesser extents by the complex microflora that resides in the large bowel. The utilization of carbohydrate by the microflora can result in changes to the numbers and types of bacteria, and more importantly to their metabolic activities in terms of the formation of genotoxins, carcinogens and tumour promoters. Resistant starches and NDO appear to be particularly effective in this respect. Both have been shown to increase the proportion of lactic acid-producing organisms (LAB; lactobacilli and bifidobacteria) in the gut, often with concomitant decreases in potentially pathogenic organisms such as clostridia and enterobacteria (Saito *et al.* 1992; Gibson *et al.* 1995; Silvi *et al.* 1999). These changes may be due to a combination of factors, namely: the ability of LAB to utilise and out-compete other gut organisms for the oligosaccharide or starch substrate (Hidaka *et al.* 1986; Wang *et al.* 1999); the ability of LAB to tolerate, better than other gut bacteria, the acidic conditions resulting from carbohydrate fermentation in the gut; and the production by LAB of growth inhibitory factors towards other gut organisms (Gibson & Wang 1994). The significance of such changes for enteric infections has not yet been elucidated.

20.4 Non-digestible carbohydrates and gut bacterial metabolism

The colonic microflora has been shown to possess a wide range of metabolic activities, resulting in the formation of substances with carcinogenic, genotoxic, tumour-promoting and anti-carcinogenic activity. These reactions include the synthesis of *N*-nitroso compounds; the activation of the food carcinogen, 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ) to a highly genotoxic derivative, 7-OHIQ; the release of carcinogens from glucuronide conjugates in the colon; and, the formation of ammonia (a putative tumour promoter) from amino acids (Rowland & Gangolli 1999). In general, species of *Bifidobacterium* and *Lactobacillus* have low activities of these enzyme activities by comparison with other major anaerobes in the colon such as bacteroides, eubacteria and clostridia (Saito *et al.* 1992). This suggests that increasing the proportion of LAB in the gut could modify, beneficially, the levels of toxicant-producing enzymes.

Such effects have been demonstrated in a number of studies in which NDO or RS have been given to humans or rats. For example, in studies in rats, oligosaccharides such as soybean

oligosaccharides, inulin and galacto-oligosaccharides have been shown to decrease the activity of bacterial enzymes and the formation of potential carcinogens in the gut (Rowland & Tanaka 1993; Rowland *et al.* 1998). In a recent EU collaborative programme, we fed groups of human flora-associated rats (germ-free rats inoculated with human faecal organisms) a high-sucrose diet, or diets containing maize (digestible) starch, or a retrograded amylomaize starch, 'CrystaLean'. Metabolic events associated with the gut microflora and thought to be involved in colon carcinogenesis (e.g. β-glucuronidase activity, ammonia concentration and the formation of 7-OHIQ) were highest in the sucrose-fed group, and lowest in the RS-fed rats.

20.5 Non-digestible carbohydrates and short-chain fatty acids

It has been proposed that many of the effects of starch, NSP and NDO on the colon are a consequence of the fermentative activities of the gut microflora. Fermentation of carbohydrates yields short-chain fatty acids (SCFA) – primarily acetic, propionic and butyric acids – which may directly influence the colonic mucosa. Of the SCFA, butyrate has attracted the most attention. It is the principal energy source for colonic mucosa, and influences cell proliferation rates, apoptosis (programmed cell death of damaged cells) and differentiation (Roediger 1996; Cummings 1997). In *in-vitro* studies, butyric acid causes a concentration-dependent slowing of the rate of growth of transformed cells and promotes expression of differentiation markers, leading to a conversion of cells from a neoplastic to non-neoplastic phenotype (Kim *et al.* 1994). Since the various SCFA have very different metabolic fates (e.g. acetate is absorbed and reaches the liver and muscles where it is used as an energy source, whereas butyrate is a preferred energy source of colonocytes and induces cellular differentiation in colon cell lines), the extent of fermentation and pattern of SCFA is likely to be of crucial importance in determining the physiological effects in the gut of a particular carbohydrate.

The amount of SCFA produced and the molar ratios of the three principal acids produced by fermentation, vary considerably depending on the carbohydrate substrate. Studies using *in-vitro* systems (batch cultures with faecal suspensions or continuous culture models of the colonic flora) have shown that the most highly fermentable NSP such as pectin, soy fibre and apple fibre, are fermented mainly to acetate (Edwards & Rowland 1991; Cummings 1995). For example, pectin yields molar ratios of acetate:propionate:butyrate of 81:11:8. In contrast, starch gives a much higher proportion of butyrate – between 20% and 30% of total SCFA (Scheppach *et al.* 1988). Wheat and oat bran also yield relatively large proportions of butyrate (up to 20%), although they are less well fermented overall than starch.

It is likely therefore that any physiological or biochemical effects mediated by butyrate in the gut will be most apparent after the consumption of starch, especially RS.

20.6 Influence of RS and NDO on gut functions related to cancer

20.6.1 *DNA damage in the colon*

The various effects of RS and NDO on gut microflora composition and metabolism, including the stimulation of butyrate formation, are likely mechanisms responsible for the changes in gut mucosal functions related to colon cancer risk seen in laboratory animal studies.

We have recently been studying the ability of NDO and RS to prevent DNA damage in colon mucosa of animals. DNA damage is considered to be an early event in the process of carcinogenesis. In the first of these studies we investigated the synthetic non-digestible sugar lactulose (Rowland *et al.* 1996).

'Human flora-associated' (HFA) rats were transferred to diets in which the sucrose or lactulose level was raised to 3% (w/w). Four weeks later, the rats from each dietary group were dosed orally with saline (controls) or with the colon carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH). At 16 h after the carcinogen dose, the rats were killed, the colon cells isolated, and the degree of DNA damage in the cells was assessed using the single-cell microgel electrophoresis (Comet) assay. Treatment with DMH induced a highly significant increase in DNA damage in both sucrose- and lactulose-fed rats. However, in rats treated with DMH, the degree of DNA damage was significantly less in the lactulose-fed animals than in those given the sucrose diet, as evidenced by a decrease in the percentage of cells with severe damage In the sucrose-fed rats, severely damaged cells accounted for 33% of the total, compared with only 12.6% in the lactulose-fed, DMH-treated animals. The results of this study indicate that lactulose consumption offered a degree of protection from the genotoxic effects in the colonic mucosa of a known colon carcinogen.

An analogous study was conducted in rats fed sucrose, digestible (maize) starch, retrograded RS, or soy fibre. After 4 weeks on the diets, the rats were given DMH and the degree of DNA damage induced in the colon mucosa cells was assessed as before. By comparison with the sucrose-fed rats, the feeding of digestible starch or soy fibre had no effect on the level of DNA damage. In contrast, in the RS-fed rats the level of carcinogen-induced damage was significantly reduced, by 50%. The results indicate that the form of the non-digestible carbohydrate is important in protecting against DNA damage in the colon, since soy fibre – a highly fermentable form of NSP – was ineffective. Whether the differential effects of starch and NSP seen in this study are a consequence of the fermentation patterns of the two carbohydrates (predominantly acetate for soy fibre, elevated butyrate for RS) remains to be elucidated.

20.6.2 *Prevention of pre-neoplastic lesions in the colon*

Aberrant crypts (AC) are putative pre-neoplastic lesions seen in the colon of carcinogen-treated rodents. In many cases, a focus of two or more crypts is seen and is termed an aberrant crypt focus (ACF). Aberrant crypts are induced in the colonic mucosa of rats and mice by treatment with various colon carcinogens such as azoxymethane (AOM), DMH and IQ. The findings of significantly more ACF with four or more crypts in rats with tumours, compared with those without tumours, suggests that large ACF may be a predictor of eventual tumour incidence (Pretlow *et al.* 1992).

Reddy *et al.* (1997) compared short- (FOS) and long-chain (inulin) oligosaccharides incorporated at a level of 10% in the diet on AOM-induced ACF in rats. The NDO were fed before carcinogen treatment and throughout the experiment, and significant decreases of approximately 25% and 35% respectively in total ACF were reported. The decreases seen were almost entirely in the smaller ACF (fewer than three AC per focus), and inhibition by inulin appeared to be more pronounced than that of FOS.

Similar results were obtained by Rowland *et al.* (1998), who reported a decrease of 41% in small ACF when inulin (5% in diet) was given 1 week after an AOM dose. No effect of inulin on large ACF was observed. Challa *et al.* (1997) demonstrated a small reduction (22%)

in total ACF in AOM-treated rats when the synthetic, non-digestible disaccharide lactulose was incorporated in the diet at 2%.

Both Challa *et al.* (1997) and Rowland *et al.* (1998) studied the effect of combined treatment with lactic acid bacteria and NDO on ACF numbers, and from these data it seems likely that the effects of the NDO on ACF are related to an increase in LAB in the colon. The combination of *Bifidobacterium longum* and lactulose resulted in a 48% inhibition of colonic ACF, which was significantly greater than that achieved by either *B. longum* or lactulose alone (Challa *et al.* 1997). Combined administration of *Bifidobacterium longum* and inulin resulted in more potent inhibition of ACF than administration of the two separately, achieving 80% inhibition of small ACF. Perhaps more importantly, the combined administration significantly decreased the incidence (by 59%) of large ACF.

20.7 Conclusions

Non-digestible carbohydrates have diverse effects on gut physiology and function that reflect their diverse physicochemical properties. Many of the effects of carbohydrates in the gut are a consequence of interaction with the complex microflora of the colon, and result from changes in bacterial metabolism or fermentation products, particularly short-chain fatty acids.

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21 Cereal Fibre and Heart Disease

Victor L. Fulgoni, III and Sharon Mueller

21.1 Introduction

It is now almost 30 years since Trowell (1972) first wrote about the lack of dietary fibre in Western diets, and the development of heart disease. There currently exists a large body of literature demonstrating the cholesterol-lowering properties of dietary fibre – and particularly of certain soluble fibres. Cholesterol-lowering is used as an indicator of the development of heart disease in most of the randomised controlled trials. The Lipid Research Clinics (LRC 1984) data indicates that in a hyperlipidaemic population, each 1% reduction in total blood cholesterol results in a 2% decrease in cardiac mortality. Thus, most clinical trials use cholesterol levels as an end-point.

21.2 Soluble fibre

Studies that have been conducted to examine the cholesterol-lowering effect of soluble fibre have used many different sources of soluble fibre, ranging from legumes and pectins to oats and psyllium. In January of 1997, the Food and Drug Administration (FDA) in the United States authorised the use of a health claim stating that in conjunction with a low-saturated fat, low-cholesterol diet, the consumption of oats reduced the risk for coronary heart disease (Department of Health and Human Services 1997). Shortly after the approval of the oat health claim the FDA decided to amend the health claim to include psyllium (Department of Health and Human Services 1998). The approval of both health claims were based on the existing scientific evidence and the scientific congruency of the relationship between these soluble fibres and their ability to lower cholesterol and thus reduce the risk of heart disease.

Recently, Anderson *et al*. (2000) published a meta-analysis of eight controlled trials that used psyllium to lower serum cholesterol in hyperlipidaemics. In all of the studies used in the analysis the psyllium was compared with an insoluble fibre placebo, and the psyllium was given at a level of 10.2 g/day over an 8-week period. The consumption of psyllium significantly lowered low-density lipoprotein (LDL)-cholesterol (by 7%), and the apoloipoprotein B (apo B):apo A-1 ratio (by 6%), compared with control subjects.

An earlier psyllium meta-analysis by Olson *et al.* (1997) demonstrated similar results. In the Olson *et al.* (1997) analysis, seven published and four unpublished studies were included. All of the studies that were included, examined the effects of ready-to-eat cereals that contained psyllium on serum lipids. The results indicated that subjects who consumed psyllium-enriched cereals had lower total and LDL-cholesterol levels (5% and 9%, respectively), when compared with subjects consuming a wheat-based control cereal.

Brown *et al*. (1999) conducted a meta-analysis on 67 controlled trials that examined different viscous fibres in their cholesterol-lowering effects. The studies included in this meta-analysis used oat products, psyllium, pectin or guar fibre, and had intervention periods that were greater than 14 days. The results of this meta-analysis were concordant with the aforementioned meta-analysis studies. Brown *et al*. (1999), found that high intakes of soluble fibre were able to significantly lower total and LDL-cholesterol. However, the authors point out that, in practical terms, the actual cholesterol-lowering effect might be quite modest. To consume 3 g of soluble fibre, an individual would need to consume three (28-g) bowls of oatmeal, which would subsequently lower serum cholesterol by ~0.129 mmol/l (5 mg/dl) or ~2% (Brown *et al*. 1999).

A meta-analysis (Ripsin *et al*. 1992) has also been conducted to determine the lipid-lowering effects of oat products. Ten clinical trials were used in the analysis. In the studies that were included, a wheat bran control was used, and there was a –0.11 mmol/l (–4.4 mg/dl) reduction in serum cholesterol which, based on the Keys scores, was not due to the displacement of fat by carbohydrate in the diet (Ripsin *et al*. 1992). The authors noted that subjects who initially had higher serum cholesterol levels (>5.9 mmol/l; 229 mg/dl), showed greater cholesterol reductions. Poulter *et al*. (1994) conducted a study on the lipid-lowering effect of oat bran. The 64 volunteers had cholesterol levels of about 6 mmol/l. Small but significant reductions in total and LDL-cholesterol were found (2.23% and 4.55%, respectively).

21.3 Epidemiological evidence

Although numerous clinical studies have examined the cholesterol-lowering properties of different types of soluble fibre, the epidemiological data are comparatively scant. One of the first prospective studies to examine the relationship between dietary fibre intake and coronary heart disease (CHD) was the Ireland-Boston Diet-Heart Study (Kushi *et al*. 1985). A diet-history recorded by a dietician was coded onto a food frequency form. After 20 years of follow-up, these researchers showed there to be an inverse association between dietary fibre intake and risk of death from CHD. Men in the upper third of dietary fibre intake had a significantly lower risk than those in the lower third (RR, 0.57; 95% CI, 0.33–0.97) (Kushi *et al*. 1985).

This relationship was also examined in the Zutphen Study (Kromhout *et al*. 1982), where men in the lowest quintile of dietary fibre intake had the highest mortality from CHD.

Rimm *et al*. (1996), in a prospective analysis, examined the relationship between dietary fibre and the risk of CHD as part of the Health Professionals Follow-up Study. Health professionals (43 757 US men) who were free from diagnosed cardiovascular disease, completed a 131-item dietary questionnaire which was used to assess their intake of dietary fibre and food sources of fibre. After 6 years of follow-up there were 734 cases of myocardial infarction (MI). Fibre intake was found to be inversely associated with risk for cardiovascular disease. Among the three main fibre sources (fruit, vegetable or cereal fibre), cereal fibre had the greatest association with decreased risk of MI (RR, 0.71; 95% CI, 0.55–0.91 for each 10 g/day increase in cereal fibre intake) (Rimm *et al*. 1996). Interestingly, insoluble fibre was the main cereal fibre consumed.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al*. 1996) was similar in many ways to the Health Professionals Follow-up Study. Men who were free of diagnosed cardiovascular disease (21 930 subjects) completed a validated dietary question-

naire and were followed for 6.1 years. The end-points of the study were MI and CHD. For men in the highest quintile of dietary fibre intake (median, 34.8 g/day), the relative risk of death due to CHD was significantly lower than for men with the lowest quintile of fibre intake (median, 16.1 g/day). Soluble fibre had a stronger association with reduced CHD-related death than did insoluble fibre, and cereal fibre had a stronger association than fruit or vegetable fibre (Pietinen *et al*. 1996).

With regard to epidemiological evidence on women, as part of the Nurses' Health Study, Liu *et al*. (1999) evaluated whether the intake of whole-grain products lowered the risk of CHD in women. In 1984, subjects completed a semi-quantitative food frequency questionnaire (SFFQ), and this was followed-up over a period of 10 years. The subjects completed further SFFQs in 1986 and 1990, and a strong inverse relationship between whole-grain intake and CHD risk was found. This relationship held firm even when lifestyle factors (women with the highest grain consumption tended to exercise more, and smoke less) were accounted for in the analysis. Liu *et al*. (1999) suggest that it would be unlikely that the CHD protection comes from only one source of grain. Rather, the protective effects were derived from the consumption of whole-grain breakfast cereals, brown rice and bran sources of insoluble fibre.

In another study using data from the Nurses' Health Study, Wolk *et al*. (1999) collected dietary data using the aforementioned SFFQ. Of the 68 782 women for whom follow-up studies were performed over 10 years, there were 591 major CHD events. As was seen in the male population in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial, the women in the highest quintile of dietary fibre intake (median, 22.9 g/day) had significantly lower risk for CHD than women in the lowest quintile (median, 11.5 g/day). Among the different sources of dietary fibre (fruit, vegetable, cereal), only cereal fibre was strongly associated with a reduced risk of CHD (multivariate RR, 0.63; 95% CI, 0.49–0.81, for each 5 g/day increase in cereal fibre intake) (Wolk *et al*. 1999).

Using data from the Iowa Women's Health Study, Jacobs *et al*. (1998), studied the effect of whole-grain intake on 34 492 postmenopausal women aged 55–69 years. Dietary intake was determined by a 127-item food frequency questionnaire. There was an inverse association between whole-grain intake and risk of ischaemic heart disease (IHD) -related death.

Kushi *et al*. (1999) recently reviewed evidence from epidemiological studies with regard to cereals, legumes and the reduction of chronic heart disease risk. The authors found that the prospective cohorts examined provided consistent evidence of the inverse association between dietary fibre intake and the development of heart disease.

21.4 Conclusions

There is considerable epidemiological evidence of a correlation between the consumption of dietary fibre and a decrease in the risk of cardiovascular mortality. There is even an indication of the increased benefit of the consumption of cereal fibre usually associated with insoluble fibre consumption (Pietinen *et al*. 1996; Wolk *et al*. 1999). Anderson and Hanna (1999) suggest that insoluble fibre provides protection against CHD due to the associated nutrients, and micronutrients that are found in these foods. In contrast, Jenkins *et al*. (1999), concluded that the protective effect of wheat bran was not due to cholesterol-lowering. Thus, the question of discerning the active components in insoluble fibre needs to be studied further.

To date, the FDA has approved three health claims for specific substances (oats, psyllium and soy) with regard to their cholesterol-lowering ability in conjunction with diet that is low in saturated fat and cholesterol. This reinforces the fact that an abundance of data exists showing the cholesterol-lowering properties of specific soluble fibres.

Jenkins *et al*. (2000) examined the effect of a soluble fibre, soy-enhanced diet on the levels of serum lipids when compared to a diet with low-fat dairy foods but low insoluble fibre. The test diet reduced the total cholesterol:high-density lipoprotein (HDL)-cholesterol ratio, and was found to further reduce the risk of CHD.

At this juncture more studies need to be conducted to examine the effect of combining these soluble fibres and to see how, ultimately, they effect serum lipid levels.

We must begin to determine the effectiveness of combinations of fibre with other cholesterol-lowering materials. After all, this is how we obtain such materials in our diet – in a combined form. Nonetheless, several other questions remain unanswered: How do other means of lowering cholesterol (beta-sitosterol, other sterols, stanols, niacin or even drugs) function with soluble fibre?… with soy protein? What is it in insoluble fibre that provides protection against CHD, and can it be isolated and combined with known cholesterol-lowering agents? Does taking β-glucan in conjunction with psyllium enhance the cholesterol-lowering ability of each soluble fibre individually? Although dietary fibre has been studied for many decades, it should be realised that we are now at a critical stage in the research that could lead to previously unthought of reductions in serum lipids, and thus to important public health benefits.

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Part 6

Technological Aspects

22 Developing Fibre-rich Foods in the Twenty-first Century

Anton J. Alldrick

22.1 Why develop fibre-rich foods?

The need to develop new dietary fibre (DF)-rich foods must be seen within the context of changes in health and prosperity experienced in the twentieth century, and which are expected to continue into the new millennium. These changes have been – and will continue to be – occurring at an increasing rate.

In terms of public health, the Western world has seen an increase in life expectancy at birth. This has been accompanied by an increasing proportion of the population that is over the age of 65 years. Increased life expectancy and a much reduced population growth has led to the prediction of the 'Demographic Timebomb.' An example of this is the prediction that, by the year 2031, the number of people older than 65 years in the UK is expected to rise from about 12 million in 1996 (20% of the population) to about 18 million, or 30% of the population (Anon. 1998a). Simultaneously, advances in public health and medicine have led to changes in disease patterns and causes of death. Using the United Kingdom as an example (population 59 million approx.), coronary heart disease, stroke and cancers now account for more than 60% of all deaths, and the cost of their treatment represents over 20% of the UK National Health Service budget (HMSO 1990). While death is one of the few certainties of the human condition, circulatory diseases and cancer are now significant causes of premature death (considered to be death before the age of 65 years). Reducing the incidence of these diseases forms a significant part of public health campaigns not only in the UK, but also in most of the Western world.

From an epidemiological point of view, lifestyle is a significant contributor to the incidence of cardiovascular disease and stroke, as well as a number of cancers. One aspect of lifestyle attracting increasing attention is the role played by diet and its components. It is not the purpose of this chapter to review the health benefits of DF. Suffice to say, there is now enough evidence to suggest that consumption of sufficient DF is linked to improved control of digestion and general gut function. In addition, DF – particularly insoluble DF – contributes to improved colon health, and reduced risk of chronic diseases of the colon, while consumption of sufficient soluble DF can lead to a reduction in blood cholesterol concentration – a known risk-indicator of cardiovascular disease. A major problem is that in a number of countries, including the UK, DF consumption has declined historically and is for many people considered to be insufficient (HMSO 1991).

In order to address this problem, it is important to realise that another feature of many Western cultures is the development of a libertarian, consumerist and often-affluent society. Within these societies, patterns of consumption for all products are in a constant state of flux. Nowhere is this more true than in the case of what the public eats. Again using the United Kingdom as an example, while disposable income rose by about 8.3% in real terms in the period 1993–1997 inclusive, expenditure on food only rose in real terms by 6.4% (Griffiths

1999). Historically the proportion of disposable income spent on food for consumption in the home is in decline, from around 30% in 1940 to 8.2% in 1998 (McClean 1991; Griffiths 1999). These social changes have also influenced what the consumer eats. For example, bread consumption in the UK has dropped by over one-half during the second half of the twentieth century, while breakfast cereal consumption has tripled (Griffiths 1999). These constraints, plus the increasingly discriminatory nature of the consumer, have led the food industry to become more innovative. The CCFRA Product Intelligence Team estimates that over 7000 new food products (excluding alcoholic drinks and confectionery) are launched through major retail outlets every year in the UK alone (Anon. 1999b).

Given that, in public health terms, a large part of the population is consuming less DF than is recommended, there is a need to either market existing products more effectively, or to develop new ones that are more acceptable to the public. This chapter seeks to highlight key factors, which need to be considered in the development of foods that will assist the public in reaching the higher DF-consumption values recommended. It is therefore a reasonable hypothesis to consider that these foods will be consumed as a consequence of a conscious choice to either maintain or increase the amount of DF eaten. With an energy value somewhere between 0 and 2 kcal per gram (Anon. 1990), DF itself has little nutritional value in classical terms. The importance of DF lies in its other physiological effects. Foods consumed for their DF content can therefore be regarded as functional foods, i.e.

> 'Processed foods containing ingredients that aid specific bodily functions in addition to being nutritious.' (Ichikawa 1994).

Functional foods provide their own unique challenges with regard to new product development and others, which overlap with the usual commercial considerations. These aspects have been more fully discussed elsewhere (Alldrick 1998) and only those aspects directly relating to DF-rich foods will be considered here. For the purposes of this chapter, the commonly held definition of DF (effectively indigestible polysaccharides and lignin) will be expanded to include oligosaccharides such as the fructan, inulin.

22.2 Key considerations in developing DF-rich foods

22.2.1 *General principles*

Product design and development should be undertaken in accordance with a defined philosophy, e.g. as described in Section 4.4 of ISO Norm 9001:1994 (Anon. 1994a). The development of novel DF-rich foods will be further assisted by the application of some of the principles first developed for the addition of essential nutrients to food published by the Codex Alimentarius Commission (Anon. 1994b). An adaptation of those principles relevant to product design and as they apply to novel DF-rich foods is given in Table 22.1.

These principles have to be applied in particular to the: customer base; physiological properties of the DF; product type; technological constraints and; how the end product will be sold. They are to a large degree inter-related, and have to be considered jointly and severally throughout the development process.

Table 22.1 Specific Considerations in The Design and Marketing of New Dietary Fibre (DF) – Rich Foods (Based on Codex Alimentarius Commission 'General Principles for The Addition of Essential Nutrients to Foods,' 1994).*

- The contribution of the new product to the consumer's total intake of DF should be consistent with good dietary practices, i.e. it neither leads to an excessive nor reduced overall consumption (principle 2).
- Consumption of the product does not adversely affect the individual's metabolism of any nutrient (principle 3).
- The DF component is stable under the normal conditions associated with similar products and is biologically active (principles 4 and 5).
- Incorporation of DF does not lead to undesirable product characteristics, including changes to organoleptic, cooking and shelf-life properties (principle 6).
- Technological and processing facilities should be compatible with ensuring that DF is incorporated in a satisfactory manner (principle 7).
- Marketing of DF-rich foods should be done in a manner which does not mislead or deceive the consumer as to the relative nutritional merit of the product (principle 8), and the nature of the product and its marketing should conform to local regulations.

* Numbers in parentheses refer to the original section numbers in the Commission's Paper.

22.2.2 *Customer base and physiological properties*

Although self-evident, it is worthwhile to remember that in order to sell a new DF-rich product successfully, appropriate motivators must be identified which will persuade the public to buy it. In developing a new DF-rich product therefore, clear understandings of not only the customer base, but also the physiological properties of DF are needed. Given the interaction between the two, these factors will be considered together.

Consumption of different types of DF is known to lead to different physiological consequences. The first question in the product design process is therefore, what motivator to select, either a generic (simply high in DF) or a specific (high in a particular type of DF which elicits a certain physiological response). Examples of the latter could include:

- the prebiotic effects of oligosaccharides such as inulin and the elaboration of a more beneficial colonic microflora, leading to a healthier bowel;
- improved bowel function and health associated with insoluble DFs such as those found in wheat bran;
- reduced blood cholesterol concentrations associated with soluble DFs, such as those from oats or psyllium.

In summary, there are two principal motivating factors to persuade customers to eat new (or existing) DF-rich foods, a desire to maintain an overall intake of DF and/or, by being part of a well-managed diet, to assist in maintaining or achieving an improved state of health. Identifying the appropriate motivating factor will depend principally on the type of product to be sold, its target market – and in turn, the nature of the DF compatible with the objective.

Having identified an appropriate market opportunity, it is necessary to develop a product that is physiologically functional. This translates into a product, which contains enough DF to satisfy local regulations for making a labelling and/or advertising claim and (where applicable), elicits the desired physiological effect in the majority of the target population. Where the motivator is simply to assist the consumer's intake of DF, appropriate analytical data will have to be provided. In the case of making a claim with regard to a particular physiological

effect, this will have to be substantiated additionally by appropriate and credible research. Such research would almost certainly have to be of a clinical nature. These can include clinical trials with the active ingredient, the product and/or epidemiological studies. Examples of the type of study required and needed to substantiate advertising or marketing claims exist in the literature (Bell *et al.* 1990; Ripsin *et al.* 1992; Roberts *et al.* 1994). Failure to perform these studies can at best lead to embarrassment and withdrawal of the product from the market, and at worse to confrontation with enforcement bodies followed by prosecution and adverse publicity.

Enthusiasm for the beneficial effects of DF must also be tempered with the realisation that high intakes are also associated with potential side effects. Two in particular that have to be considered are:

- the microbial fermentation of DF in the colon, leading to personal discomfort and/or social embarrassment;
- textural and other attributes that make consumption difficult by certain groups (e.g. the elderly).

There is both apocryphal and clinical evidence indicating that for a large proportion of the population, increased DF consumption can be accompanied by increased risk of unpleasant side effects, including gut distension, flatulence and loose stools (Bolin & Stanton 1998). Formulating DF-rich products must take into account these potential side effects. Even mild symptoms, which might be considered to be acceptable by experimental scientists, will probably not be by members of the general public.

A second concern is the suitability of the product for the elderly. As discussed in the introduction, the twenty-first century will see a substantial increase in the proportion of elderly persons (currently defined as people aged greater than 65 years). Developing acceptable products will more and more have to take into account the specific nutritional and other physiological demands of this group. DF is known to have satiating effects and certain types of DF, e.g. of cereal origin, are known to contain components capable of chelating essential minerals (e.g. phytate). This raises the possibility that, unless done in an informed manner, increasing DF intake has the potential of contributing to micronutrient deficiency. A recent survey (Finch *et al.* 1998) on the nutrition of the elderly in the UK showed that average intakes of DF were lower than those recommended. It also showed that a significant proportion of those studied, were clinically anaemic and had low iron stores. Current UK guidelines (Anon. 1991) recommend that the elderly should exercise a degree of caution in the consumption of phytate-rich sources of DF. A further factor to consider is the overall weakening in oral health that accompanies the ageing process. Traditional foods, rich in DF tend to be hard-textured and often difficult to chew for persons with below-average masticatory performance (Laurin *et al.* 1994). Since ease of chewing is a factor determining food choice in many of the elderly, traditional DF-rich foods tend to be avoided by them. The development of new DF-rich foods should therefore be performed in a holistic manner. It should consider both organoleptic and nutritional aspects and address, where necessary, the question of vitamin and mineral supplementation (see also Table 22.1).

22.2.3 *Product type and technological constraints*

It has been claimed that the ancient Greeks recognised the benefits of a high DF diet. One quotation from Hippocrates, the fourth century BC Greek physician translates as:

> 'To the human body it makes a great difference whether the bread be made of fine flour or coarse, whether of wheat with bran or wheat without bran' (Anon. 1990).

Modern interest in these foods began in the nineteenth century with the views of such as Graham and Kellogg in the US, and Allinson in the UK. Until very recently, most DF-enriched foods were farinaceous, often based on wholemeal flours, addition of bran fractions or whole grains. The chemical diversity of dietary DF (ranging from plant-derived oligo- and polysaccharides through to microbial exudates and to amylase-resistant starch) and their consequent different physical-chemical properties has led to new DF-enriched products being marketed. Some of these challenge established perceptions of what is, or is not considered to be, a good source of DF. Such products include soft drinks and dairy products, where DF has been added to facilitate a health claim (Mellentin 1999; Wouters 1999).

In developing new high-DF products the most important question is that of functionality. In reality, this is actually two questions: physiological, assuring that both the raw material and the finished product achieve the desired end-point beneficial to health; and technological functionality, assuring that addition of dietary DF does not compromise the organoleptic qualities of the finished product. There is a potential two-way interaction between how food technology interacts with DF, leading to changes in the physiological properties of the DF, and the interaction between DF and the technology leading to changes in the properties of the product and means of production.

22.2.4 *Physiological functionality*

It has been known for some considerable time that thermal and mechanical processing of raw materials leads to changes in the physicochemical properties of DF. For example, work by Ralet *et al.* (1990) demonstrated that extrusion of wheat bran (rich in insoluble DF, low in soluble DF) leads to the conversion of insoluble to soluble DF, the degree of conversion being dependent on the mechanical energy supplied. Process-linked solubilisation of cereal dietary DF has had a major impact on the way that cereals are incorporated into animal-feeds. Soluble fibre is generally considered to be an antinutrient in broiler chickens (Annison 1991). Subsequent studies by Sundberg *et al.* (1995) demonstrated that addition of appropriate enzymes to the feed (e.g. β-glucanases in the case of barley) overcame these effects. While this might be considered an extreme example (the average consumer does not normally consume a single type of pelleted feed containing in excess of 60% coarsely milled grain), it does highlight the need to be aware that the effects of processing on the physicochemical structure of the DF-containing raw-material can have serious effects on the desired physiological effects in the consumer.

Some of the more recently discovered beneficial properties of DF appear to be particularly related to their physicochemical properties. Thus the blood cholesterol-lowering effects associated with consumption of dietary soluble DF are dependent on its viscoelastic properties. The significance of this can be seen in work studying the effect of drinking fruit juices containing soluble DF fractions with different gel-forming properties (Davidson *et al*. 1998).

Volunteers drinking fruit juice preparations with low viscosities, containing soluble dietary DF and which had poor gel-forming properties did not experience the hypocholesterolaemic effects normally associated with consuming an equivalent amount of soluble DF with good gel-forming properties (leading to a juice with an increased viscosity). Ingredient origin and processing are therefore two factors that need to be considered and controlled when developing a novel, DF-rich, functional food.

22.2.5 *Technological functionality*

DF-rich foods can be divided into a number of types: (1) those whose basic ingredients inevitably lead to a product with a high DF content, e.g. certain breakfast cereals containing whole or partially processed grain, muesli bars and wholemeal bread; and (2) those enriched with DF – effectively high DF analogues. Manufacturing high-DF products brings its own challenges both in relation to adapting technology and maintaining desired organoleptic properties. These challenges reflect the need to supply a minimum amount of DF in the product and relate mainly to the physicochemical properties of the DF itself, or to its diluent effects.

The first challenge to be faced is the need to provide a product that will contain sufficient DF to achieve a physiological effect when consumed in reasonable quantities. Guidelines and regulations for the exact amount of DF required vary from country to country. For example, the United Kingdom Government guidelines (Anon. 1999c) acknowledge three different categories of claim for DF in foods. Similarly, in order to make claims compatible with the requirements of United States Regulations (Code of Federal Regulations) regarding the potential benefits soluble DF in the form of either oat or psyllium DF, the product must contain specified amounts of DF or DF-containing material (Anon. 1999d).

Some of the potential difficulties facing anyone in product development producing a functional DF-rich food are encapsulated in the example of the experimental study referred to previously using high-DF, low-viscosity fruit juice preparations (Davidson *et al.* 1998). Typically, fruit juices are perceived by the consumer to have low viscosities. The challenge therefore, is not only to provide a drink with a (physiologically) functional DF content, but also one that is acceptable to the consumer (low viscosity). It must therefore be recognised that an initial product concept may lead to inherent contradictions between a desired physiological end-point and a commercially acceptable product. In developing a new functional DF-rich product, the new product development technologist must therefore, reconcile the physicochemical attributes associated with the desired physiological effects effected by the particular DF, with those attributes having an effect on the end-product's final characteristics. Thus, producing a fruit drink with a high DF content capable of contributing to a lowered blood cholesterol concentration (potentially more viscous than the traditional product) may provide far more commercial challenges than, for example, a fruit drink containing a DF with a proven prebiotic activity and low viscosity.

A second challenge is to ensure that the recipe is compatible with existing technology. An example of the considerations that need to be employed with regard to reconciling technology, ingredients and desired product is that of wholemeal (100% extraction rate, flour) bread, baked in plant bakeries using 'no-time' dough-making techniques (e.g. Chorleywood Bread Making Process). Studies performed at the author's institute (Collins & Hook 1991) have demonstrated that production of a loaf with those characteristics preferred by the consumer is dependent on a number of parameters. Particle size distribution within the flour was impor-

tant not only for baking quality but also to the overall appearance of the crust and crumb. Addition of dried gluten protein was found to be a suitable method for improving the hedonic parameters including loaf volume and crumb-score.

Despite its ability to supply substantial amounts of DF, the market share of bread baked with wholemeal flour (100% extraction) tends to be smaller than that of bread baked with white flour that has a lower DF content. Consequently, there has been much attention on development of white or speciality breads with elevated DF contents. In the case of bread, the effects of the different DF fractions on technological parameters depend on the DF-source itself. Thus in one study (Pomeranz *et al. 19*77) where up to 15% of flour was replaced with DF, wheat bran and cellulose were observed to increase dough water absorption, while oat bran decreased it. Similarly, dough mixing times were unaffected by the addition of wheat bran, but increased by the addition of either oat bran or cellulose.

Of particular interest has been the relatively recent development of dietary DF preparations that are highly purified, colourless and tasteless. These preparations can be added in quantities that are high enough to make a significant dietary contribution, but low enough to ensure a product which does not challenge manufacturing technology and the product characteristics preferred by the consumer. Initial concepts originally addressed the question of insoluble DF in bread (Anon. 1986). More recently, attention has focussed on the use of other sources of DF, in bread, including soluble DFs such as those derived from oat (Gormely & Morrisey 1993) and oligosaccharides such as inulin (Silva 1997). The relative ease with which these purified DF preparations can be used has led to them being exploited in other goods, including flour confectionery (Anon. 1998b) and breakfast cereals (Anon. 1995).

22.3 Selling the product

Given the expanding spectrum of health benefits associated with DF, DF-rich foods will increasingly be considered to fall in the category of functional foods. An analysis (Young 1999) of the European situation in 1997, valued the functional foods market at US$1.24 billion. In terms of market share, the largest was held by dairy products (65%), followed by spreads (23%), bakery and cereal products (9%) and drinks (3%). In the final analysis, the only successful product is one that contributes to the profits of the producing company. Thus, no product is better than the marketing and sales programme that supports it, and the question of the methods by which the customer is to be both made aware of – and persuaded to purchase – the product has to be addressed. Advertising and labelling of these products must be in compliance with local legislation. Generally speaking, individual foods for general public consumption cannot be considered to be medicines, i.e. in themselves they do not alleviate, prevent or cure a disease. Product advertisements should be 'legal, decent, honest and truthful' (Anon. 1999a)

Being considered as a functional food will provide mixed blessings for DF-rich foods. Functional foods attract considerable interest from consumer lobby groups. In the United Kingdom alone, the UK Advertising Standards Authority upheld 21 complaints against functional food products during the period 1994–1997 (Winkler 1998). Enthusiasm for DF-rich foods should be tempered with a sound marketing policy. Even with such a policy, novel DF-rich foods are no guarantee to commercial success, as evidenced by the withdrawal of a range of psyllium fibre-enriched products following unsuccessful pilot sales trials during 1999 (Buss 2000).

22.4 Conclusions

Changes in demographics and public health suggest an increasing role for dietary management in the betterment of the individual health. DF is one dietary factor that can contribute to this improvement. Achieving an increased public consumption of DF will require the development of new DF-rich foods. These will either be enriched variants of existing foods, or totally new ones. Most importantly, any claims made for such products will have to be capable of clinical substantiation.

In undertaking product design, one must bear in mind dietary, organoleptic, technological and commercial considerations. Examples of these considerations include:

- The potential diluting and satiating effects of DF, which may lead to the overall reduced intake of essential nutrients in the diet. It might therefore be necessary to fortify the product with additional vitamins and minerals (this might also be considered to be a commercial/marketing concern).
- Production of DF-rich analogues of existing products leading to changes in organoleptic properties (e.g. increased 'chewiness' and reduced loaf volumes).
- Processing itself can change the physicochemical attributes of DF and also be affected by DF leading to changes in performance. New DF-rich products should therefore, not only be compatible with existing technology and customer aspirations but should also remain physiologically functional.
- Care needs to be taken as to how the product is marketed and sold, identifying the appropriate motivators.

With the exception of the last point, these considerations can be achieved within an informed product development strategy, incorporating principles enunciated in the ISO norm 9001 and Codex Alimentarius guidelines. To some degree, these can also meet commercial considerations. However, until the product is launched, the overall success of the product will always be – to one degree or another – a matter of chance.

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23 Technological Aspects of Dietary Fibre

Friedrich Meuser

23.1 Introduction

At first sight, the subject of the technological aspects of the occurrence of dietary fibre in food and its use in food production appears to be clearly defined. Consequently, it should be necessary only to decide which aspects to consider, how to select them from the host of sensory characteristics of a wide variety of foods, and to distinguish between them in order for a clear picture of those characteristics of dietary fibre that are of relevance in food production to emerge. However, as both the definition of the term 'dietary fibre' and its analytical characterisation are still in a state of flux, the manner in which this affects the subject to be dealt with in this chapter must be considered.

23.1.1 *Nutritional versus technological aspects*

Today, it is generally accepted that dietary fibre is taken to mean remnants of the edible parts of plants that are neither degraded nor absorbed in the human small intestine (Meuser & Suckow 1982; Wisker *et al.* 1985; GDCh 1989; Jones 2000). The same applies to analogous carbohydrate-based food ingredients. Polysaccharides, oligosaccharides, lignin and associated plant substances are therefore classified as dietary fibre (Table 23.1). These substances are either partially or wholly fermented when they reach the large intestine. The nutritional effects of dietary fibre, which are listed together with the technological characteristics of dietary fibre products in Table 23.2, derive from the indigestibility and fermentation of these substances. The characterisation of dietary fibre must be seen in connection with its analyti-

Table 23.1 Selected constituents of the remnants and carbohydrates of the edible part of plants resistant to human digestion.

Constituent of the	
Remnants	Carbohydrates
Cellulose	Araban
Pectins	Arabinogalactan
Hemicelluloses	Arabinoxylan
Inulin	Fructan
Resistant starches	β-glucan
Lignin	Glucuronan
Tannins	Mannan
Waxy substances	Xylan

Table 23.2 Physiological effects and technological properties of dietary fibre products.

Physiological effects	Technological properties
Laxation	Water-binding capacity
Faecal bulking and softening	Water-swelling capacity
Frequency	Water-retention capacity
Regularity	Water-solubility
Attenuation	Fat-binding capacity
Blood cholesterol	Viscosity
Blood glucose	Particle size
Mouthfeel	
Taste	
Colour	

cal determination, which is most frequently based on either chemical or enzymatic methods (Hellendorn 1975; Thomas & Elchazly 1976; Schweizer & Würsch 1979; Meuser *et al.*1983b). These methods, to a certain extent, imitate the process of digestion in and before the small intestine. Some of them have been standardised (Bundesgesundheitsamt 1988; AOAC 1997) and are applied in statutory food checks in some countries, for example in Germany (Bundesgesundheitsamt 1988).

The classification as dietary fibre of all substances not degraded in the small intestine, in conjunction with its unspecific determination, has resulted in a quantitative and qualitative discrepancy between classification and analytical determination. This hinders efforts to determine the nutritional effects of dietary fibre, as it is not possible to establish a clear relationship between the analytical determination of dietary fibre and its nutritional effects.

For example, low degree-of-polymerisation carbohydrates may not be detected as dietary fibre either as they cannot be precipitated by ethanol (AOAC 1997) owing to their low molecular weight, or as they are not retained on the membrane during membrane filtration (Bundesgesundheitsamt 1988). However, this type of carbohydrate is fermented by microorganisms in the large intestine, thus producing the effects ascribed to dietary fibre.

In addition, substances added to manufactured foods may be detected analytically as dietary fibre, although their contribution to the nutritional effects is either insignificant or nonexistent owing to the small quantities used in the recipes. However, such substances may be essential for the formation of the sensory characteristics of the food of which they are an ingredient. Examples of such substances are those with a high water-binding capacity that are detected as dietary fibre when the analytical methods referred to above are applied to either the food that contains them or to the dietary fibre products themselves. Consequently, they may be considered as dietary fibre. In some cases, it may be difficult to categorise such substances as either additives, which have to be declared, or ingredients, which do not. One of the most important criteria may be the contribution of such substances to the total dietary fibre intake in the average consumption of food.

Finally, it should be mentioned that although substances produced from vegetable raw materials or derived from material occurring during food production, for instance, may have a nutritional effect similar to that ascribed to dietary fibre, they may have no technological function. Resistant starches can be regarded as an example of such substances.

There are many more problems that arise from the definition of dietary fibre and affect the way in which the subject is dealt with. At this point it should also be mentioned that the word 'fibre' does not correctly describe the physical structure of the substances that is covers. The term 'fibre' gives the impression that the substances have a fibrous structure. Yet this only applies to the part of their mass that is derived from the cell-wall material of the edible part of plants and consists mainly of polysaccharides and lignin. The term 'fibre' therefore needs to be broadly interpreted so that it also includes higher polymer, soluble carbohydrates. These are referred to as soluble dietary fibre, and they are detected as such by the analytical methods. The term implies that the soluble substances derived from vegetable materials have a fibrous structure, which is quite misleading. Semantically, it is therefore also interesting to note that the German term *Ballaststoffe*, which corresponds to the term 'dietary fibre', avoids any association with fibrous materials altogether.

Categorising the substances based on carbohydrates and the associated substances of vegetable origin found in food as 'dietary fibre' can only be tolerated from a nutritional point of view. However, this only applies if the term 'dietary fibre' is interpreted as a collective term for companion substances of vegetable origin that are resistant to digestion, are not absorbed and develop their nutritional effects as a result of their indigestibility and subsequent fermentation.

Viewing dietary fibre collectively as companion substances of food comes closest to the English term 'roughage' which also corresponds to the German term *Ballaststoffe*. Both terms – *Ballaststoffe* and roughage – avoid considering the different components of the mixture of substances separately and thus do not differentiate between their effects on the digestion process and their contribution to good health.

23.1.2 *Dietary fibre in food production and consumption*

The main advantage of considering dietary fibre collectively as 'roughage' is that it provides a basis for a logical concept for healthy nutrition that also includes the ground-breaking findings of Burkitt and Trowell in particular (Burkitt 1973, 1979; Burkitt & Trowell 1975; Trowell *et al.* 1976). Such a concept only requires food to be produced from raw materials of vegetable origin and for the original composition of the raw materials to be retained as far as possible during processing into the basic ingredients of foods. The methods of producing wholemeal products, including breakfast cereals and wholemeal bread, are excellent examples of such production processes. For example, it has been sufficiently proven that consumption of substantial quantities of such products aids faecal bulking and softening, thus contributing to stool frequency and regularity (Eastwood *et al.* 1973; Findlay *et al.* 1974; Feldheim *et al.* 1982). There are major benefits to health if such products are eaten together with fruit and vegetable products in preference to animal products and those that are rich in fat. This type of diet, which is based on mainly unprocessed foods, is known in Germany as *Vollwertkost*, or wholefood (Koerber *et al.* 1985).

Unfortunately, acceptance of such forms of nutrition in Germany and in many other highly civilised countries is very low. Diets in these countries are based on foodstuffs with preferred sensory qualities. Foods of vegetable origin made of highly refined basic ingredients such as white flours, starches, sugar, fats and oils have found particularly wide acceptance. Such foods have only a low dietary fibre content which, considered in relation to the overall food intake, is too small to cover the intake recommended as a result of the findings of nutritional research.

My own surveys show, for example, that the dietary fibre intake in the German Federal Republic in 1984 was, on average, 18% below the assumed requirement of 30 g dietary fibre per person per day (Meuser *et al.* 1984). The survey also showed that bread accounted for 42% of the calculated average overall intake of around 9 kg dietary fibre per person per year. Essentially, the results of the calculations performed at that time are still valid today. On the basis of the consumption of the foods that are already generally accepted it can therefore be assumed that the required dietary fibre intake could be covered solely by changing eating habits and consuming more wholemeal bread. This is because the dietary fibre content of such bread is nearly twice as high as that of bread made of white flour, and as the percentage of wholemeal bread eaten is only 10% of total bread consumption which is around 83 kg per person per year. Although bread consumption has on the whole risen slightly since 1984, the amount of wholemeal bread eaten has unfortunately declined in relative terms.

The same calculations can be performed for other countries to obtain similar information on the nutritional situation of the respective populations. The results largely depend on which figures for the dietary fibre requirements of individuals are used in the calculations as a basis for ensuring adequate or optimum regulation of the digestive process. The intake of dietary fibre, calculated as being 18% lower than the average requirement of 30 g dietary fibre per person per day in 1984, would correspond to 150 000 tons per annum dietary fibre for the present population of Germany of around 80 million. This would increase to 500 000 tons per annum if the calculations were based on a requirement of 40 g dietary fibre per person per day. This means that covering such a high additional requirement can provide manufacturers of dietary fibre products with a substantial and lucrative line of business.

23.1.3 *Food legislation aspects of dietary fibre*

Both the preferred eating habits and the above figures provide the background to the description of the technological aspects of dietary fibre. The aspect of staying healthy by eating a diet rich in dietary fibre and the technological aspect of preserving the sensory quality of those foods that are most widely accepted need to be reconciled. The most important approach to solving this problem is to reprocess dietary fibre-rich by-products from processing vegetable raw materials into dietary fibre products and to manufacture substances that act in the same way as dietary fibre from carbohydrates of vegetable origin for use as ingredients in food production. The use of such products and substances is subject to national and international regulations on food and, in the EU, to the recently introduced EU Novel Food Directive (Meuser *et al.* 1984; Verordnung (E.G.) Nr. 258,1997).

Legal regulations on foodstuffs may make an important distinction between dietary fibre products used as food ingredients and those used as food additives. Different countries have different regulations that are of crucial significance not only for the approval of the products for use in enriching food but also for the degree of enrichment permitted in food supplementation. Regulations of this type are usually based on the principle that food supplements are added to food for nutritional reasons, not for technological ones. However, dietary fibre products are examples of products that may be of benefit in the manufacture and quality of foods thanks to their functional properties. However, this cannot be taken to mean that any product (substance) with technologically interesting functional properties that can be determined analytically as dietary fibre ought to be approved as an additive.

As far as food production is concerned, however, the approach by which dietary fibre products are used as ingredients or additives in the manufacture of food in order to achieve

an adequate dietary fibre intake with the food differs entirely from that of altering the composition of vegetable raw materials as little as possible. While the latter can be described by the slogan 'Back to nature', the former focuses on technical and scientific advances in food manufacture. It includes the concept of producing foods that affect health in a functional way thanks to certain ingredients and are thus able to either prevent illnesses or cure them. Functional food, as it is known, is thus about to blur the boundary between food and medicine.

At this point it can be seen that, owing to the way dietary fibre is categorised as a result of the analytical methods used to determine it in foods, the substances identified as dietary fibre may be added to food either in the form of largely unaltered by-products of raw material processing or as dietary fibre products derived from by-products with modified compositions or as chemically pure carbohydrate-based substances of vegetable origin. Specific nutritional effects that are dependent on chemical structure and biological characteristics can be obtained by adding dietary fibre products and pure substances, in particular those with the same function. The dietary fibre products and substances whose properties are of relevance to food manufacture will be dealt with below.

23.1.4 *Scope of the article*

The terms of reference for investigating the technological aspects of dietary fibre with regard to their nutritional effects have thus been defined. The foods covered range from those made of raw materials whose composition is largely unaltered and are thus rich in dietary fibre, to those enriched with by-products from the production of basic materials that are rich in conventional dietary fibre and those to which dietary fibre products and substances are added during the production process. As regards the first type of food, the technological challenge involves developing the sensory characteristics in the best possible way, taking account of the physicochemical characteristics and flavour of the dietary fibre components of the raw material. The challenge presented by the other two types of food is largely how to avoid altering the accepted sensory characteristics of well-known foods when adding dietary fibre products or substances to the recipes or at most to alter them only within tolerable limits. It is rare for entirely new foods to be developed with the aim of making dietary fibre a key component of an acceptable product. The most important task in food technology is therefore to enrich foods with dietary fibre within the possibilities considered here in order to increase the dietary fibre intake for nutritional reasons. The focus of attention in the following is on this technological aspect in addition to the manufacture and characterisation of dietary fibre products. The present state of knowledge will first be described in order to ensure clarity and consistency within the article. This will be followed by a description of two approaches taken from my own field of research by way of example in order to highlight technological aspects that are of relevance to the development of dietary fibre products and their application in food production.

23.2 Latest developments in the production and use of dietary fibre products

A great deal of research has been carried out into dietary fibre products derived from seeds, fruits, husks, peel, leaves and the support organs of plants, above all for nutritional reasons.

The product characteristics that are of relevance in food technology have only been considered to a limited extent even though they provide the key to the economic success of such products.

23.2.1 *Functional properties of dietary fibre*

Dietary fibre products can give foods texture, firmness, mouthfeel and other sensory characteristics. Their physical properties – from which the technological characteristics are derived – (see Table 23.2) make them suitable for use as water-binding agents, thickeners, suspending agents, gelling agents, film formers, emulsifying agents and stabilisers. The most important characteristics of dietary fibre products, such as their swelling capacity, water-binding and water-retaining properties and the viscosity of their salt-containing solutions or dispersions depend on the molecular structure of their individual components. In addition, there are synergy effects between components resulting in certain characteristics being either emphasised or suppressed. These characteristics are influenced by the concentrations and production conditions (temperature, pH, ionic concentration, etc.) employed when incorporating dietary fibre products in recipes in food production. The complex interaction of these factors shapes the characteristics of dietary fibre products and finally determines their use as texture-forming agents. A sound knowledge of the separate components that make up dietary fibre products, including their molecular structures and physicochemical characteristics, is therefore required in order to be able to determine the relationships between structure and characteristics and thus enable dietary fibre products to be used for specific purposes.

23.2.2 *Raw material basis for dietary fibre products*

The raw materials used to manufacture dietary fibre products are the by-products of processing cereals, fruits and vegetables such as bran, husks, peel and pomace. Dietary fibre products in common use today are derived from wheat, oats, barley, maize, rye, apples, citrus fruits, carrots, potatoes and sugarbeet. Fibres derived from legumes such as smooth peas and lupins have been gaining in importance for some time now. Another recent development is the extraction of fibres from wrinkled peas (Lindhauer 1994; Meuser *et al.* 1997).

The production methods are divided into dry and wet processes (grinding cereals and starch extraction respectively). For example, the resultant dietary fibre raw materials consist of the outer fibres of the support organs of various plants, the bran and husks of cereals and legumes or the inner fibres of the cotyledons of legumes. The outer fibres mainly contain water-insoluble polysaccharides and pectin. The inner fibres consist of cell wall polysaccharides of varying degrees of solubility.

Most dietary fibre products are predominantly mixtures of cell-wall polysaccharides depending on their source and the processes used in their manufacture. They contain not only cellulose, lignin, hemicellulose, pectins and associated products in various proportions, but also starch, proteins, lipids and minerals (Olson *et al.* 1987; Schweizer & Edwards 1992; Lämmche *et al.* 1999; Klotz *et al.* 2000) (Tables 23.3 and 23.4). The various extraction, cleaning and drying processes used in the manufacture of dietary fibre products, including fractions of the dietary fibre raw materials, have a decisive influence on their composition, the ratio of fibrous to non-fibrous material, their particle size, and their physicochemical characteristics. For example, finely ground dietary fibre products behave quite differently from coarsely ground ones on rehydration. The same applies not only to untreated products and

Table 23.3 Analytical composition of dietary fibre products.

	Analytical composition (% d.m.)							
	Dietary fibre							
Dietary fibre product	Total	Insoluble	Soluble	Starch (α-Glucans)	Protein (N × 6.25)	Fat	Total sugars	Ash
Wheat bran*	48.2	47.1	1.1	16.3	18.4	5.2	5.0	6.0
Oat bran*	21.2	12.7	8.5	42.1	20.5	10.5	3.9	3.8
Barley bran*	19.9	15.9	4.0	52.8	15.9	4.5	3.4	3.0
Lupin†	81.9	–	–	0.2	17.6	–	–	1.8
Wrinkled pea†	76.3	–	–	12.3	10.6	–	–	2.3
Vitacel®†‡	76.3	–	–	0.2	0.2	–	–	0.7

* Source: Klotz et al. 2000.
† Dietary fibre products extracted from cotyledons of lupins and wrinkled peas (source: Lämmche et al. 1999).
‡ Commercial product made from wheat fibre. (Source: Lämmche *et al*. 1999.)

Table 23.4 Relative composition of the insoluble part of dietary fibre of different sources.

	Relative composition (%)		
Insoluble dietary fibre	Cellulose	Hemicellulose	Lignin
Wheat bran	37.3	55.2	10.5
Brewer's spent grains	35.2	49.8	15.0
Maize bran	23.8	79.9	2.4
Soya hulls	79.9	2.4	12.2

Source: Meuser *et al*. 1985.

those treated with alkalis but also to products manufactured by means of dry and wet processes.

Most of the fibres are yellow to brownish in colour and have a bitter, fruity or characteristic flavour, which means that they are only suitable for use as food ingredients to a limited extent. Only highly purified or chemically treated fibres are white or slightly yellow in colour and free from any unpleasant taste. All such dietary fibre products are used to enrich a wide variety of foods (Seibel & Hanneforth 1994; Endreß & Schinz 1998) (Table 23.5).

23.2.3 *Polysaccharides of plant cell walls*

The polysaccharides of the cell walls are divided into insoluble, colloidally swelling and soluble materials depending on their degree of solubility. This classification is crucial not only for the manufacture but also for the application of dietary fibre products. It is particularly important to note that the boundary between colloidal swelling and dissolving is fluid. Polysaccharides that do not dissolve in water or diluted alkalis are termed 'insoluble'. They include cellulose and the high-molecular weight arabinoxylans, xyloglucans and arabinogalactans. These polysaccharides constitute the raw material of dietary fibre products after separation from the soluble components, and the commercially valuable components such as

Table 23.5 Selected applications of selected dietary fibre products.

Application	Dietary fibre product									
	Apple	Aronia berry	Citrus	Strawberry	Raspberry	Currant	Oat bran	Pea	Small carrot	Beetroot
Bread	X						X	X		
Baked goods	X		X				X	X	X	
Ready-to-serve meal									X	
Fruit preparations		X		X		X				
Beverages	X	X	X		X		X			X
Gluten-free products	X									
Chewing tablets	X	X	X			X	X			
Food supplement	X	X	X			X		X		
Bars	X		X						X	X
Confectionery		X				X				
Soups and sauces							X	X		X
Pasta						X	X	X		

Source: Endreß & Schinz 1998.

starch, protein and lipids. Hydrocolloidally swelling polysaccharides are composed of various neutral monosaccharides and uronic acids, and form a wide variety of structures. Also known as vegetable gums, they can be removed from plant extracts by ultrafiltration, selective precipitation or ultracentrifugation. Polysaccharides that are soluble in water or in diluted alkalis include xylans, xyloglucans, glucomannans, galactomannans, β-glucans, fructans, galacturonans, arabinans and arabinogalactans. They can be obtained from plant extracts by selective precipitation or dewatering.

In this connection it should be mentioned that there is a wealth of knowledge about the molecular structure, gelling and swelling capacities, water-retention capacity and the rheological properties of dietary fibre and its components (Renard *et al.* 1992; Viëtor *et al.* 1992; Quemener *et al.* 1993; Westerlund *et al.* 1993). However, neither manufacturers nor users of dietary fibre products have so far shown sufficient interest in applying it to derive relationships between structure and properties.

23.3 Dietary fibre products obtained from cereals, and enrichment of cereal products

Being a basic foodstuff, cereal products already make a major contribution to dietary fibre intake (Meuser *et al.* 1983a). They therefore provide an opportunity for further enrichment as the acceptance threshold can be moved more easily for cereals than for more highly refined foodstuffs. The spectrum ranges from sophisticated baked products to black breads, for example. Other examples are pasta, all types of breakfast cereal, and snacks. It would therefore seem reasonable to enrich such products, especially with dietary fibre products derived from cereals.

23.3.1 *Cereal-based dietary fibre products*

The most commonly used dietary fibre product is wheat bran (Seibel 1975). Furthermore, there is a certain tradition of using brewer's spent grains (Meuser *et al.* 1982), barley and oat husks (Bhatty 1993; Ganβmann 1990) in order to enrich baked products and cereals with dietary fibre. The use of barley bran derived from huskless (Bhatty 1986) or dehusked (Vorwerck 1990) barley and oat bran derived from dehusked oat kernels (Wood 1991), which represents a new source of dietary fibre, is also becoming more widespread thanks to their high soluble dietary fibre content, in particular their β-glucan content. A factor common to all cereal-based dietary fibre products is their characteristic flavour, a product-specific composition and the corresponding physicochemical properties. There are limits to enriching foodstuffs with cereal bran, as it has a detrimental effect on sensory characteristics, appearance, mouthfeel, flavour and chewing characteristics when certain concentrations (which vary according to the foodstuff) are exceeded. Enriching foods with high levels of untreated cereal bran is therefore problematic.

In principle, it is possible to use other fibrous materials such as wheat fibres (Vitacel) (Bollinger 1994), orange or apple fibres, dietary fibre products derived from carrots, beets and potatoes, or those derived from legumes, to enrich cereal products with dietary fibre. However, the problems are the same as for cereal dietary fibres. In addition, the particularly high water-binding capacity of some of the dietary fibre products mentioned, can be highly detrimental to the processing characteristics of the cereal products.

23.3.2 *Composition and functional properties of cereal dietary fibre products*

The insoluble dietary fibre of wheat, barley and oat bran is composed mainly of cellulose, insoluble arabinoxylans, gluco- and galactomannan, pectin-like polysaccharides and lignin. Barley and oat bran also contains insoluble (1→3), (1→4)-β-D-glucans (Knuckles *et al.* 1992) which are the main constituents of the cell walls of the outer coverings of the cereal grains. Depending on the degree of mechanical disintegration, they occur either in intact cell structures, in cell walls or as single fibres.

The soluble dietary fibre of the three types of bran is also composed of arabinoxylan, gluco- and galactomannan and pectin-like substances, although wheat bran also contains very small amounts of arabinogalactan in the form of glycoprotein complexes. Compared with wheat bran, barley and oat bran have the additional feature of a relatively high soluble β-glucan content. These substances are located in cell walls or the extracellular matrix where they act as storage polysaccharides. The proportion of these substances present in the bran depends on the extraction process used.

The hydration behaviour of the polysaccharides is also determined by their molecule size, the degree of branching, and their intermolecular aggregation. Intermolecular bonds such as hydrogen bridge bonds can be disrupted and intramolecular bonds destroyed by the action of heat. The former causes macromolecules to disintegrate, thus weakening the cell-wall aggregates, while the latter results in molecular degradation. The relationship between specific composition, molecular structure, aggregation and particle size distribution as a macroscopic quantity, determines the hydration properties of brans, such as their water-binding capacity, water retention capacity and swelling capacity. These physicochemical parameters affect

the sensory characteristics such as texture, mouthfeel and chewing characteristics to a large extent.

The other principal components of bran are starch, low-molecular weight saccharides, proteins, lignin, minerals and fats, including their companion substances. Some of these components are important for the development of flavour and aroma when brans are used as ingredients in foodstuffs. A high fat content can have a detrimental effect on the sensory characteristics and shelf-life; this is especially true of oat bran.

Other sensory characteristics of brans, such as their mouthfeel, chewing and swallowing characteristics are largely governed by the size and shape of their particles. The accepted sensory quality characteristics of the foodstuffs restrict the amount of bran that can be added to the recipes. Adding bran to baked products generally reduces their volume, alters the texture of the crumb and has a detrimental effect on the mouthfeel (Dreher 1995). These negative sensory changes can be compensated for by optimising recipes and production processes (Altrogge *et al.* 1980; Sluimer 1987; Seibel & Brümmer 1991). By way of example, Table 23.6 lists the quantities of bran that can be substituted for wheat flour with a low dietary fibre content depending on the type of end-product, so that products with acceptable sensory characteristics can be manufactured (Nestl & Seibel 1990; Seibel *et al*. 1991; Jotova & Seibel 1992).

23.3.3 *Modification of cereal dietary fibre products*

Another way to increase the amount of dietary fibre added to foodstuffs is to modify brans mechanically, chemically, enzymatically, thermally or thermomechanically (Caprez *et al.* 1986, 1987; Cadden 1987; Sievert 1987; Galliard & Gallagher 1988; Pfaller *et al.* 1988; Ralet *et al.* 1990; Camire & Flint 1991; Posner 1991; Rasco *et al.* 1991; Spicher & Zwingelberg 1991; Auffret *et al.* 1994; Meister *et al.* 1994; Kunzek *et al.* 1995).

The suitability of the modified products has hitherto usually been assessed by considering the changes in the composition, water-binding capacity, swelling and water-retention capacity of the original products, and partly by considering their new processing characteristics. However, less attention has hitherto been devoted to the new sensory characteristics of the bran – and the foodstuffs in which they are incorporated – that result from modification, in particular the changes in hydration behaviour.

Table 23.6 Possible percentage of flour exchange against untreated dietary fibre products in the recipe of baked and extruded products.

Product	Possible flour exchange (%)	Dietary fibre products	Reference
Wheat (flour) bread	10–15	Wheat bran	Seibel & Brümmer 1991
Wheat rolls	15	Oat husks, oat bran	Seibel & Brümmer 1991
Toast	5–10	Wheat bran	Seibel & Brümmer 1991
Pasta	5–20	Wheat bran	Jotova & Seibel 1992
Hard cookies	20	Oat bran, oat husks	Seibel *et al*. 1991
Crumbly cookies	5–20; 100	Oat bran, oat husks	Seibel *et al*. 1991
Extruded products			Nestl & Seibel 1990
Rusks	20	Oat bran	Meuser *et al*. 1983
Flat breads	20	Brewer's spent grains	Seibel *et al*. 1979
Bran-enriched extrudates	20–30; 50	Wheat bran, soya hulls	Pfaller *et al*. 1988

The mechanical methods used include, first and foremost, particle size reduction processes combined with separation processes. These bring about changes in the fibre matrix and in the composition, that in turn affect the water-binding properties. For example, particle size reduction and air classification of wheat bran resulted in a significant increase in the dietary fibre content of the coarse fraction (Posner 1991). At the same time, the flavour of the coarse fraction was less bitter and astringent than that of the basic material, and its phytic acid content was also lower. Accordingly, there was a higher concentration of proteins, ash and vitamins in the fine fraction. Unfortunately, particle size reduction reduced the shelf-life. This meant that the sensory quality of the modified bran deteriorated more rapidly than that of the unmodified material (Galliard & Gallagher 1988). Comminution also altered the nutritional effect of the bran, as changes in the particle size affected its physicochemical and thus functional characteristics (Wisker *et al.* 1980; Caprez *et al.* 1986).

Furthermore, it should be mentioned that an attempt was made to modify wheat bran and thus improve its sensory qualities by treating it chemically with acid, alkali or ethanol, either on their own or combined with enzymes such as amylase and protease (Rasco *et al.* 1991). As regards dough-forming and baking, none of the properties of the chemically modified brans was superior to those of the unmodified brans, while brans treated with α-amylase or α-amylase and calcium hydroxide produced the best results in respect of the crumb and colour of the baked products in which they were incorporated. Although the colour of the bran was improved by bleaching it with hydrogen peroxide, the bran itself developed an unpleasant flavour so that it was no longer suitable for use in baked products.

Attempts to modify the chemical composition and physical characteristics of wheat bran by various thermal processes such as boiling, steaming, autoclaving, roasting, infrared light roasting or extrusion cooking showed that processes employing dry heat had only a marginal effect. By comparison, boiling, steaming and autoclaving resulted in a significant increase in the total dietary fibre content, presumably owing to the formation of fibre–protein complexes. There was an increase in the protease-resistant protein fraction owing to aggregation of the proteins, while the starch content decreased slightly due to thermal degradation. Gentle roasting, infrared light roasting and extrusion cooking scarcely altered the total dietary fibre content. Boiling the bran increased its water absorption capacity owing to the partial gelatinisation of the starch present in it (Caprez *et al.* 1986).

The influence of baking and extrusion on the dietary fibre content and the composition of the dietary fibre of ground maize flakes, rolled oats and potato peel depended primarily on the respective raw material. The total dietary fibre content of rolled oats and potato peel increased slightly after such treatment, probably owing to the formation of resistant starch. The ratio of soluble to insoluble dietary fibre was altered, with an increase in the proportion of soluble components. This was true for extrusion in particular. Furthermore, the water-binding capacity of extruded maize flakes and rolled oats was considerably higher than that of the untreated materials (Camire & Flint 1991).

These examples show that the physicochemical characteristics of bran or dietary fibre products can be modified by thermal processes. Few studies have yet been conducted into how the characteristics of the modified dietary fibre affect the sensory quality of the foodstuffs in which they are incorporated. However, to sum up these remarks on the modification of dietary fibre products based on cereal brans, it can be said that the large number of modification processes used hitherto include innovative methods of influencing the functional characteristics of these products in specific ways.

23.4 Research approaches to developing dietary fibre products

The description of the latest developments in the manufacture and use of dietary fibre products shows that in most cases only isolated approaches have been selected in order to develop such products with reference to their nutritional effects and to study their application in specific foodstuffs. The motivating force behind this is usually the desire to increase the value of the dietary fibre-rich by-products obtained during processing of the raw materials – which are used predominantly as animal feed – by means of suitable reprocessing methods so that they can be used as foods or food supplements. Attention has focused on enriching foods with dietary fibre products and thus increasing dietary fibre intake. In doing so, it has been particularly important to maintain the accepted quality characteristics of the foods selected for enrichment. Apart from several extreme cases resulting from these good intentions, which have given rise to concepts that have been implemented mainly for the purposes of advertising foods, enrichment has definitely led to noticeable improvements in dietary fibre intake. However, the goal of a sufficient intake is still a long way off. It is therefore necessary to search for new ways of enriching foods with dietary fibre and to develop new dietary fibre-enriched products.

The two possibilities that we recently explored may prove suitable. They differ in approach and are in marked contrast to the procedures used in the past. One of them involves applying the appropriate combination of technical processing steps and raw materials to derive dietary fibre products that can be added to known foods in higher concentrations than has hitherto been possible. The other approach aims to produce new dietary fibre products by using wet-processing methods to treat raw materials that have so far been used scarcely or not at all in the production of basic materials for food. Both approaches are described in brief below. With regard to the first of these, attention will focus on the systematic procedure used rather than on the description of the results obtained. It should be mentioned that the second approach developed from the problem of how to assess whether wrinkled peas and lupins are suitable for industrial amylo-starch extraction and the production of protein products respectively.

With regard to the intended use of these raw materials, it is interesting to note that, unlike the bran obtained when milling cereals, the inner fibres that can be extracted from cotyledons are, as far as quantity is concerned, not so much a by-product as a second main product when compared with the quantity of commercially valuable components produced. This is of crucial importance when considering the economic viability of the methods used to process this type of raw material and explains the high level of interest in deriving dietary fibre products from them (Elers *et al.* 1997). However, the details of the processes used to make such products will not be described here. Instead, only the approach used to characterise the products with a view to their potential applications and to compare them with a product derived from wheat fibre (Vitacel) will be described.

23.4.1 *Modified dietary fibre products from cereal brans*

Wheat, barley and oat brans were modified according to the schedule shown in Fig. 23.1, in which all levels and stages are interconnected. In addition to the combination of processing methods and raw materials used, the integrated system can be regarded as a completely new approach to manufacturing dietary fibre products. The different proportions of the substances associated with dietary fibre that were present in the bran provided the starting point for the

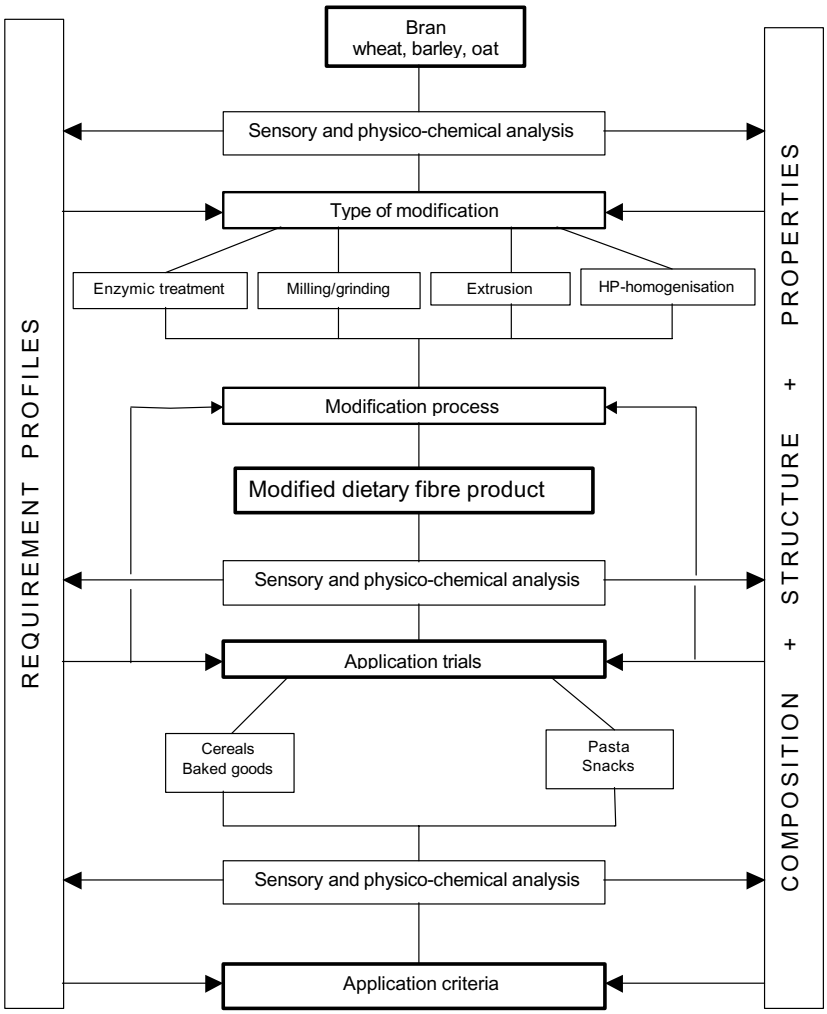

Fig. 23.1 Requirement profiles for development and application of dietary products.

modification process (see Table 23.3). It is these substances in particular that can be relatively easily degraded or converted, enabling the sensory characteristics of the products in which they are incorporated to be altered as outlined above. However, modification must be managed in such a way as to prevent the bran losing its nutritional effects, which are mainly based on indigestible carbohydrates.

Figure 23.1 illustrates the three requirement profiles for the raw material, the dietary fibre product and its use in food, which finally result in the application criteria. The requirement profiles can be defined according to composition, structure and properties by sensory and physicochemical analysis of the substances present at each processing stage.

An example of the analysis of the sensory profiles of the three types of bran given in Table 23.3 is shown in Fig. 23.2 (Klotz *et al*. 2000). The profile analysis reveals that, as expected, there were considerable differences between the sensory characteristics of the differ-

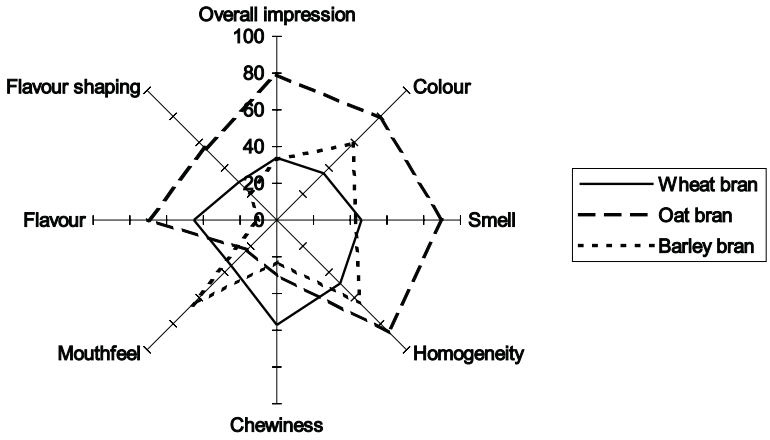

Fig. 23.2 Sensory profile analysis of the different brans.

ent types. The method used to modify the bran can now be selected on the basis of the profile analysis in order to produce the required modified dietary fibre product. One example of this is the change in the sensory analysis of wheat bran following extrusion cooking, as shown in Fig. 23.3 (Kretschmer & Meuser 1999). It can be seen that extrusion resulted in a product with a better rating for each of the criteria shown.

The cycle that the raw materials undergo to obtain dietary fibre products is then repeated for the relationship between the dietary fibre product and its application in various foods. The foods thus produced are also characterised according to a requirement profile in order to achieve the application criteria required for the use of a particular dietary fibre product in certain foods.

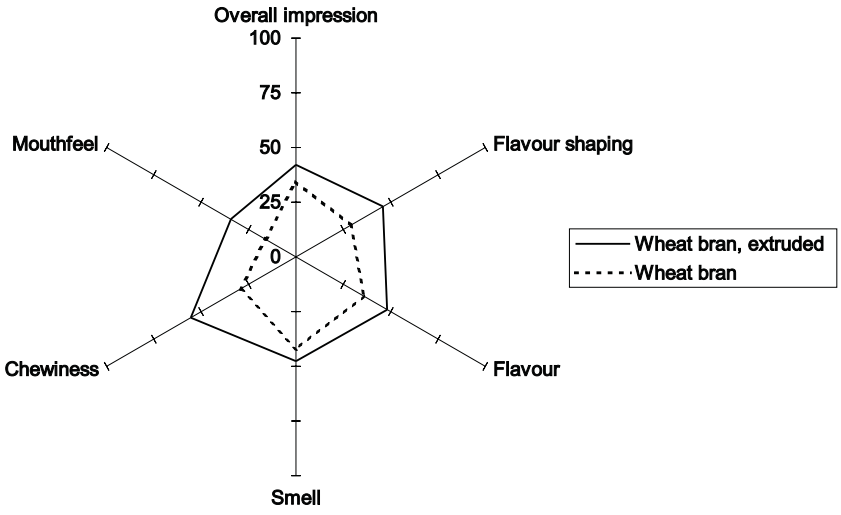

Fig. 23.3 Sensory profile analysis of the treated and untreated wheat bran.

This approach enabled us to demonstrate that either a single process or a combination of processes can be used to obtain dietary fibre products from bran and mixtures of different types of bran and that these can be added to baked products, pasta, fried snacks and extrusion cooked products in higher concentrations than is possible for non-modified products. The comparison showed that processing did not result in any significant loss of quality. This means that it is possible to satisfy one of the most important conditions for expanding the use of bran-based dietary fibre products so that more cereal products can be enriched with dietary fibre than is possible using current technology.

23.4.2 *Dietary fibre products from wrinkled peas and lupins*

Despite the possibilities permitting their requirement profiles to be modified, the fact remains that most dietary fibre products are only suitable for a limited number of applications. Certain applications remain closed to many dietary fibre products for the simple reason that they are either not neutral in flavour, or have a very distinctive colour. Neutrality of flavour, in particular, is a major criterion governing the range of applications. In this respect, the inner fibres extracted from the cotyledons of lupins and wrinkled peas have the distinct advantage of being particularly neutral in flavour and exhibiting outstanding water-binding characteristics.

The dietary fibre products characterised analytically in Table 23.3 were extracted from wrinkled peas and lupins by methods based on well-known extraction processes (Meuser *et al.* 1997; DE 13207.7-41, 1981). The products were mainly derived from the cotyledons of the raw materials. In addition to their composition being analysed, they were tested for the relevant physicochemical characteristics (water-binding capacity, viscosity) in the same way as the wheat fibres (Vitacel) that were analysed by way of comparison (Lämmche *et al.* 1999; Meuser & Niemann 1999). In addition, the dry matter of the insoluble non-cellulose and non-starch components of the dietary fibre of these materials was subjected to acid hydrolysis and the monosaccharide content of the hydrolysates was determined. The dietary fibre products were used to make a variety of foods. The results of their experimental use in soups will be described below.

Table 23.3 shows that the dietary fibre preparations derived from wrinkled peas, lupins and wheat fibre had a very high total dietary fibre content. Although the concentrations of companion substances were mainly due to the source of the preparations, they also depended on the production process used. The dietary fibre found in the preparations was predominantly of the insoluble kind. Only products derived from wrinkled peas and lupins contained around 6–8% soluble dietary fibre in relation to their total mass. This was partly a result of the production process, in which parts of the soluble dietary fibre of the raw materials remained in the washed residue owing to the limited quantity of washing water or extraction water used in production. These soluble components were dried onto the solid components when the preparations were dried.

The composition of the products reveals that their physicochemical characteristics are governed essentially by the insoluble dietary fibre component. This is comprised of non-starch polysaccharides, which in turn consist of various monomers (depending on the source). The polysaccharides of the wrinkled pea, lupin and wheat products consisted mainly of arabinose, galactose and glucose respectively (Table 23.7). The dietary fibre product derived from the Salout variety of wrinkled pea had by far the greatest cold water-soaking capacity (CWSC) and cold water-binding capacity (CWBC) of the four products analysed

Table 23.7 Monosaccharide content of the dry matter of the insoluble non-cellulose and non-starch components of dietary fibre of different sources and relative composition of the Neutral Detergent Fibre of these sources.

	Monosaccharide content of the dry matter of the extracted material (%)*			
			Wrinkled pea†	
Monosaccharide	Vitacel	Lupin† fibre	Markana	Salout
Arabinose	0.5	6.1	35.5	37.8
Galactose	<0.1	39.9	2.3	3.0
Glucose	8.3	6.2	14.9	11.9
Xylose	8.3	5.2	4.3	1.3
Galacturonic acid	–	4.5	2.6	3.4
Component	Relative composition of the Neutral Detergent Fibre (%)			
Hemicellulose	17.1	13.2	24.3	26.8
Cellulose	75.1	1.9	4.6	8.3
Lignin	5.0	0.3	0.3	0.2

* After acid hydrolysis.
† Dietary fibre products extracted from cotyledons of lupins and wrinkled peas.
Source: Meuser & Niemann 1999.

(Table 23.8). The dietary fibre product derived from lupins had a lower CWSC than the other products, although it should be pointed out here that both physical characteristics evidently depend to a considerable extent on the drying conditions. Drying the dietary fibre products at temperatures above 60°C resulted in a considerable decrease in the CWSC and CWBC.

The rheological behaviour of the aqueous dispersions of the products (10% solids content) varied to a large extent (Table 23.9). The degree to which dispersions formed particle gels de-

Table 23.8 Physical properties of different dietary fibre products.

Dietary fibre product	Cold water soaking capacity (CWS C) (ml/g)	Cold water binding capacity (CWB C) (g/g d.m.)	Cold water retention capacity (CWR C) (g/g d.m.)	Cold water solubility (CWS) (% d.m.)
Vitacel	21.7	12.3	4.6	2.4
Lupin*	14.0	10.1	4.5	14.6
Markana*				
<60°C†	17.8	10.5	4.0	2.0
>60°C	9.8	5.9	3.6	7.0
Salout*				
<60°C	32.4	17.0	5.0	3.0
>60°C	13.2	7.9	6.2	6.3

d.m., dry matter.
* Dietary fibre products extracted from cotyledons of lupins and wrinkled peas.
† Temperature regiment at which the products were air-dried.
Source: Meuser & Niemann 1999.

Table 23.9 Steady stress sweep test of different dietary fibre products.

Dietary fibre product	τ_0 (Pa)	η_0 (Pa s)	τ_{strain} (Pa)	η_{equ} (Pa s)
Vitacel				
30 min†	503	52 500	687	164
90 min†	334	38 500	488	52
Lupin*				
30 min	8	1 260	120	0.4
90 min	16	1 680	157	0.6
Markana*				
30 min	97	10 400	174	26
90 min	210	17 400	378	70
Salout*				
30 min	255	13 100	499	53
90 min	412	43 000	748	109

τ_0 = yield stress; η_0 = zero-shear viscosity; τ_{strain} = stress of mechanical disrupture; η_{equ} = steady shear viscosity.
*Dietary fibre products extracted from cotyledons of lupins and wrinkled peas.
†Time of swelling (min); concentration 10% d.m.; temperature 25°C.
Source: Meuser & Niemann 1999.

pended on the swelling time and temperature. Although gel formation occurred immediately in Vitacel irrespective of the temperature, the degree of viscosity and the yield stress of the gel decreased considerably during the swelling period. In contrast, the viscosity and yield stress of the wrinkled pea products increased significantly over the swelling period so that the values for the product derived from the Salout variety of wrinkled pea surpassed those of all the other preparations at the end of the swelling period. The lupin product only assumed a structure similar to that of particle gel after a relatively long swelling period and/or an increase in temperature.

These investigations permit the conclusion to be made that water is not bound very firmly in the cavities of the cellular skeletons consisting of cellulose and hemicellulose, such as those present in Vitacel. It is therefore released on mechanical stressing after a state of saturation has been reached. This is demonstrated by the degeneration over a relatively long swelling period of the particle gel structure that had been formed initially. In contrast, the non-starch polysaccharides of the products derived from the cotyledons of legumes are matrix-forming hydrocolloids. Although the rate at which they bind water is slow, they do so more strongly than the skeletons comprising cellulose or hemicellulose.

The findings on the relationships between the composition and the physicochemical characteristics of dietary fibre products were used to determine the relationships between characteristics and applications in selected model formulations for food products. First and foremost, this involved investigating the texture-forming and viscosity-stabilising effects of the dietary fibre products. A model experiment in which they are incorporated in a dried soup mix is described below.

The results in Fig. 23.4 (Lämmche *et al*. 1999) show that all samples of the soups made to a recipe without starch had a far lower initial viscosity than those made with starch although in some cases the proportions of dietary fibre products used were far higher than those for starch. Gelatinisation of the starch therefore evidently governed viscosity development when

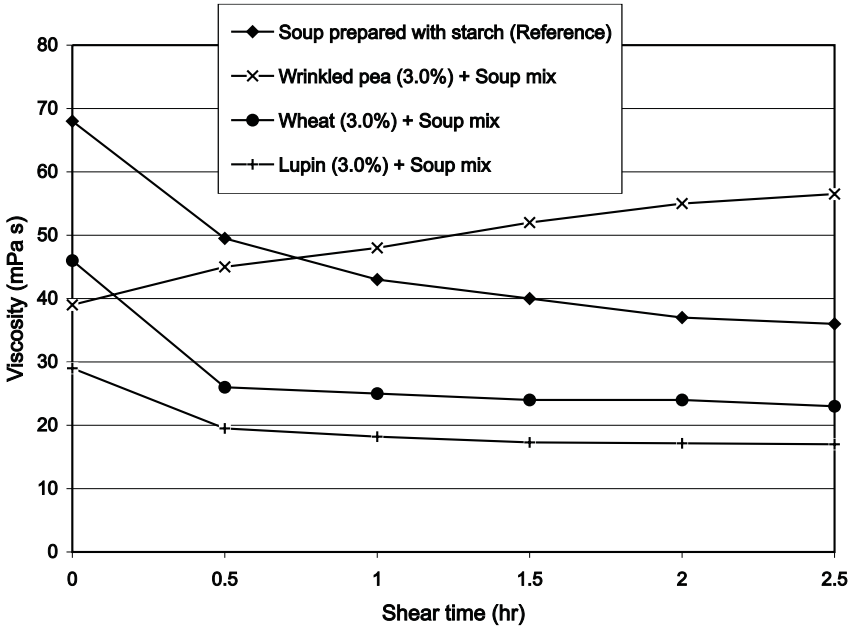

Fig. 23.4 Stability of the viscosity of soups made with dietary fibre products from different sources.

the soup was prepared. However, it was interesting to observe that the viscosity of the soups rose continually after the addition of 3% wrinkled pea products. The result was comparable to that obtained when starch is added.

As regards the suitability of dietary fibre products as food additives to improve the quality, provide texture and stabilise the viscosity of foods, it may be concluded from these results that products derived from wrinkled pea cotyledons are suitable for use in food formulations requiring cold- and warm-swelling characteristics and a high shear stability, including for relatively long periods of time. Dietary fibre products derived from lupin cotyledons are suitable for adding to food formulations requiring moderate swelling, hot water swelling characteristics and shear stability, including over relatively long periods. Dietary fibre products derived from wheat fibre (Vitacel) are particularly suitable for use in the manufacture of products subjected to shearing and, where appropriate, to heating during manufacture but which are intended to be consumed as soon as possible if they have a medium to high water content.

23.5 Summary

To begin with, attention is drawn to the lack of any clear relationship between the analytical determination of dietary fibre and its nutritional effects. It is for this reason that categorising the indigestible components of foodstuffs as dietary fibre can only be regarded as tolerable from a nutritional point of view. The term is a collective one covering different types of substances. Its advantage is that it provides the basis for a logical concept for a healthy diet. The concept can be based on foods made of raw materials whose composition is largely

unaltered during processing. However, eating such foods is not compatible with the type of diet normally preferred. The aspect of staying healthy by eating a diet rich in dietary fibre and the technological aspect of maintaining the sensory quality of those foods that enjoy the widest acceptance therefore need to be reconciled.

The most important approach to solving this problem is to use dietary fibre products that can be derived from the by-products obtained when processing vegetable raw materials. It is the technological product characteristics that are of particular interest in processing. Dietary fibre products are able to give foods texture, firmness, mouthfeel and other forms of sensory appeal. Their physical properties make them suitable for use as water-binding agents, thickeners, suspending agents and gelling agents as well as film-forming agents, emulsifying agents and stabilisers. These properties depend in the main on the molecular structures of the individual components of dietary fibre. In addition, there are synergy effects between components. The complex interaction of all the properties of any dietary fibre product determines its application as a texture-forming agent.

The methods used to manufacture dietary products can be divided into dry and wet processes. Most dietary fibres are predominantly mixtures of cell-wall polysaccharides depending on their source and the process used to obtain them. They contain cellulose, lignin, hemicellulose, pectin, and associated products in varying proportions.

Being a basic foodstuff, cereal products already make a major contribution to dietary fibre intake. Such products provide good opportunities for further enrichment, as their acceptance threshold can be moved more easily than that of more highly refined foods. The most frequently used dietary fibre product is wheat bran.

All cereal dietary fibre products have a characteristic flavour, a product-specific composition and corresponding physicochemical properties in common. These characteristics therefore mean that there are strict limits to using cereal brans to enrich foods. However, brans can be modified mechanically, chemically, enzymatically, thermally or thermomechanically so that food can be enriched with greater quantities of modified products than is possible in the case of non-modified raw materials. It is shown that the raw materials can be modified in accordance with a specially designed schedule.

In spite of the possibilities available for modifying the requirement profiles of dietary fibre products, the fact remains that most of them are only suitable for certain applications. Many dietary fibre products are unsuitable for particular applications as they are either not neutral in flavour or have a distinctive colour. Neutrality of flavour in particular is a major criterion determining the range of applications. It is shown that, from the technological point of view, fibres derived from wrinkled peas, lupins and wheat straw have major advantages over cereal brans. For example, the non-starch polysaccharides of the dietary fibre products derived from legume cotyledons are highly matrix-forming hydrocolloids that bind water slowly but firmly. All three dietary fibre products form structures similar to particle gels.

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24 Oatrim and NutrimX: Technological Development and Nutritional Properties

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Disclaimer

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24.1 Introduction

Dietary fibres obtained from cereal grains can contribute health benefits for many people. In the USA, if the population were to modify their diets based on existing knowledge of nutrition, it is likely that the $950 billion spent annually on health-care could be drastically reduced.

Excessive consumption of saturated and *trans*-saturated fats can contribute to an elevation of blood cholesterol levels, a condition which is recognised as being a risk for heart disease. Oats have been shown to be effective in lowering blood cholesterol levels since 1963 (deGroot *et al.* 1963), and a recent meta-analysis of several studies has shown a consistent efficacy of oats as a hypocholesterolaemic agent in humans (Ripsin *et al.* 1992). The soluble fibre from oat products are known to be biological factors in the lowering of blood cholesterol levels, making them both nutraceutical and functional substances. The Food and Drug Administration has recently recognised the importance of soluble fibre – generally referred to as β-glucan – from oat flour and bran, by allowing a health claim on food labels (FDA 1997). The effectiveness of oats in lowering cholesterol levels is dependent on the consumption of a sufficient quantity of oatmeal or oat bran, over an extended period of time. Oatrim and NutrimX, both of which are soluble fibre powders derived from oat flour or bran, can be easily incorporated into commonly consumed foods, such as meats, dairy and baked items (Inglett *et al.* 2000). More extensive use of soluble fibre materials in food applications should help make a sustainable consumption of oats possible for broader nutritional benefits. The most recent technological developments and nutritional properties of these two oat soluble fibre products, Oatrim and NutrimX, will be reviewed in this chapter.

24.2 Oatrim

Oatrim is a soluble fibre that contains oat product, and which is helping industrial organisations to focus on foods that are being generated for people seeking a healthy lifestyle, with

increased longevity and vitality, and less chronic diseases. Oatrim was discovered (Inglett 1990) and patented (Inglett 1991) by the US Department of Agriculture, with licenses granted to industrial organisations.

The process involved the α-amylase conversion of gelatinised starch from oat flour or bran to amylodextrins, while retaining the biologically activity of its soluble fibre. The recovered product, soluble fibre (β-glucan) and amylodextrins from the processing, has been called oat β-glucan-amylodextrins, hydrolysed oat flour, or Oatrim. Barley and other cereal grains have also been used to prepare other amylodextrin compositions containing their soluble-fibres (Inglett 1992). Components in Oatrim, other than amylodextrin and β-glucan, include protein, lipid and minerals in small amounts, as compared with the flour or bran (Inglett 1993).

Since the combination of β-glucan and amylodextrins produces an excellent fatty textured material, Oatrim was found to be useful as a fat replacement in preparing food products. The powder can be converted to a shortening-like gel by heating and cooling a 25% Oatrim dispersion. The fat-like gel has an energy content of 1 kcal/g, compared with 9 kcal/g for fat (Inglett & Grisamore 1991). The gel is heat-stable for baking and pasteurising operations, and replaces shortening in food recipes on an equal weight substitution basis. The amount of Oatrim powder used in recipes can be modified to achieve the intended functional and textural properties desired. Both powder and gel give the sensory attributes of fatty texture and natural-tasting products. Generally, the replacement of saturated and *trans* fat are the intended use, but besides replacing unusually high levels of saturated and *trans* fat in foods, it also lowers calorie content and adds soluble fibre to the diet. These properties make it possible for the reduced-fat foods to both look and taste like a traditional, higher-fat food.

Oatrim powder, or its gel, can be used in most food applications, including processed meats, pasteurised cheeses, milks and baked products. More healthful designed products containing Oatrim are appearing frequently in foods that are considered to be more nutritious. Some major products that are either 'reduced-fat' or 'fat-free' are currently under active development, including meats, frozen desserts, salad dressings, sauces, gravies, soups, mayonnaise, margarine, breads, waffles, granola bars, muffins, cookies, brownies, beverages and cakes (Inglett & Warner 1992; Inglett & Newman 1994; Inglett *et al.* 2000). The Oatrim content in food items is noted on ingredient labels as hydrolysed oat flour or Oatrim.

The blood cholesterol-lowering properties of Oatrim have been demonstrated in studies with both chickens (Inglett & Newman 1994) and hamsters (Yokoyama *et al.* 1998b). In the first clinical trial in humans, Oatrim received a 'Two Thumbs Up for Oatrim' review in the December 1993 issue of *Agricultural Research* published by the USDA (McBride 1993), which reported on some of its preliminary nutritional properties. Subsequent nutritional research revealed that the consumption of 5 g of oat β-glucan per day, in the form of Oatrim, gave a wide variety of health benefits (Behall *et al.* 1993, 1997; Scholfield *et al.* 1993; Hallfrisch *et al.* 1995). These nutritional benefits were reported as follows:

(1) Oatrim could totally substitute or partially replace fat in foods.
(2) The soluble fibre in Oatrim has a hypocholesterolaemic property (lowers total blood cholesterol).
(3) Oatrim can be used for weight reduction.
(4) Oatrim has strong antioxidant properties, and thus greatly reduces the amount of oxidation products in the urine.

Research on the nutritional properties of Oatrim as a source of soluble fibre in the diet (Behall *et al.* 1997) is ongoing.

The rheological properties of Oatrim suspensions have been studied over the past few years (Carriere & Inglett 1998a, 1999). Depending on the β-glucan content of the material, Oatrim suspensions can exhibit shear-thinning, or shear-thickening behaviours (Carriere & Inglett 1999). At high levels of β-glucan, Oatrim suspensions have been shown to exhibit double shear-thickening regions, with concomitant double stress overshoots observed in the transient experiments (start-up of steady-state shear flow). The response of Oatrim suspensions (5% by weight in deionised water, with varying levels of β-glucan) during thixotropic loop experiments at 25ºC are shown in Fig. 24.1. For Oatrim-5 (5% by weight β-glucan) the suspension exhibits shear-thinning behaviour across the shear rate range. For Oatrim-10 (10% by weight β-glucan), the suspension displays shear-thinning behaviour up to a shear rate of roughly 20 s^{-1}. From 20 to 80 s^{-1} a region of shear-thickening behaviour is observed, the phenomenon being most likely due to the partially solubilised materials in the suspension. Above 80 s^{-1}, shear-thinning behaviour is again evident. For Oatrim-24 (24% by weight

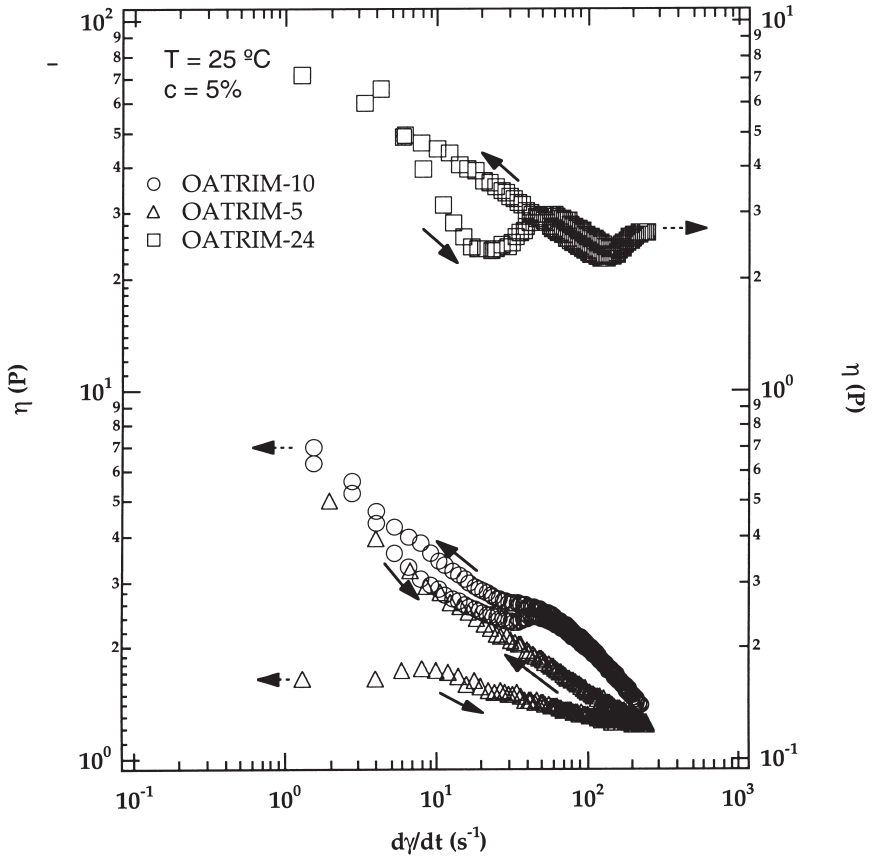

Fig. 24.1 Rheological response (shear viscosity versus shear rate) of Oatrim suspensions with varying β-glucan levels to a thixotropic loop experiment. The solid arrows denote the direction of the applied shear flow.

β-glucan), the suspension displays shear-thinning behaviour up to a shear rate of 20 s^{-1}, where shear-thickening behaviour is observed in accord with the behaviour observed for Oatrim-10. At 100 s^{-1}, a second region of shear-thickening behaviour is observed. The exact cause of this unusual rheological behaviour is currently unknown.

24.3 NutrimX

NutrimX is a new generation of soluble β-glucan products having important health benefits for inclusion in functional foods and nutritional supplements (Inglett 1998a). These new natural ingredients were prepared from oat and barley grains for the potential healthy, 'good-for-you' foods by overcoming some of the principal oppositions to traditional oat and barley products.

The NutrimX family of mechanically solubilised β-glucan products all have lower bran cellulosic components and lipids than do the starting materials (Inglett 1998b). They are prepared in high yields by a wet process that removes the crude fibre components (hull and miscellaneous cellular fragments), along with some of the lipids. The ingredients can be used in the liquid or dried form. NutrimX is generally dried to give high yields of the products. A patent application was filed August 7, 1998, and this has recently received allowance for issue in 2000 (Inglett 1998b).

NutrimX can be used in various formulations of functional foods or nutritional supplements. It can also be used to increase the levels of soluble β-glucan, typically to about 0.75 g soluble β-glucan per serving. NutrimX can also reduce fat, especially excessive saturated and *trans* fats, in various foods. NutrimX imparts moistness, softness and cohesiveness in baked foods such as muffins and cookies (Warner & Inglett 1998), in addition to improving the texture of a calcium-fortified 'smoothie' beverage containing NutrimX and imparting 0.75 g β-glucan from oat flour per serving. Many new-type foods were made using NutrimX as a texturiser or a binder, including frozen desserts, breakfast foods, beverages, meats, non-dairy creamers, canned soups, and bakery items such as muffins and cookies.

NutrimX can be used as a substitute for dairy cream or coconut cream (including milk) (Maneepun *et al.* 1998a, b). A 5% solids dispersion of NutrimX OB from oat bran (OB) has the consistency of cream. NutrimX OB can replace dairy cream or coconut cream at 100% replacement for some foods; indeed, coconut cream replacement was especially successfully at 60–80% levels in eight food desserts that have been evaluated. NutrimX ingredients imparted increased moistness, smooth texture and cohesiveness to various other functional foods. A limiting factor in replacing butter and coconut cream was the decreasing flavour from fat with increasing NutrimX substitution. Generally, about 50% of these fats can be replaced in desserts without any serious effect on the sensory properties of the food products. Higher replacement levels could be achieved if some of the butter or coconut flavours are added, or other flavourings used.

At the current level of development, NutrimX compositions are available in a 1–15% range of β-glucan levels. NutrimX OF from oat flour (OF) is called Nutrim-5 (the 5 represents the percentage of β-glucan), and has a composition as follows: moisture, 5.83%; protein, 5.01%; lipid, 1.06%; ash, 2.98%; β-glucan, 4.45%. Currently, Nutrim-5 is available only in pilot plant quantities, but may be used in foods or nutritional supplements in order to obtain a health claim.

The solubilised oat products contain lower molecular weight β-glucans than are found in the uncooked material (Knuckles *et al.* 1998). Reduced β-glucan molecular weights have been identified in the ordinary cooking of oatmeal. Contrary to reducing the biological activity of its soluble fibre, the processing appears to make the β-glucan more biologically active (Yokoyama *et al.* 1998a). The NutrimX products also maintained about the same antioxidant properties as the starting oat materials (Prior *et al.* 1998).

The NutrimX products have unusually high viscosities at 5–15% solids and ambient temperatures (Carriere & Inglett 1998b), the viscosities being many times greater than that of Oatrim, the enzyme-hydrolysed flour (Carriere & Inglett 1998c). NutrimX materials produced from defatted oat fines (DFOF), oat flour (OF), barley flour (BF) and oat bran (OB) were found to exhibit shear-thinning throughout the shear rate range studied during a thixotropic loop experiment, as is illustrated in Fig. 24.2 (Carriere & Inglett 2000). No regions of shear-thickening behaviour were found for any NutrimX materials. The rheological responses of the various NutrimX suspensions during either cycle of a thixotropic loop experiment, can be modelled using a power law constitutive equation. The NutrimX suspensions behave as pseudoplastic materials, with power law exponents ranging from 0.42 to 0.71.

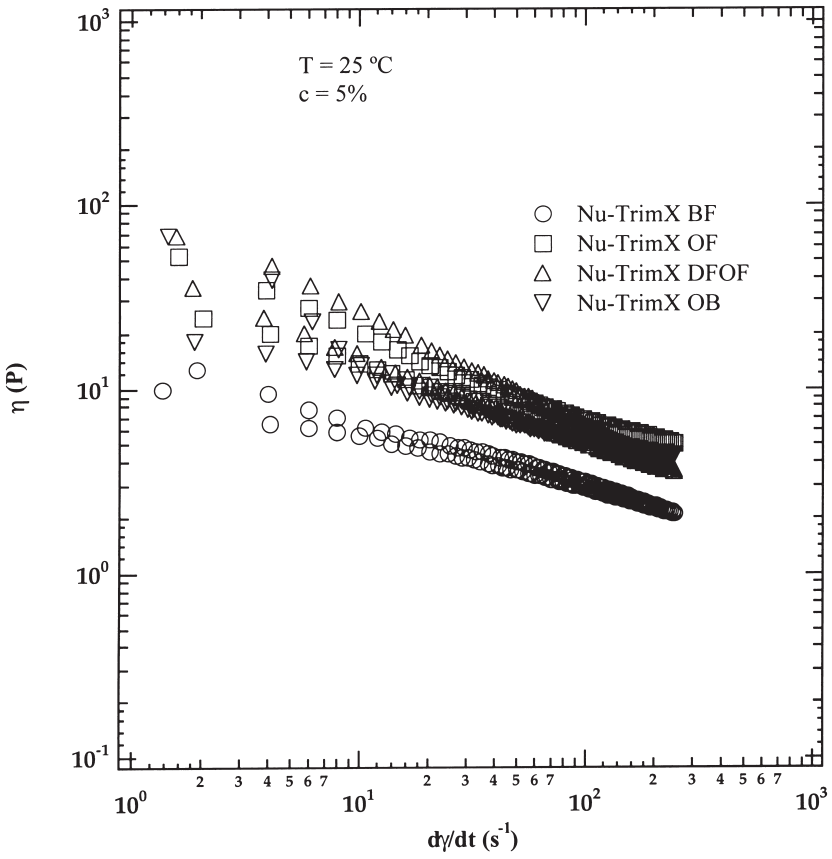

Fig. 24.2 Rheological response (shear viscosity versus shear rate) of NuTrimX suspension.

The number of NutrimX products can be expanded by co-processing with oilseed, wheat, corn and various starches. These products can have unusual functional properties and nutritional properties for the health food markets. Major benefits might also be obtained in manufacturing costs and yields of the desired products, making the licensing of NutrimX technology a much more attractive proposition (Inglett 1999).

24.4 Summary

The technological development of Oatrim has produced a unique soluble fibre material derived from oats that has the potential to improve public health on a world-wide scale. The consumption of foods high in saturated and *trans* fats appears to be an increasing public health problem, and for this reason alternative foods and nutritional supplements are becoming more popular. Oatrim illustrates the availability and use of a soluble fibre ingredient that may add health benefits and nutritional properties to foods. At present in the United States, foods containing Oatrim attract sales on a multi-million dollar scale. Oatrim is used in a wide variety of health- and nutrition-related foods, and particularly in fat-reduced cookies, cereal bars, muffins, beverages and meats. NutrimX is a new source of soluble β-glucan ingredients for use in nutraceutical and nutritional supplement products. NutrimX from oat flour (Nutrim-5) is a new product, pilot plant quantities of which are currently available for use in nutraceuticals or nutritional supplements. Moreover, in addition to improving nutritional quality, Nutrim-5 can increase the health benefits of foods.

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25 Effect of Processing on the Properties of Dietary Fibre

Kaisa Poutanen

25.1 Introduction

A large part of dietary fibre intake results from the consumption of processed food. The dietary fibre in food products either originates from natural raw materials, or increasingly, from industrial ingredients tailored to serve as specific sources of fibre. Hence, the process-induced changes at different steps of the production process are relevant to the nutritional and functional properties of dietary fibre in the final food product.

Since the introduction of the dietary fibre concept of Trowell in 1972, much epidemiological research has been conducted, showing the presence or absence of correlations between dietary fibre intake and the risk of certain diseases. The sometimes controversial results have led to new discussions being conducted about the definition of dietary fibre, and there is increasing demand to be able to analyse not only the quantity of dietary fibre, but also its quality. This is especially true when referring to process-induced changes. Even though the amount of dietary fibre would remain the same, physiological functionality in the small and large intestine – as well as the bioavailability of the associated bioactive, non-nutrient compounds – may vary according to the processing strategies chosen.

25.2 Processing

Cereals, vegetables and fruits are the major sources of dietary fibre in consumer foods, and also, they are the raw materials for the manufacture of fibre-rich ingredients. In the processing of plant foods, there are various steps and unit operations that influence the sensory and nutritional quality, safety and stability of the product. Important characteristics of dietary fibre amenable to changes during processing include molecular, structural and functional properties. The two major reasons behind the effects of food processing on dietary fibre are hydrolytic enzymatic reactions and chemical degradation or crafting reactions, which are affected by the amount of water and thermal energy used. In addition, changes in the physical properties of dietary fibre preparations and food products, such as particle size, surface area and porosity, are caused by shear forces in mechanical and physical processing, and influence especially the extractability and rheological properties of fibre in the food matrix.

It is important to remember that, in addition to the nutritional properties, the different fibre constituents have many effects on the functional properties, sensory quality and stability of food (Tables 25.1 and 25.2). The structure–function relationship and process-induced changes are, in this respect, better understood than the influence of dietary fibre properties on physiological effects. It seems that there has often been a ‘black box’ between food technologists and nutritionists: both have spoken about the same components but with different terms. With the increasing interest in the relationships between food and health, the situation is

Table 25.1 Different properties of dietary fibre.

Molecular properties	Chemical structure Degree of polymerisation
Structural properties	Molecular interactions Cell walls Cell and tissue structures
Functional properties	Viscosity Water binding Solubility

Table 25.2 Different effects of dietary fibre.

Effects on food quality	Sensory properties Stability
Physiological effects	Intestinal transit time Nutrient absorption Colon fermentation
Health effects	Lipid and sugar metabolism Cardiovascular disease Cancer

changing. It is, however, easier to analyse changes in dietary fibre properties during processing than to assess the nutritional importance of these changes. More predictive methods are needed to link the technological and nutrition research, and to relate the molecular and structural properties to physiological effects.

25.3 Effects of mechanical processing

As the chemical composition of dietary fibre components may vary in different parts of the raw material tissue, fractionation of plant raw materials by milling produces fractions with quite different dietary fibre characteristics. This has been clearly demonstrated, for example in rye (Härkönen *et al*. 1997; Glitso & Bach Knudsen 1999). Milling also changes important physical properties of the fibre-containing material, such as particle size and hydration properties, which are important both from technological (Zhang & Moore 1999) and nutritional (Yiu 1989) points of view. Mechanical energy has, in several cases, also been shown to have an influence on polysaccharide structure. Shear forces caused by decanter and disc centrifuges are one reported cause of depolymerisation of oat β-glucan and subsequent loss in viscosity of oat fractions (Wood *et al*. 1989).

25.4 Effects of endogenous and added enzymes

The level of endogenous enzymes is one of the reasons for variation in the quality of plant raw

materials. In cereal processing, both non-desired (sprouting) and desired (malting) synthesis of cell-wall hydrolases and enzyme-catalysed reactions occur during germination of the grain. The role and properties of cell wall-degrading enzymes, such as xylanolytic enzymes or β-glucanases, as well as their inhibitors in grains, are being elucidated. Since the dietary fibre components act as substrates for these enzymes, depolymerisation and solubilisation of fibre occurs when suitable conditions are created for the enzymes to be active. In particular, wet processes at moderate temperatures, such as wet milling, extraction or prolonged baking procedures (e.g. sour dough baking) may activate the enzymes in grains. In fruits and vegetables, enzymes active in the degradation of pectin cause similar effects.

The molecular weight of β-glucan in oat-based dietary fibre preparations has seldom been reported in nutritional studies, but variation in molecular weight and hydration properties of β-glucan may partly explain the differences reported in cholesterol-lowering effects of oat products, as reviewed by Ripsin *et al.* (1992) and Kahlon & Chow (1997). In the processing of oats, even very low levels of endogenous β-glucanase can partially depolymerise β-glucan, leading to products with much lower viscosity than expected (Jaskari *et al*. 1995). Because surface sterilisation of oat groats yielded more viscous oat flour slurries (Zhang *et al.* 1997) than non-sterilised groats, it has been suggested that β-glucan hydrolases on the surface of the oat groats caused the viscosity losses as observed in raw and kilned oats. Also an enzyme-deactivated oat bran concentrate yielded an oat gum dispersion with increased β-glucan content and solution viscosity; however, the yield was lower (Beer *et al*. 1996). The relationship between depolymerisation, hydration capacity and viscosity of the extractable dietary fibre components is a complex one, and process optimisation is needed to obtain the desired physiological responses.

Endogenous cell wall-degrading enzymes influence the baking properties by changing the cell wall structure and hydrolysing polymers when whole meal flour is used, as in the baking of Finnish rye bread (Fabritius *et al*. 1997; Autio *et al*. 1998), or when bran fractions are used to increase the fibre content of wheat bread (Laurikainen *et al.* 1998). Water-extractable polysaccharides of higher molecular weight could be extracted from autoclaved rye bran than could be from normal bran, indicating the role of endogenous enzymes in determining the degree of polymerisation of soluble fibre (Laurikainen *et al*. 1998). The degradation of cell wall polymers and cell wall structures, and thus the amount and size of insoluble particles in the dough, depend on the levels of endogenous enzymes (Fabritius *et al*. 1997). If the level of endogenous enzymes in cereal raw material is high, depolymerisation of added fibre preparations may occur (unpublished results at VTT Biotechnology).

In baking, the use of hemicellulases – especially xylanolytic enzymes – has clearly increased in recent years (Poutanen 1997). Even though pentosanases are used for technical reasons (increased loaf volume, improved crumb structure, longer shelf-life), they obviously also change the characteristics of the dietary fibre. Most of the enzymes have a higher activity towards soluble substrates, and hence may hydrolyse part of the dietary fibre components to small oligomers, which are no longer precipitated in the classical fibre assays. A reduced fibre content of bread, due to the use of xylanase-containing enzyme mixtures, was recently demonstrated by Laurikainen *et al*. (1998). On the other hand, enzymes releasing high-molecular weight polymers from the cell-wall matrix might increase the amount of soluble, highly viscous material.

25.5 Effects of thermal processing

Thermal processing, such as steaming, cooking, baking, extrusion and microwave treatment, changes the properties of dietary fibre in many ways (Nyman *et al*. 1994). Both increases and decreases in the total dietary fibre content have been reported, as well as changes in extractability, leading to redistribution between the relative amounts of soluble and insoluble fibre (McDougall *et al*. 1996). Svanberg (1997) showed the effects of processing on dietary fibre in vegetables to be complex, with heat processing generally degrading the dietary fibre polysaccharides. The lower molecular weight and the lower viscosity of the fibre showed this. For example, in carrots the degree of depolymerisation and the loss of intermolecular association of water-soluble polysaccharides depended on the severity of heat treatment (Svanberg *et al*. 1995, 1997). Solubilisation and depolymerisation of polysaccharides was also observed with repeated microwave treatments of green peas; however, the molecular weight was again similar after passage through the stomach and small intestine of rats (Svanberg *et al*. 1999).

In cereal processing, a slight increase in soluble fibre content has been reported due to flaking of oats, rye and wheat (Plaami 1996). Increased solubility and partial depolymerisation of β-glucan in wheat bread containing oat bran has been reported by Sundberg *et al*. (1996). Increased solubilisation of the major dietary fibre component, arabinoxylan has been observed during baking of both wheat (Westerlund *et al*. 1989) and rye bread (Autio *et al*. 1996). The water-soluble arabinoxylans in wheat bread had a higher degree of arabinose substitution than those in the flour and dough, indicating increased solubilisation of especially highly substituted arabinoxylans during baking (Westerlund *et al*. 1990). The total amount of arabinoxylans was smaller in wheat bread crust than in the crumb, probably due to increasing degradation caused by more severe thermal conditions (Westerlund *et al*. 1989).

Extrusion, which is widely used in the production of breakfast cereals and snack foods, combines thermal and mechanical energy. Here, extensive degradation of the dietary fibre polysaccharides has been reported, resulting in increased solubility and possibly also, reduced viscosity of the dietary fibre (Camire & Flint 1991; Ralet *et al*. 1991). Increased solubility of the dietary fibre in wheat flour was also observed during extrusion, and as compared with wheat flour, the fibre in the extruded product was more extensively degraded in the rat intestine (Björk *et al*. 1984). The effects may be controlled by the main extrusion variables: water content, temperature, extruder geometry and screw speed. Extrusion has also been shown to increase fibre content by increasing the Klason lignin value of potato peels and wheat, possibly by catalysing the Maillard reaction (Theander *et al*. 1993; Camire *et al*. 1997).

25.6 How to optimise the properties of dietary fibre in food processing

In most countries, dietary recommendations suggest that consumers should increase their dietary fibre intake. Fibre also has a positive health image, which should encourage consumers to increase their purchase of foods rich in fibre. However, despite these points, why is the intake of dietary fibre still so low?

One reason lies in the eating habits of modern consumers: not enough fibre sources are available in popular product categories, such as convenience and snack food. Another reason lies in the fact that, often it is not easy to produce palatable foods with high levels of dietary

fibre. For example, in the baking of wheat bread, addition of bran to increase the fibre content is known adversely to affect the texture, crumb structure and loaf volume of the final product (Rao & Rao, 1991; Zhang & Moore 1999). Improved results have been obtained through different milling (Zhang & Moore 1999), and chemical and enzymatic pre-treatments (Rasco *et al*. 1991) of the bran particles, or by the use of enzymes during baking (Laurikainen *et al*. 1998). In all of these studies the main objective has been to improve the sensory quality, and little attention has been paid to the nutritional properties of the dietary fibre.

When aiming at foods with positive health effects, processing should optimise both technically and physiologically important raw material properties. The tendency to consider the sensory properties and consumer acceptability in the first instance is, however, logical from the nutritional point of view. Only foods which are eaten will have any physiological functions, and also the dietary fibre in foods not chosen by consumers remains insignificant for public health.

When specific nutritional or health properties of a food are used in marketing to increase purchase intentions – as in the case of health claims – special care should be taken to ensure the claimed health benefits of the food. As shown above, the addition of adequate amounts of an ingredient rich in oat β-glucan does not necessarily mean that the product will retain the anticipated health effects of the ingredient. Even though the food is carefully processed, storage conditions (e.g. deep-freezing) can change dietary fibre functionality by decreasing the solubility of, for example, β-glucan, reflecting changes in molecular organisation and crystallisation (Beer *et al*. 1997).

Food processing has an important role in manufacture of palatable, nutritious foods with high levels of dietary fibre. Good collaboration between food technologists, chemists and nutritionists as well as consumer scientists is needed to understand and exploit the mechanisms by which dietary fibre properties change and influence end-product quality in processing.

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26 Fibres and Fibre Blends for Individual Needs: a Physiological and Technological Approach

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26.1 Definition and origin of dietary fibres

By definition, dietary fibres are organic components of plants which cannot be degraded by human digestive enzymes, and thus remain unabsorbed in the small intestine. On the basis of their physiological definition, dietary fibres are composed of different groups of materials, the most important being those of the plant cell walls (cellulose, hemicellulose, lignin and pectin). Other categories of dietary fibres are non-starch reserve polysaccharides (guar, locust bean gum, konjac flour), wound-inducible plant gums (gum arabic, gum tragacanth) and several minor components such as waxes or condensed phenolics (Schulze *et al*. 1993).

From the nutritional point of view, components of the plant cell walls are the main sources of dietary fibre, the main components being carbohydrates and lignin (a polymer of coniferyl or related alcohols). Depending on the species of the plant, its age, the type of tissue and the plant's growth conditions, the fibre content – as well as the relative composition – will differ. Analytical methods according to German food law (§ 35; LMBG 1985), or to AOAC methods (enzymatic-gravimetric methods) (AOAC 1995), distinguish between soluble and insoluble fibres and include cellulose, hemicellulose, lignin, pectin and resistant starch.

Traditionally, those parts of plants selected for human consumption were chosen based on their nutritional value, and included both energy-rich and fibre-depleted raw materials. Dietary fibres were regarded as superfluous for nutrition, and attempts were made to reduce their levels in food. In nature, the highest fibre content is located in the outer regions of cereal grains, and in the peels or husks of fruits and vegetables. Here, the protective cell walls are more developed than around internal cells. This, when enhanced by processing methods, means that today's food is relatively poor in fibre content. In contrast, lignified tissue is rich in fibre, but is not normally used as food.

For many hundreds of years, the fibre content of cereals and other seeds ensured that humans had a high fibre intake, but changes in nutrition during recent decades (increased sugar and meat consumption, reduced bread consumption) as well as improved milling technology have led to a continuous fall in fibre intake (Ulbricht and Pfaff 1993; DGE 1996). Nowadays, the starchy endosperm of seeds can be effectively separated from the fibre-rich seed coats, and people prefer to prepare food from white flour. Some food regulations, such as the German law for pasta articles, even state an upper limit for the degree of milling of pasta flour. However, it should be noted that these developments have occurred based mainly on sensory considerations.

Since the studies of Trowell and co-workers (Trowell *et al*. 1985) (Table 26.1) which drew connections between dietary fibre intake and the occurrence of diseases in modern civilisations, fibres have been viewed in a new light, and their properties have spawned many dietary

Table 26.1 Diseases resulting from a nutrition low in dietary fibre, hypotheses according to epidemiological studies (Trowell *et al*. 1985).

- Constipation
- Diverticular disease of the colon
- Cancer of the large bowel
- Appendicitis
- Crohn's disease and ulcerative colitis
- Functional gastrointestinal disorders: irritable bowel and other syndromes
- Duodenal ulcer
- Hiatal hernia and gastro-oesophageal reflux
- Obesity: interaction of environment and genetic predisposition
- Diabetes mellitus (non-insulin-dependent diabetes)
- Gallstones
- Lipid metabolism and coronary heart disease
- Varicose veins, haemorrhoids, deep-vein thrombosis and pelvic phleboliths
- Renal stones

fibre-related research programmes. Following the acceptance that dietary fibre improves the health-promoting properties of food, a major focus of research has been directed towards improving their sensory properties. With tailored fibres, it is possible to enrich food with dietary fibre without changing the food's known and preferred texture and flavour. By contrast, fibres can be added to improve the quality parameters of foodstuff, such as freshness or mouthfeel. Additionally, special dietary fibre isolates can be used as functional ingredients, for example to enhance flavour, colour or texture (see Table 26.5).

26.2 Raw material for the isolation of dietary fibres

Raw materials containing dietary fibre are available in large quantities; indeed, the classification of such fibres is often based on the traditional use of the raw material for nutrition (Table 26.2):

- parts of plants that are separated during food processing but are usually eaten;
- parts of plants that normally do not serve as food;
- plants in which non-starch polysaccharides are accumulated in large quantities; and
- gums that are secreted in response to wounding.

Depending on the origin, there is a difference in rating of dietary fibres. For the image of a product, and hence its marketability, it is better to use a natural ingredient derived from a raw material that is generally accepted as being 'healthy'. For this reason, the first group above is the most interesting. The European law for novel food (European Parliament and Council 1997) places an emphasis on the importance of the origin of the foodstuff for nutritional acceptance. Dietary fibres that belong to the second group would fall into the class of so-called 'novel food'. Thus, the marketability and value of a dietary fibre directly correlates with the origin of the material. For example, the image of a dietary fibre derived from stems or straw is much lower than that derived from cereal grains or potato pulp.

Table 26.2 Raw materials for industrial dietary fibre production.

Source for dietary fibre isolation/production	Examples	Products
Edible co-products of food processing		
Residues of fruit- and vegetable juice processing	Fruit or vegetable pomace	Pectin, liquid pectin extract, apple fibre, carrot fibre, aronia fibre, raspberry fibre, etc.
Co-products in starch, sugar, oil and protein isolation	Endosperm of cereal grains	Wheat grain fibre
	Endosperm of grain legumes	Lupin fibre, pea (cotyledon) fibre
	Potato pulp	Potato fibre
Non-edible residues of food production		
Separated parts or tissues of plants that usually were not consumed by humans	Senescent stems	Wheat stem fibre, bamboo fibre
	De-sugared beetroot pulp	Sugarbeet fibre
Indigestible storage or skeleton carbohydrates		
Polyfructan-containing tubers	Jerusalem artichoke, chicory	Inulin, oligofructose
Non-starch reserves of endosperm	Locust bean, guar bean, konjac	Locust bean gum, guar gum, konjac gum
Reserves/skeleton of aqueous plants	Red or brown marine algae	Agar-agar, carrageenan
Lignified or wooden material	Straw, trees, nut shells	Microcrystalline cellulose, modified cellulose
Exsudates		
Plant gums that form after wounding cell tissue	Acacia trees	Gum arabic
Microorganism	*Xanthomonas* spp.	Xanthan gum

26.3 Physical-chemical aspects

The interaction of dietary fibres with other food ingredients is very important in food processing. These interactions (e.g. dough rheology) depend on the chemical properties as well as the macromolecular structure. The number of charged and/or polar groups on the surface of the polymeric chains influence their interactions with other food components. Linkage type and branching or substitution of the main polymer chain influence solution properties.

Macromolecular structure is another important factor that influences the functionality of carbohydrate polymers. Insoluble dietary fibres are a consequence of inter-chain linkage of numerous polymer chains. The soluble fibre pectin, which exists as a structural element in primary cell walls, can be extracted from cellular matrix. The composition of plant cell walls undergoes changes during the development of the cell. Thus, fibre isolated from senescent stems where cell walls stabilise the plant against environmental influences (e.g. wheat fibres), differ from fibre isolated from endosperm cell-wall material (e.g. Herbacel AQ Plus Wheat grain fibre). Dietary fibre with high water binding capacity can be isolated especially from cells that are involved in the water or nutrient transport system in the plant. If the capillaries in the plant material remain intact during processing, the dietary fibre isolate resembles a sponge in water. A comparison of some common fibre isolates is given in Table 26.3.

In contrast to the insoluble fibres, most soluble fibres (except polyfructose) produce aqueous solutions of high viscosity, or form gels in which liquid is immobilised.

As well as this important interaction with water, the combined charged, hydrophilic and hydrophobic properties of various soluble fibres leads to a range of reactions with other components such as cations and cholesterol. Thus, different dietary fibres have different physiological properties (Table 26.4).

26.4 Physiologically nutritional properties of dietary fibres

Hippocrates, in the fourth century BC remarked that, 'For the human body there is a big difference if the bread is made from fine or coarse flour, and if made with flour containing bran or not'. Later, Hakim, a Persian doctor from the eleventh century noted that, 'Chuppatis containing more bran are digested faster. Those containing less bran need a longer time to get excreted'.

Table 26.3 Comparison of the dietary fibre contents of some fruit and cereal-based dietary fibres.

	Total dietary fibre content (%)	Soluble dietary fibre content (%)	Water-binding capacity (g/g fibre)
Classic Apple Fibre	60	15	3–5
Classic Citrus Fibre	68	28	7–8
Herbacel AQ Plus Apple Fibre*	87	15	13–15
Herbacel AQ Plus Citrus Fibre*	92	17	14–17
Herbacel AQ Plus Wheat grain fibre*	88	27	11–13
Wheat Fibre (straw)	95	3	6–8
Cellulose (E460)	99	–	2–3

*Trade name of Herbafood Ingredients GmbH.

Table 26.4 Physiological aspects of dietary fibres.

Physiological effects	Commercial dietary fibres
Water-holding capacity (saturation of the surface, swelling)	
• fibrous dietary fibres (cellulose type) with hydrophilic polar groups	Carboxymethylcellulose, Methylcellulose
• particles with capillary cavities	Fruit fibres, wheat grain fibre, lupin fibre
Gel formation or strong viscosity building	Pectin, agarose, carrageenan, xanthan, guar, locust bean gum
Binding of cations	
• acidified polysaccharides	Pectins, alginates, carrageenans
• lignin	
• phytic acid	
Binding of sterols	
• polysaccharides with hydrophobic groups	High-methoxyl pectin
• surface-active substances	Gum arabic
• high-viscosity polysaccharides	Guar
Microbiological usability	
• di-, oligo-, and polysaccharides	Pectin, inulin, pentosan concentrate, gum arabic, guar

Today, much research is performed on the diseases resulting from diets low in dietary fibre. In the book *Dietary Fibre, Fibre-depleted Foods and Disease*, Trowell *et al*. (1985) devoted a complete chapter to each disease (see Table 26.1).

The physiological effects of dietary fibres vary, depending on their chemical structure. These effects can be categorised into water-holding capacity, gel formation, binding of cations, binding of sterols and microbiological usability (Table 26.4). These physiological effects of dietary fibres can be expressed in different sections of the gastrointestinal tract, for example the mouth and stomach, the small intestine and colon, as well as outside the gastrointestinal tract.

26.4.1 *Effects of dietary fibre in the mouth and stomach*

In the mouth and stomach, dietary fibre prolongs the time of chewing and dilutes the energy content of the food. This also results in an extended time of consumption, which influences the amount of foods consumed, and results in a reduced intake of calories. Dietary fibres that increase the viscosity of the digestion mass delay the speed of excretion, which leads to a prolonged feeling of satiety, and therefore reduce further consumption. The positive effect of pectin on the dumping syndrome, i.e. uncontrolled emptying of the stomach, has been described by Behall and Reiser (1986).

26.4.2 *Effects of dietary fibre in the small intestine*

Dietary fibre influences the transit time of the digestion mass within the small intestine in different ways. Soluble dietary fibres prolong transit by increasing the viscosity, while insoluble

dietary fibres reduce transit time by increasing the contraction frequency and by extension of each contracting intestinal section. Pectin and guar as dietary fibres are purported to reduce the rate of absorption and digestion. They supposedly bind enzymes non-specifically, and thus reduce their activity by preventing the formation of enzyme–substrate complexes. These dietary fibres strengthen the so-called unstirred water layer in the gut, which leads to a higher diffusion barrier and therefore to a reduced rate of absorption. Polyionic dietary fibres shift the pH profile, which in turn influences the secretion of acids and bases and the excretion of enzymes and hormones. This leads to a reduced hydrolysis of carbohydrates within the small intestine, and thus to a reduced absorption of monosaccharides and flattening of the postprandial serum glucose level curve; for this reason, soluble fibres are successfully used in the therapy of diabetes mellitus type II. The affinity of polyanionic dietary fibres (e.g. pectin) for cations has several positive effects, one of which is the binding and removal of poisonous heavy metals (Endress 1997). Negative effects related to the binding and removal of essential minerals have been debated in the past, although no difference has been found in the mineral balance of vegetarians compared with non-vegetarians. Furthermore, mineral dietary fibre complexes can be used as fortifiers of minerals. The affinity of dietary fibres for sterols has been examined widely (Endress 1991). Soluble fibre intake may result in the reduced absorption of bile salts from the small intestine into the liver, leading in turn to increased excretion of nutritive and endogenous cholesterol, glycerides and fatty acids, as well as phospholipids and bile acids.

26.4.3 *Effect of dietary fibre in the colon*

In the colon, the presence of dietary fibres results in an increased water-holding capacity which, depending on the type of dietary fibre, may promote or reduce flatulence under the corresponding conditions. Microbial decomposition of dietary fibre leads to an increased stool volume (bulking), the overall amount of bacteria being increased. Due to the increased metabolic activity of these bacteria however, more short-chain fatty acids are formed, and this results in a reduction of colonic pH that may have positive benefits. High metabolic activity of the colonic microorganisms leads to the formation of gases that may (negatively) cause flatulence, but this effect will diminish or even disappear as the subject adapts to a fibre-rich diet. Such gas formation may have positive benefits, however, by delaying the growth of colorectal cancer cell-lines, and also by helping to form light-consistency stools. In some cases, there is an increased formation of *n*-butyrate, which influences the growth rate of cells and improves the nutrition of colon-enterocyte cells (Schulze & Zunft 1993).

26.4.4 *Effects of dietary fibre outside the gastrointestinal tract*

Dietary fibres also exert an effect outside the gastrointestinal tract, for example in the reduction of the serum glucose levels after a carbohydrate-rich meal. In addition, fibre may reduce the rate of transit of ammonia and amines into the blood, and thus relieve the associated liver and kidney function. Dietary fibre also has a positive effect on arteriosclerosis by reducing serum cholesterol level through an influence on the enterohepatic pathway. Also, polyanionic dietary fibres (mainly low methoxylated pectin) assist in the excretion of toxic heavy metals via the urine, with an overall positive effect on the body. Finally, reduced energy input and reduced absorption of nutriments lead to a reduction in body weight.

26.4.5 *Dietary fibres as prebiotics*

One important property of some dietary fibres is that they can be metabolised by colonic microorganisms, leading to an enhanced bacterial growth and increased stool mass. Certain microbial metabolic products are also health-promoting; if the composition of the colonic flora is influenced positively (e.g. by stimulation of lactobacteria or suppression of *Clostridia*), then fibrous material may have a prebiotic effect.

26.5 Additional properties of dietary fibre

Advantages may be gained in using some dietary fibre concentrates in food products by utilising the natural combination of these materials with other secondary plant metabolites or flavour compounds (see Table 26.5). In the case of chokeberry or blackcurrant, colouring is very distinctive. Due to their integration in the fruit tissue, these anthocyanin colourings are stable to both heat and light. Even more importantly, members of plant phenolics act as antioxidants, which are active in protecting against cardiovascular and coronary diseases (Bitsch 1999). Several dietary fibres have a distinctive flavour, for example citrus fibres or fibres made from berries or vegetables. Other nutrients are often also present in combination with dietary fibres, for example bioflavonoids in citrus fibres, polyunsaturated fatty acids (PUFA) in blackcurrant fibre, or provitamin A in carrot fibres (Herbafood Ingredients 2000).

26.6 Technological aspects of dietary fibre as functional ingredients in foods

As mentioned above, the properties of a dietary fibre isolate or concentrate depend on its chemical composition and structure. Some properties, such as water retention, are equally important from both physiological and technological viewpoints. Generally speaking, soluble fibre is able to immobilise large amounts of water and, under certain conditions, to form a gel. In contrast, insoluble fibres bind less water, but have a more or less fibrous structure and can be used as a filler with a stabilising function in food systems. The sensory perception of insoluble fibres is 'rough', but they become smoother with decreasing particle size or breakage of compact molecular linkages through thermomechanical techniques.

Today, the behaviour of fibres can be tailored to fulfil criteria that are ideal for individual needs. Different processing steps exist to obtain special properties, namely:

(1) Physical processing of dietary fibres:
- Milling and sieving
- Extrusion
- Cooking

(2) Extraction media and drying process:
- Enzymatic catalysed or chemical modification of dietary fibres
- Degradation of molecular mass
- Methylation, amidation, etc.
- Debranching

Very few, if any of these modifications have been applied to every dietary fibre or raw material, and thus a great potential still exists in this area. For example, apple pomace has been studied extensively in the past to produce a number of dietary fibre materials, including:

- classic apple pectin (with different functionalities e.g. high and low methoxylation);
- low-viscosity apple pectin;
- pectin-enriched apple fibre;
- classic apple fibre with defined particle size; and
- apple fibre isolates.

Different treatments of apple fibre produces materials that differ widely in their water-binding capacities. Extruded apple fibre was developed especially for baking products, where enhanced water-binding correlates to prolonged freshness. Physiological effects are in relation to particle size. Generally, with decreasing size, microbiological digestibility increases, and with this the stool volume increases. Also, if a specific binding capacity exists, it increases with surface area, which is negatively correlated to particle size.

New products can be produced simply by mixing components. For example, for a fibre-rich instant drink, liquid pectin extract can be mixed with a microfine fruit fibre such as chokeberry before drying, to enhance the cold dispersibility of the final product, a fibre-enriched pectin extract. Other combinations can also be designed for special needs with regard to colour, flavour, fibre content, viscosity, etc.

26.7 Application fields of dietary fibres in food and food supplements

Dietary fibres are often used for reasons other than simply enrichment of food with soluble or insoluble fibre components. Additional advantages, either technological or marketing-orientated, can be achieved by using dietary fibres as ingredients. Success in the market today requires that the product must not only be healthy, but must also fulfil the expectation of the consumer in regard to goodness, taste and/or convenience. Unlike the situation in the past, when bran or classically milled pomace was used to fortify food, high-fibre isolates are available today in a wide product range. Although, fibres with tailored functions can be used for different food systems and to obtain certain properties, two main product types can be prepared by incorporating dietary fibre:

(1) Products that are enriched in dietary fibre but do not differ significantly in terms of sensory or visual properties from traditional products. These products represent an appropriate way to reduce the deficit in fibre by about 10 g per capita, as identified by nutrition scientists (DGE 1996). These could also help to reduce the risk of the diseases, as listed in Table 26.1.
(2) Products in which additional properties of the fibres such as colour or texture (Table 26.5) are used to design innovative products that differ from the traditional product. The dietary fibre content can be referred to as an additional marketing element.

Food ‘engineers’ can use dietary fibres either as a single ingredient or in combinations in order to produce different properties and synergistic effects. For example, in sponge cakes

Table 26.5 Tailored dietary fibres for each application field in the food industry.

Properties	Application field	Dietary fibres
Flavour	Cakes and biscuits, beverages, delicacies, fruit bars	Apple pectin extract, orange fibre, raspberry fibre, carrot fibre, beetroot fibre
Colour	Fruit yoghurts, beverages	Chokeberry fibre, black currant fibre
	Cereal/fruit bars	Mandarin fibre, chokeberry fibre, apple fibre
	Delicatessen	Apple pectin extract, beetroot fibre, carrot fibre
Water-binding	Sausages, salty fillings	Wheat grain fibre, lupin fibre
	Cream cheese	Citrus fibre, apple pectin extract
	Supplement for weight reduction	Citrus fibres, low-viscosity pectin
	Fruit preparations	Citrus fibre, apple pectin extract
	Heat-stable sweet fillings	Fruit fibres, lupin fibre
Binding of cholesterol or bile salts	Food supplement	Apple pectin extract, high-methoxylated pectin
	Beverages, sour milk products	Low-viscosity pectin
Binding of heavy metals	Food supplements, beverages	Low-methoxylated pectin, algin acid
Texture-forming	Ice cream, sauces, desserts	Fruit fibres
	Pasta, salty fillings	Wheat grain fibre
	Gluten-free bread	Pectin-enriched apple fibre, fruit fibres
Lowering of fat, starch or calories	Fillings, soups, creams	Fruit fibres, potato fibre, lupin fibre
	Snacks, extrudates	Oat fibre, pea fibre
	Desserts	Wheat pentosan concentrate, lupin fibre

apple pectin can be used to improve the product's viscoelastic properties (which reduces its fragility), while the water binding properties of wheat grain fibre can be used to enhance the product's freshness.

26.7.1 *Dietary fibres in bakery products*

Fibre-enriched white bread

Bread is still the main source of dietary fibre intake. The dietary fibre content in the starchy endosperm of wheat is low compared with that in the seed coat, but whole-grain products are eaten only in some countries. The dietary fibre content of the most commonly used wheat flour (T550) is about 4 g/100 g, which leads to a final content of below 3 g/100 g bread. This fibre content can be doubled by the addition of only 5–6% of dietary fibre isolate calculated on flour. Consequently, white bread or toast can be labelled as 'rich in fibre', and is physiologically comparable with whole-wheat grain bread. Compared with normal white bread, fibre-enriched bread has the following advantages:

- an enhanced dough stability;
- an enhanced bread yield;
- reduced bakery losses;
- improved bread crumb; and
- prolonged fresh-keeping due to the water binding of fibres and reduced retrogradation of starch.

A number of fibres are available for this enrichment, with fruit fibres, wheat grain or lupin fibres all increasing the dough qualities. For higher fibre fortification it is better to use fibres with high water-binding capacity (>10 g/g fibre) up to a concentration of 2%, and beyond that level to add low water-binding fibres such as oat or pea fibre. By doing this it is possible to produce low-calorie bread that can be recommended for weight-reduction diets.

Bread for special diets

For the production of gluten-free bread it is important to replace or compensate the dough-stabilising function of cereal glutens. A healthy alternative is the use of fruit fibres in combination with apple pectin extract. The pectin chains of these products can stabilise the dough by the formation of a heat stable, three-dimensional network. The viscosity-enhancement of high water-binding fruit fibres also acts as a kind of stabiliser which guarantees volume and fine crumb of the bread. Expanded apple fibre can also be used as a filler.

Bread with prebiotic effects (supporting the growth of certain colon bacteria) of an excellent quality can be produced using dietary fibre blends. For example, mixtures of about 60% of prebiotic soluble fibres (inulin and pectin) together with texture-supporting fruit and pea fibres, have synergistic effects on the dough and hence on bread quality.

Low-calorie baked goods

Until recently, bran was used in the preparation of low-calorie baked goods. Today, calorie- or fat-reduced products with improved quality can be produced using high-fibre isolates (e.g. wheat grain fibre, lupin and fruit fibres or oat fibre). For example, the fresh-keeping, pore-

structure of the crumb (or juiciness) can be enhanced in sand cake-like recipes. The use of these fibres can also lead to a reduction of butter in recipes (without changing the organoleptic properties) with an increased yield of product, and thus to a lowered recipe cost. Colour or flavour enhancement can be achieved by adding classic fibres such as mandarin, chokeberry or carrot fibre in the range of 1–2%, in place of flour.

26.7.2 *Dietary fibres in beverages*

Dietary fibres are added to beverages mainly for nutritional reasons, but can also be an important element in developing functional products. Soluble fibres influence the viscosity, but should not change the character of a beverage. Low-viscosity pectins have been developed for the fortification of beverages. An important property of these soluble fibres is that they can reduce serum cholesterol, elevated levels of which are seen as a major cause of coronary heart disease (Behall & Reiser 1986; Cerda *et al*. 1988). Insoluble fibres, because of their particle size, influence the organoleptic properties of products. Consequently, it is possible to create new products by the inclusion of these fibres, for example drinks in which visible particles such as small apple flakes remain suspended. The swelling properties of these fibres (in combination with soluble fibres) can form the basis for products that promote weight-reduction, within the framework of a balanced diet. They can also promote digestion, cure constipation and improve the balance of the intestinal microflora. Combinations of single ingredients added to other healthy products such as whey, yoghurt or fruit juice have resulted in the launch of new types of beverages during the past few years.

26.7.3 *Dietary fibres in meat products, sausages or fillings*

Until recently, consumers knew little of the possibilities of plant fibres in meat processing. From a nutritional point of view, meat products probably will not be accepted as a dietary fibre source. However, the high functionality of new dietary fibre isolates has opened up new fields for industry. Neutral fibres with high water and oil binding capacity, combined with texturing properties, are interesting natural ingredients for multi-purpose use. General advantages are:

- reduction of caloric value (substitution of fat)
- improvement of sensoric impression due to enhanced juiciness
- improvement of texture and stability

Depending on the fibre and special application, additional advantages also exist.

Minced meat products (doner-kebab like)

Adding 2–3% of high water-binding fibres to minced meat improves the processing in many ways. A cost reduction of about 9% due to the enhanced yield, can be achieved. As well as the economic and quality factors (juiciness, texture, calories), reduced losses also lead to less environmental pollution.

Boiled sausages

Fruit fibres (because of their higher soluble fibre content) can effectively support the gel matrix formed by solubilised meat proteins and associated fat. Reduced cooking loss and a more stable, but juicy matrix result, with a positive effect on mouthfeel. The dietary fibres (addition of 1–3%) can be taken as a natural alternative to phosphate, milk or soy proteins.

Salami-like cured sausages

Dietary fibres increase the speed of ripening of cured sausage, which is a critical point in production. In particular, the intact capillaries of the fruit fibres permit faster water release. The addition of just 1% of fibre improves the texture, making the product more firm, and prevents the drainage of oil.

Fillings

Viscosity and texture enhancement makes the handling of fillings easier. Unlike ingredients such as breadcrumbs or flour, neutral fibres support the flavour of the main filling components. As is the case with salami, the production of the dry product is improved because the speed of drying is faster and more regular. On the other hand, with dried, filled pasta such as ravioli, re-hydration is improved by the capillary behaviour of the fibre, which increases the soaking speed. In the case of fresh products such as minced meat, economic advantages exist in the inclusion of some fibre.

26.7.4 *Frozen products*

Fruit fibres in ice cream or sorbet

An interesting application field for dietary fibre is in ice cream. Due to the increasing consumption in Western countries, ice cream can be seen as a good tool to develop functional foods. Because of low storage temperatures, it is ideal for sensitive ingredients such as polyunsaturated fatty acids. Based on technological, qualitative and marketing aspects, dietary fibres can be used in this product. Some of the advantages of using high water-binding fibre isolates in ice cream are summarised in Table 26.6. For the development of fibre-fortified or low-calorie products, it should be mentioned that an increase in the dietary fibre content up to 30 g/kg is possible without having negative effects. In particular, the improved melting properties (Fig. 26.1) are very interesting, while fibre also reduces ice crystal growth during storage and transport.

Frozen convenience products

Similar to the situation with ice cream and sorbet, quality improvement results from enhanced freeze–thaw stability, which leads to a prolonged shelf-life. The quality of convenience foods such as pizzas, cakes (especially gateaux) and pasta can be improved substantially.

26.7.5 *Dietary fibres in dairy products and protein-stabilised emulsions*

The major technological challenge with these products (yoghurts, curd cheese preparations,

Table 26.6 Advantages of high water-binding dietary fibre* in ice cream and sorbet.

Technological advantages
- Stabilisation of ice milk or sorbet mix without additive.
- Enhanced mix viscosities
  - permit freezing with an higher overrun without negatively affecting the ice crystal sizes
  - lead to more homogenous air-bubble formation
- The consistency after freezing is more dry and structured
- Enhanced melting stability ensures stability of the final product during transport to distributors
- Prolonged shelf-life because of retarded recrystallisation

Quality improvements
- Improved melting properties
- Improved body due to the fibrous framework (mouthfeel)
- Reduction of cold impression
- Enhanced freeze–thaw stability reduces the recrystallisation, leading to sensorically detectable ice crystals
- Enhanced nutritional value (replacement of fat)

Marketing advantages
- Label-friendly ingredient (without E-code)
- Positive image of apple or citrus fruits
- Acceptance of dietary fibre as healthy ingredient

*For example, Herbacel AQ Plus Citrus fibre or Herbacel AQ Plus Wheat grain fibre

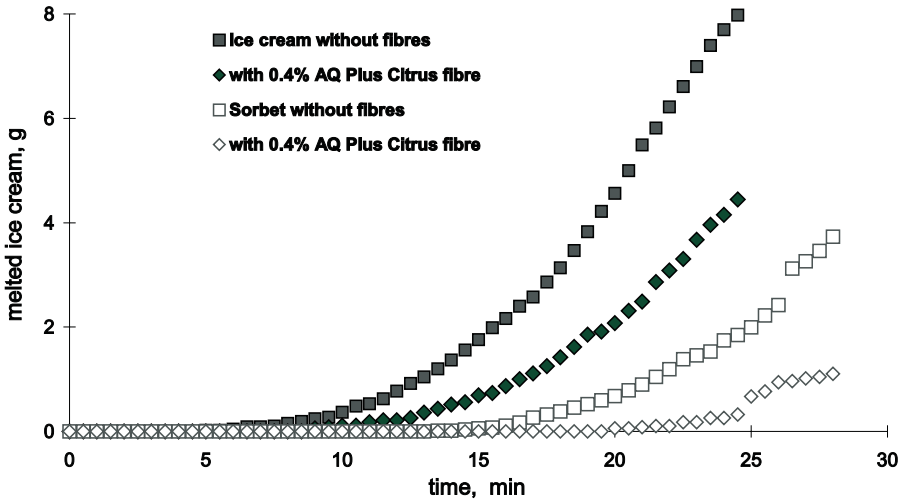

Fig. 26.1 Melting properties of ice cream and sorbet with dietary fibres at 25°C.

milk desserts, sauces, dressings) is the prevention of phase separation or syneresis. Protein-based emulsions and gels tend to become unstable near the isoelectric point of the proteins. In the case of milk, casein coagulates under acid conditions, whereupon the immobilised whey can separate from the protein aggregates or networks. The same is seen with rennet-coagulated products.

Gelatin or modified starch are added as stabilising ingredients, but these can be replaced by dietary fibre materials of high water-binding capacity such as Herbacel AQ Plus fruit

and wheat fibres. Hydrocolloids such as guar and locust bean gum are widely used, but the amounts which can be added is limited, and this is therefore of little physiological interest. As an example, if a homogenisation step is employed, it is possible very simply to produce fruit juice-yoghurt drinks. Adding 1.5–3% of fibre to a product is a concentration which is physiologically significant. The fibre also adds stabilising properties, protects proteins from coagulation, and reduces problems of syneresis. Appropriate fibres are readily dispersible, even in cold water, and increase the viscosity. This viscosity is stable against shear and high temperatures, and is independent of either pH value or salt conditions. Due to the texture-forming properties of fruit fibres, it is possible to produce low-fat products without reducing creaminess or mouthfeel. The products have more body and are not rough and crumbly, as is the case on enrichment with cereal bran.

26.7.6 *Dietary fibres in pasta and extruded products*

Pasta

Pasta is a very popular food in many countries, and is accepted by the young and old, and the active and convalescent alike. In order to achieve a successful fibre enrichment of pasta, it is necessary that there are no deleterious effects on either taste, colour or texture. To produce pasta without negatively affecting organoleptic properties, fibre isolates obtained from oat, citrus fruits or wheat grain are the first choice.

Several additional advantages exist, namely:

- enhanced yield due to improved water-binding properties, leading to an economic advantage in the case of fresh products or frozen pre-cooked meals;
- reduced cooking time;
- improved drying properties;
- enhanced cooking stability and tolerance; and
- enhanced water-binding capacity improves and stabilises products such as lasagne or cannelloni.

An interesting field for future development is the design of pasta for special diets. For example, as part of a diet which positively influences cholesterol levels, by adding low-viscosity pectin or β-glucan-rich oat fibre.

Extruded products (chips, crisps, crackers, snacks)

Neutral, low water-binding types of oat or pea fibre improve the crispiness, reduce breakage in production and shipping and improve workability. The fibre serves as a filler, reducing the calorie content of the final products. Additionally, the fat-repelling properties of fibre make it an ideal choice to reduce the fat content of fried snacks. Between 20–40% less fat is bound during frying as compared with controls without fibre.

26.7.7 *Dietary fibres in fruit preparations or fruit spreads*

Dietary fibres can be used in place of hydrocolloids to stabilise fruit preparations (for yoghurt or ice cream) and fruit spreads, and to standardise their viscosity.

Fruit preparations made with these fibres can be mixed homogeneously with sour milk products, because there is no reaction with calcium and thus no gel matrix has to be sheared. The products have thixotropic properties, with a reversible viscosity after shearing. This is important for resistance against pumping processes.

26.8 Dietary fibres for a healthy future

The nutritional benefits of dietary fibres are not only accepted scientifically, but surprisingly, are well understood by the consumer. It is now widely accepted that a well-balanced dietary fibre intake not only has a positive influence in preventing diseases of modern civilisation, without any side effects, but also can remedy diseases in the digestive system resulting from poor nutrition

The food industry now has dietary fibres that meet the highest demands of the consumers and promise marketing advantages. For almost any application, the food technologist can find ideal dietary fibres with versatile functionalities.

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Part 7

Cereal Cell-wall Polysaccharides

27 Cereal Arabinoxylan: Occurrence, Structure and Properties

Roger Andersson and Per Åman

27.1 Occurrence

Cereal grains (caryopsis) are composed of different structures, including the bran (pericarp, testa, aleurone and part of the outer starchy endosperm), germ and flour (mainly starchy endosperm). Covered cereals like barley and oats are surrounded by the hull (glumes), which for these cereals remain attached after threshing. Glumes, pericarp and testa are dead tissues and consist mainly of lignified cell walls, while the aleurone is metabolically active with thick, resistant cell walls. The starchy endosperm is a storage tissue with thin unlignified cell walls which are easily degraded in order to, when required, release stored energy. The composition and structure of the cell walls of cereals thus varies with the function of the wall. Major components of the cell walls are cell-wall polysaccharides, cell-wall protein, lignin and substituents, including cinnamic acids, but the detailed structures remain to be elucidated (Fincher & Stone 1986; Carpita & Gibeaut 1993). Arabinoxylan is one of the major polysaccharides in all major cereal cell walls, and is part of so-called hemicellulose that is generally believed to act as a cement between cellulose fibres within cell walls. Interactions between alkali-extracted barley arabinoxylan and mixed linkage β-glucan were recently indicated by the fact that an arabinoxylan precipitated together with β-glucan fragments after lichenase treatment of the mixture (Izydorczyk & MacGregor 2000).

Most of the examples given in this overview deal with arabinoxylan in wheat, rye, barley and oats, which are the major cereal crops of northern Europe. The quantitative determination of arabinoxylan in cereal food is not straightforward, since it coexists with other polysaccharides that also include xylose and arabinose residues. Total arabinoxylan content in wheat has been estimated to be 5.5–7.8% ($n = 22$) in whole grain (Saulnier *et al.* 1995b) and to an average of 2.1% ($n = 49$) in flour (Andersson *et al.* 1992). These values are calculated as the sum of arabinose and xylose residues, and may be slightly overestimated because of the contribution from arabinogalactan-protein. Loosveld *et al.* (1998) corrected for the contribution from arabinogalactan-protein in the calculation of the content of water-extractable arabinoxylan and reported a range of 0.31 to 0.69% for a set of 18 European wheat flours.

Rye has a high content of arabinoxylan. Nilsson *et al.* (1997) analysed seven Scandinavian rye cultivars and found a range of 8.2 to 10.0%, of which on average 23% was extractable in water. Åman (1987) determined the cell-wall composition in oats ($n = 16$) and found a large variation in the contents of arabinose (1.1–2.0%) and xylose (2.4–10.6%) residues and an average content of 6.9% arabinoxylan. It should be noted that it is difficult to compare the whole-grain arabinoxylan content of oats to that of wheat and rye, since most oat cultivars are covered and include the hull that is very rich in arabinoxylan. The content of arabinoxylan in barley has been analysed for a set of ten covered and six naked barley cultivars covering a wide variation in chemical composition (Oscarsson *et al.* 1996). The average content of arabinose residues did not differ between covered and naked cultivars, but the content of xylose was

higher in the covered than in the naked (5.5 and 3.3%, respectively). The total arabinoxylan content among all cultivars ranged between 4.8 to 9.7%, of which on average, 7 and 12% was water-extractable in covered and naked cultivars, respectively.

27.2 Structural elements

The structure of wheat arabinoxylan was first studied by Perlin (1951a, b), who isolated a water-extractable fraction and suggested that it was a polysaccharide with a main chain of (1→4)-linked β-D-xylopyranose residues with terminal α-L-arabinofuranose residues attached at O-2 and/or O-3. Since then, many investigators have studied arabinoxylan in cereal grain and concluded that there are mainly four differently linked xylose residues in the polymer chain (Fig. 27.1) (Hoffmann *et al.* 1991b, c; Gruppen *et al.* 1992b; Cleemput *et al.* 1993; Andersson *et al.* 1994b). The relative abundance of 2-monosubstituted xylose (2mXyl) is generally low but significant amounts have been found in barley arabinoxylan (Viëtor *et al.* 1992), as well as in isolated fractions from rye (Ebringerová *et al.* 1990; Vinkx *et al.* 1995a). There are also other sugar residues, such as galactose and glucuronic acids included in more complex heteroxylans and glucuronoarabinoxylans (Saulnier *et al.* 1995a; Verbruggen *et al.* 1998a).

The molecular weight of plant cell-wall polysaccharides is very difficult to determine since the extraction and purification procedures generally include steps leading to partial degradation of the polymers. The molecular weight distribution of water-extractable arabinoxylan from rye was very wide (15–650 kDa) with a weight average of 124 kDa and a polydispersity index of 2.0, as determined by multiple angle laser light scattering (Nilsson *et al.* 2000). Hoffmann *et al.* (1991b) reported a mass range of 25 to more than 1000 kDa for wheat arabinoxylan determined by size-exclusion chromatography calibrated with pullulan standards.

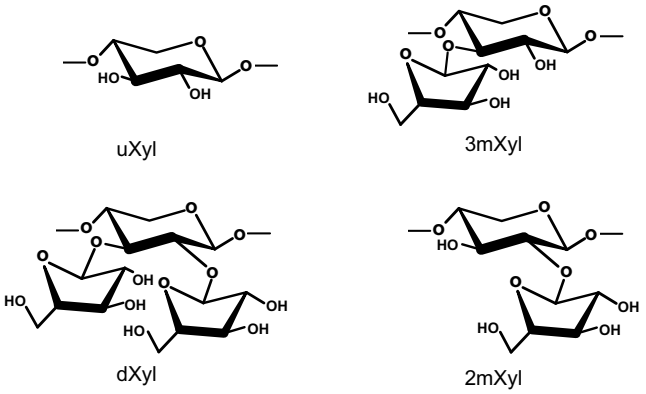

Fig. 27.1 Main structural elements of arabinoxylan: (1,4)-linked β-D-xylopyranose residue (uXyl), (1,3,4)-linked β-D-xylopyranose residue (3mXyl), (1,2,3,4)-linked β-D-xylopyranose residue (dXyl) and (1,2,4)-linked β-D-xylopyranose residue (2mXyl) substituted with terminal α-L-arabinofuranose residues.

27.2.1 *Phenolic components*

Hydroxycinnamic acid derivatives, such as *p*-coumaric and ferulic acid (Fig. 27.2) present in cereal cell walls have recently been reviewed (Ishii 1997). The phenolic acid residues are ester-linked to position O-5 of arabinosyl side chains. It has been shown that ferulic acid substituents are involved in the oxidative gelation of water-soluble arabinoxylan by formation of dehydrodiferulic acid units (Geissmann & Neukom 1973), Markwalder and Neukom (1976) found small amounts of this dimer in water-insoluble cell-wall material from wheat endosperm, indicating that diferulic acid cross-links between adjacent polysaccharide molecules could reduce their solubility. Nordkvist *et al.* (1984) showed that ferulic acid residues in barley grain were present, with the highest content in the aleurone layer (1.2% of cell walls), by studying pearled fractions. The highest levels of *p*-coumaric acid were observed in the outermost husk-rich fraction (0.6% of cell walls), while very low amounts were found in the starchy endosperm fractions.

Recently, the phenolic components in maize bran heteroxylans have been thoroughly studied (Saulnier & Thibault 1999; Saulnier *et al.* 1999). Both ferulic acid (2.9%) and its dehydrodimers (2.5%) were released from maize bran by alkaline treatment. The 5-5', 8-O(4)', 8-5' dimers dominated, but a 8-8' dimer was also present. Oligosaccharides with the ester-linked phenolic residues still attached were isolated after controlled mild acid hydrolysis. In one of the compounds, both phenolic acids of the 5-5' dimer were linked to arabinose and in another compounds, one phenolic acid was linked to arabinose and the other to arabinose which was further substituted with a 2-linked xylose residue. The structure of these compounds indicates that the heteroxylans in maize bran are covalently cross-linked through dehydrodiferulates.

27.3 Structure heterogeneity

Structure heterogeneity of arabinoxylan was demonstrated very early by Preece and Mackenzie (1952) and Preece and Hobkirk (1953) using ammonium sulphate precipitation of gums from barley and other cereals. This is one of the most important features of

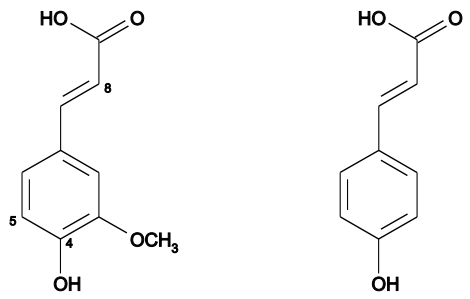

Fig. 27.2 Structure of ferulic and *p*-coumaric acids. Atom numbers depict the binding positions of the ferulic acid dehydrodimers.

arabinoxylan, and means that there is a variation in structure, viz. degree of substitution, distribution of substituents, and degree of polymerisation. These variations have a large effect on physical properties. Thus, in order to understand the function of arabinoxylan in various products, it is important to know the fine structure, defined as the content and distribution of differently linked sugar residues.

Arabinoxylans isolated from different tissue types show variation in structure. Nyman *et al.* (1984) analysed soluble and insoluble dietary fibre in milling fractions of different extraction rates from wheat, rye, barley, sorghum, rice and maize. The content of soluble dietary fibre was independent of extraction rate, while the insoluble fibre increased at high extraction rate for all cereals studied, indicating that the extractability of cell-wall polysaccharides in the outer layers is much lower than in the starchy endosperm. Similar trends have recently been found for rye arabinoxylan in milling fractions enriched in pericarp/testa, aleurone and endosperm, respectively (Glitsø & Bach Knudsen, 1999). The endosperm fraction contained 4.2% arabinoxylan and the pericarp/testa fraction as much as 39.9%. The water-extractability was 70% and 14% in the endosperm and pericarp/testa, respectively. Delcour *et al.* (1999) studied the distribution and structure of arabinoxylans in wheat roller milling streams. The total content of non-starch polysaccharides increased with increasing ash levels, which implies the existence of an arabinoxylan gradient toward the outer layers of the wheat kernel. Water-extractable arabinoxylan from the outer layers was less substituted with arabinose residues than that from the inner endosperm. This was due to a higher proportion of unsubstituted and a lower proportion of disubstituted xylose residues in the outer fractions, while the proportion of monosubstituted was similar in all milling streams.

Bengtsson *et al.* (1992b) isolated water-extractable arabinoxylan from 28 rye grain samples originating from four different countries. The average yield of arabinoxylan ranged from 1.4% in samples from Canada to 1.7% in Swedish samples. The content of uXyl, 3mXyl and dXyl followed the linear relationship:

$$\mathrm{uXyl} = b_I\,\mathrm{3mXyl} + b_{II}\,\mathrm{dXyl}$$

where the constants $b_I = 1.17$ and $b_{II} = 0.75$ depict the average number of uXyl residues per 3mXyl and dXyl, respectively. This indicates that fractions rich in 3mXyl also contain more uXyl than those rich in dXyl. Linear relations between the relative contents of structural elements of rye water-extractable arabinoxylan fractions and their Ara/Xyl ratio have also been reported (Vinkx & Delcour 1996). The fact that the variation in structure of the water-extractable arabinoxylans from rye follow linear relationships indicate that the variability of the biosynthesis is limited.

A relationship between arabinoxylan structure and protein was reported for a set of 20 Swedish wheat flour samples (Andersson *et al.* 1994b). There was a positive correlation between the content of water-extractable dXyl and content of crude protein. Later, the same relationship was found in a set of seven rye grain samples (Nilsson *et al.* 1997). Such relationships are very difficult to explain since the storage protein, which is the major part of the crude protein, and the arabinoxylan are located in separate parts of the cell structure. It does, however, indicate that the endosperm cell walls of protein-rich grain differ from those in grain with less protein. One can speculate that there may be a genetic link between cultivars high in protein and specific cell-wall structures, respectively. Oscarsson *et al.* (1996) studied 16 barley cultivars but could not detect any correlation between dXyl and protein for those samples.

27.3.1 *Heterogeneity studied by fractionation*

Fractionation of water-soluble arabinoxylan has been performed with several techniques, based mainly on precipitating agents or chromatography. Ammonium sulphate, as mentioned above, has been used to purify arabinoxylan from contaminants such as β-glucan, based on the fact that arabinoxylan remains soluble at higher concentrations of ammonium sulphate (Westerlund *et al.* 1993). Water-extracts of wheat contain arabinogalactan-peptide. This can be removed from the arabinoxylan because it remains soluble at concentrations of ammonium sulphate higher than those required to precipitate most of the arabinoxylan (Fincher & Stone 1974; Westerlund *et al.* 1990; Hoffmann *et al.* 1991b). Fractionation of the water-extractable arabinoxylan of rye gave fractions with increasing Ara/Xyl ratio and molecular weight, with increasing ammonium sulphate saturation (Vinkx *et al.* 1993). Izydorczyk and Biliaderis (1992a) fractionated the water-extractable arabinoxylan of wheat and found a similar relationship between Ara/Xyl ratio and ammonium sulphate saturation, but a reversed trend for molecular weights in the fractions.

Several investigators have used graded ethanol precipitation to fractionate arabinoxylan extracts. Hoffmann *et al.* (1991b) fractionated cold-water-soluble arabinoxylan from wheat flour and found that the proportion of branched xylose residues was higher in fractions precipitating at higher ethanol concentrations. Increasing Ara/Xyl ratios in fractions precipitated with increasing ethanol concentrations have also been found for water-extractable as well as barium hydroxide-extractable wheat arabinoxylans (Gruppen *et al.* 1992b). Gel permeation chromatography has shown that fractions obtained by graded ethanol precipitation of water-extractable wheat arabinoxylan cover the same molecular size range, but those precipitated with the highest ethanol concentrations had a larger proportion of low molecular weight components (Cleemput *et al.* 1995). This indicated that there may be a correlation between molecular weight and Ara/Xyl ratio.

DEAE-cellulose anion-exchange chromatography based on the procedure of Neukom *et al*. (1960) has been used to fractionate water-soluble arabinoxylan. Lineback *et al.* (1977) fractionated wheat arabinoxylan and compared the results with those of several previous investigators and concluded that they agreed reasonably well. The elution of polysaccharide material from the DEAE-cellulose is carried out stepwise with water, two or three concentrations of sodium borate and sodium hydroxide, with each solvent yielding a separate polysaccharide fraction. Generally, the first two fractions (water and diluted borate) contain different arabinoxylans, with the latter having a higher degree of substitution (Lineback *et al.* 1977; MacArthur & D'Appolonia, 1980; Westerlund *et al.* 1990). Fractions eluted with higher borate concentrations are enriched in arabinogalactan-peptide and those eluted with sodium hydroxide are mixtures of polysaccharides and protein. Recent results indicate that the protein part of the rye arabinoxylan extract influences the interaction between the column material and the arabinoxylan (Nilsson *et al.* 1999).

27.3.2 *Substituent distribution*

The distribution of arabinosyl substituents within the arabinoxylan has been studied by degradation of the xylan chain, followed by fractionation and isolation of the released oligosaccharides. Such degradation can be obtained by periodate oxidation or by using specific enzymes. Periodate oxidation has been applied to a water-extractable arabinoxylan from rye (Åman & Bengtsson, 1990). This oxidation attacks all xylose residues that are free of substit-

uents, while those that carry arabinose residues are resistant. The number of xylose residues in the isolated oligomers thus indicates the number of substituted xylose residues that appear in sequence. Results showed that the branch points of this arabinoxylan (water-extractable rye arabinoxylan) were present predominantly as isolated residues (36%) or as small blocks of two residues (62%).

Structural studies on enzymatically generated arabinoxylan-oligosaccharides have resulted in detailed information about the chemical structure of the polysaccharide, as well as the substrate specificity of the applied enzymes (Hoffmann *et al.* 1991a; Gruppen *et al.* 1992c; Kormelink *et al.* 1993; Verbruggen *et al.* 1998b). Recent advances in enzyme research have resulted in increased availability of useful enzymes, including different types of *endo*-xylanases, arabinofuranosidases and glucuronidases. Further advances in chromatography, NMR spectroscopy and mass spectrometry have simplified the fractionation and structural determination of the oligosaccharides released. A structural model for water-unextractable arabinoxylan from wheat has been suggested by Gruppen *et al.* (1993), based on enzymatic degradation followed by oligomer isolation on Bio-Gel P2 and anion-exchange chromatography. It was concluded that the arabinoxylan contained highly branched regions enriched in dXyl and 2mXyl substituted xylose residues and less branched regions devoid of the latter xylose residue. In a separate study by Verbruggen *et al.* (1998a), the glucuronoarabinoxylans from sorghum was subjected to enzymatic degradation and oligosaccharide characterisation. The authors proposed a tentative model of the polysaccharide built of eight structural elements.

Another approach to the structural characterisation of arabinoxylan was employed by Nilsson *et al.* (2000). *Arabinoxylan I*, isolated from the rye cultivar Amando, was subjected to incomplete enzymatic degradation to produce relatively large arabinoxylan fragments, which could reveal an uneven or blockwise distribution of arabinosyl substituents. The fragments obtained had a molecular size distribution covering the range for Biogel P-6 and a wider range of Ara/Xyl ratios than had been found for the untreated arabinoxylan. These results indicated that within the arabinoxylan, regions with different substitution density existed. Whether these different regions are parts of the same polymer or not, remains to be elucidated.

27.4 Arabinoxylan classification by extractability and structure

Arabinoxylan can be released from the cell wall by different solvents such as water or alkali. Extractability is an important parameter that can provide information on the linkages between the components in the cell wall. The fine structure, defined as the content and distribution of differently linked sugar residues, is only partly responsible for extractability. Since the fine structure is not the only criterion, isolated extracts are mostly mixtures of different polymers and polymers with similar structure can be found in different extracts. Sequential extractions with water, barium hydroxide, water and sodium hydroxide have been performed on wheat (Gruppen *et al.* 1991, 1992a), barley (Viëtor *et al.* 1992) and rye (Vinkx *et al.* 1995b; Nilsson *et al.* 1996). Aqueous barium hydroxide was used by Meier (1958) to fractionate hemicellulose isolated from spruce wood and he found that the fraction which had not precipitated was enriched in arabinoxylan. Later, this agent was reintroduced by Gruppen *et al.* (1991), who found that it could be used as a selective extractant for arabinoxylan from water-unextractable cereal cell-wall material. The barium hydroxide prevents solvation of the β-glucan that instead can be extracted by water in a subsequent step after neutralisation. The arabinoxylan

fractions obtained by such sequential extraction show very large differences in structure. Extraction selectivity is partly determined by interactions between the polysaccharides and other cell-wall components such as proteins and phenolic acids. If proteins are linked to the arabinoxylans, they could serve as a criterion for selective extraction and fractionation.

Rye arabinoxylans can be classified based on extractability and structure (Fig. 27.3). There are four distinctively different classes, but their extractabilities partly overlap. The water extractable arabinoxylan fraction in rye is dominated by a high-molecular weight arabinoxylan denoted *Arabinoxylan I*, which consists mainly of 3mXyl and uXyl, is very low in dXyl and has an Ara/Xyl ratio of 0.5 (Bengtsson & Åman 1990; Vinkx *et al.* 1993). This fraction can be isolated from the water extract by DEAE-cellulose anion-exchange chromatography. The second class is very high in dXyl and has a very high Ara/Xyl ratio of 1.4. This fraction, denoted *Arabinoxylan II*, is partly water-extractable and has been isolated after *endo*-xylanase treatment (Bengtsson *et al.* 1992a). A similar structure was later isolated without enzymatic degradation by Vinkx *et al.* (1993), but this fraction also contained a significant amount of 2mXyl (Vinkx *et al.*, 1995a). This shows that *Arabinoxylan II* is present as an individual polymer and not only as a highly substituted region in a polymer that is otherwise less substituted.

Arabinoxylan fractions containing mono- as well as di-substituted xylose residues are present in both water- and alkali-extracts (marked with stripes in Fig. 27.3). These fractions are not homogeneous, and can be fractionated into several subfractions with different degrees of substitution. ^{1}H-NMR on the water-extractable part of this fraction from rye has shown that the microenvironment of the di-substituted xylose residues varies, indicating that polymer structures with a range of Ara/Xyl ratios exist (Vinkx *et al.* 1993). A highly substituted glucuronoarabinoxylan (Ara/Xyl ratio greater than 1) has been isolated as a major polysaccharide from the barium hydroxide extract of wheat bran (Schooneveld-Bergmans *et al.* 1999). The complexity of this polymer was shown by poor enzymatic degradability and the presence of branched arabinose and terminal xylose residues. The xylose residues of the backbone were not only substituted at O-3 and at O-2 and O-3 by terminal arabinose residues, as well as at O-2 by (4-O-methyl) glucuronic acid residues, but also through dimeric arabinose, xylose and possibly galactose-containing side chains, as well as through 2,3-linked arabinose residues.

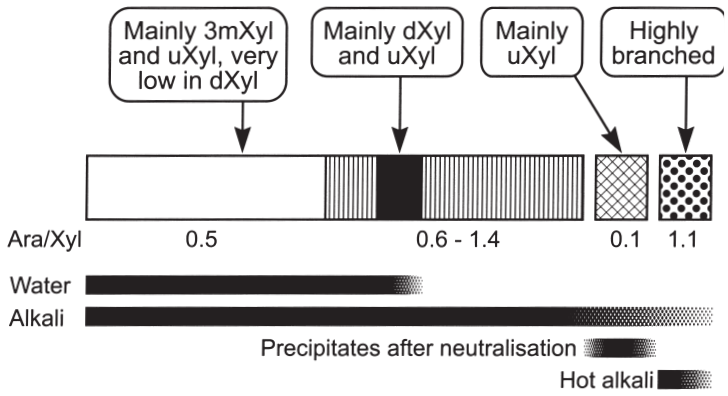

Fig. 27.3 Schematic representation of rye arabinoxylan classes.

The third class of rye arabinoxylan (Fig. 27.3) is very sparsely substituted and isolated as a precipitate after neutralization of an alkali extract (Nilsson *et al.* 1996). The highest yield of this arabinoxylan class was found in the bran. An arabinoxylan with a very low degree of substitution (Ara/Xyl about 0.2) has also been isolated from the barium hydroxide extract of wheat bran (Schooneveld-Bergmans *et al.* 1999). In this polymer, one out of seven or eight xylose residues of the backbone was mono-substituted at O-3 by terminal arabinose residues. Enzymatic degradation showed a random distribution of the substituents, which gives sequences of six or more unsubstituted xylose residues. Methylation analysis showed that low amounts of di-substituted xylose residues were also present in this arabinoxylan fraction.

Finally, the fourth class of rye arabinoxylan is a highly branched heteroxylan with a complex structure. The anomeric region of the proton NMR spectrum of this polysaccharide fraction compared very well with that of an isolated heteroxylan from maize bran. Structural studies of the latter heteroxylan has revealed a 4-linked xylan backbone, of which about 80% of the residues are mono- or di-substituted (Chanliaud *et al.* 1995; Saulnier *et al.* 1995a; Saulnier & Thibault, 1999). The side chains contain terminal arabinose or glucuronic acid residues, as well as dimeric and oligomeric chains with arabinose, xylose and sometimes galactose residues. Some of the arabinose residues, which always seem to be linked directly to the backbone, carry feruloyl units at O-5.

27.5 Properties of arabinoxylan solutions

An important property of arabinoxylan is its ability to form highly viscous solutions in water. Andrewartha *et al.* (1979) investigated the properties of a water-extractable arabinoxylan isolated from wheat flour. They found that highly substituted regions became rod-like, while regions with less arabinose tended to be more flexible. When the arabinose residues were partially removed, regions without substituents formed aggregates and became insoluble. The conclusion from this is that arabinoxylan is water-soluble because of its substituents. Izydorczyk and Biliaderis (1992a) measured intrinsic viscosity and concentration dependency of 'zero shear' specific viscosity $(\eta_{sp})_0$ for wheat arabinoxylan, and concluded that the arabinoxylan behaved as a relatively stiff and extended polymer rather than a random coil. The intrinsic viscosity ranged from 2.8 to 4.2 dl/g for water-extractable arabinoxylan from eight wheat varieties, indicating differences in molecular weight or conformation (Izydorczyk *et al.* 1991). The viscosity dependency of molecular weight has also been studied on fractions obtained by preparative gel permeation chromatography (Izydorczyk & Biliaderis 1992b). The intrinsic viscosity varied between 3.4 and 8.5 dl/g, and all fractions showed a shear thinning behaviour at a concentration of 2%. Shear thinning behaviour was observed for a 0.2% solution of the fraction with the highest viscosity. Nilsson *et al.* (2000) compared *Arabinoxylan I* isolated from different rye cultivars. Viscosity measurements showed that the fraction from cultivar Muskate had a higher viscosity than that from cultivar Danko (Fig. 27.4). Differences in molecular size distribution were confirmed by size-exclusion chromatography which revealed a much higher proportion of high molecular size polymers in Muskate than in Danko.

Ebringerová *et al.* (1994) studied a water-insoluble arabinoxylan with a very low Ara/Xyl ratio (0.14) from rye bran. The molecular weight was determined as 62 600 Da, and light scattering measurements showed that the polysaccharide formed aggregates of two to eight mol-

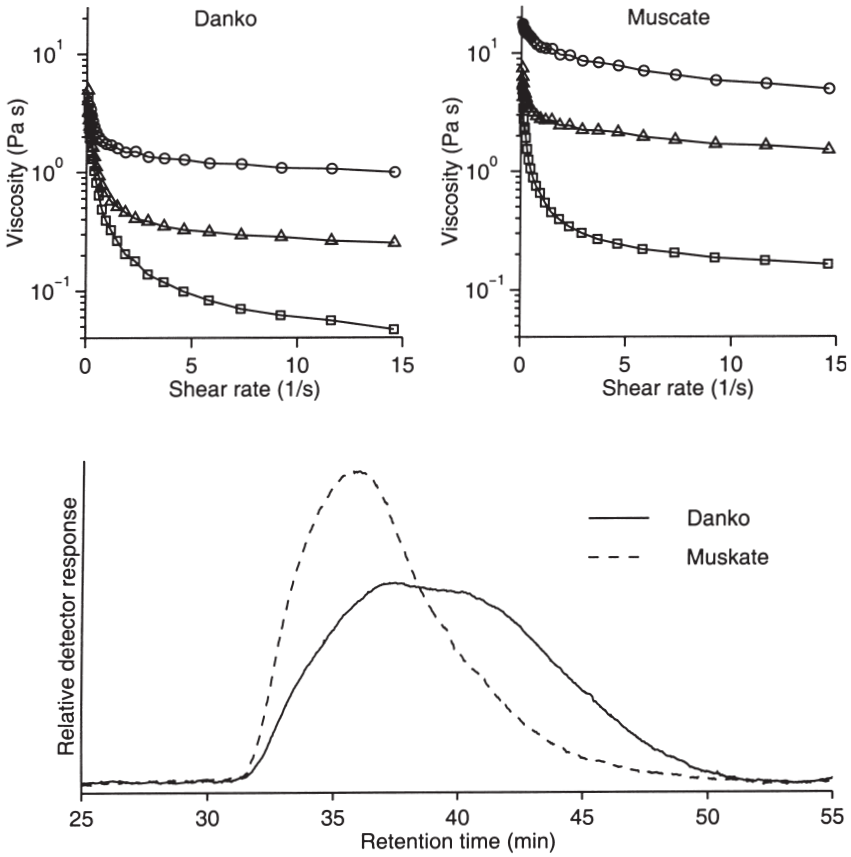

Fig. 27.4 Viscosity (top) and size-exclusion chromatography profiles (bottom) of *Arabinoxylan I* isolated from the rye cultivars Danko and Muskate. Water solution concentrations 0.5 (squares), 1.0 (triangles) and 1.5 % (circles). (From Nilsson *et al.* 2000.)

ecules in dimethyl sulphoxide (DMSO) and complexing solvents. Recently, an arabinoxylan-containing extract with novel rheological properties was prepared from wheat bran (Cui *et al.* 1999). Extraction conditions were adjusted to minimise degradation of the polysaccharides and extraction with 0.5 M NaOH at 25°C yielded a fraction with 77% arabinoxylan and 23% β-glucan. The fraction showed shear thinning behaviour at low concentration (0.5%). An interesting feature of this arabinoxylan was that it formed a thermally reversible gel upon cooling (2% solution) from 25°C to 4°C. Such gelling behaviour is not typical for arabinoxylan, but may be due to the low Ara/Xyl ratio (0.3) (Cui *et al*. 1999). Another possibile explanation is that the gelling is due to the β-glucan in the sample.

In a study of the water-soluble arabinoxylan of 22 wheat cultivars grown in France, a poor correlation between the content of water-soluble arabinoxylan and the relative viscosity of grain extract has been found, indicating a natural variation in molecular size (Saulnier *et al.* 1995b). This was confirmed by size-exclusion chromatography and by analysis of the intrinsic viscosity that ranged between 198 and 589 ml/g. Bengtsson *et al.* (1992) measured the viscosity of water slurries of 28 rye samples after enzymatic starch degradation. There was a large variation in apparent viscosity, 80% of which could be explained using a mathemati-

cal model based on the type of arabinose substituents in the water-extractable arabinoxylan. The model indicated that a high proportion of di-substituted xylose residues increased the viscosity of the polymer. Another potentially important property of arabinoxylan in solution is its ability to inhibit ice-crystal growth (Kindel *et al.* 1989). Water-soluble arabinoxylan was isolated from one rye and two wheat cultivars and further fractionated by size-exclusion chromatography. The rye arabinoxylan was a better inhibitor than the wheat arabinoxylan, and the best rating was obtained for the fractions with the highest molecular weight.

27.5.1 *Effect on baking performance*

Arabinoxylan has a very high water-holding capacity estimated at 15 g water/g arabinoxylan (Bushuk 1966). This means that almost 25% of the water in wheat dough is associated with just 1.5% of the flour (McCleary *et al*. 1986; Eliasson & Larsson 1993). Thus, the arabinoxylan could influence the water distribution in the dough as well as dough consistency because of its rheological properties. There are several ways to study the influence of flour components on baking properties. A strategy commonly used to study the role of arabinoxylan in breadmaking is the addition of purified fractions to the dough. This usually results in improved bread volume. One problem with such an approach is that the added arabinoxylan may behave differently than the arabinoxylan already present in the flour. The effects of arabinoxylan on breadmaking can also be studied by addition of an enzyme that selectively affects the native arabinoxylan. A further strategy is to study the natural variation in chemical composition in a large set of samples and relate this variation to baking performance. However, such investigations are often difficult to interpret since there may be covariation between the parameters studied.

Roels *et al.* (1993) investigated the influence of mixing time and baking absorption on the baking performance of wheat flour and found that flours of poor quality had higher contents of water-soluble arabinoxylan than good flours. A negative correlation between contents of water-soluble arabinoxylan and the Zeleny sedimentation value, was also found for six European wheat flours, indicating that the arabinoxylan disturbs the flocculation of the gluten proteins. Cleemput *et al.* (1993) further investigated the arabinoxylan in the same sample set and reported that the best correlation was found between the content of disubstituted xylose residues and baking absorption. Mono- and unsubstituted residues were also negatively, but much less, correlated to baking absorption. Positive correlations between farinograph water-absorption and water-extractable as well as unextractable arabinoxylan were found for 20 Swedish wheat flours (Andersson *et al.* 1994a). A model mainly based on polysaccharide characteristics was able to explain 92% of the variance in water absorption. The strongest correlations were found for un- and mono-substituted water-extractable xylose residues, while no correlation was detected for the di-substituted ones.

Addition of water-soluble, non-starch rye polysaccharides (mainly consisting of arabinoxylan) to wheat flour dough increased the consistency in the farinograph, as well as the initial stress relaxation modulus (G_0) (Girhammar & Nair 1995). The effect was increased further by addition of an oxidising agent inducing oxidative gelation of the rye arabinoxylan. The authors concluded that oxidative agents may be used to improve dough consistency, and that it may counteract dough stickiness caused by arabinoxylan-degrading enzymes. Biliaderis *et al.* (1995) compared the effects of high- and low- molecular weight arabinoxylan fractions on breadmaking. The fractions were highly purified (protein content <2%) from two different spring wheat cultivars and their molecular weights were estimated to 202 and

135 kDa, respectively. An increase in farinograph water absorption and dough development time was observed upon addition of both arabinoxylan fractions, but the effects were greater for the high-molecular weight fraction. Loaf volume was also affected by the arabinoxylan addition, and the maximum increase was obtained at a level of 0.5% of the high-molecular weight fraction. Breads with added arabinoxylan exhibited a greater rate of starch retrogradation, probably because of their higher moisture content. Nevertheless, these breads had a softer breadcrumb than the controls over a 7-day storage period. Courtin and Delcour (1998) studied the effects of two arabinoxylan fractions with apparent weight-average molecular weights of 50 and 555 kDa, respectively. They found that the baking absorption, as measured by mixograph, decreased with addition of the low-molecular weight fraction, while an opposite effect was obtained with the high-molecular weight fraction. Both arabinoxylan fractions gave increased loaf volumes, although the effect was larger for the high-molecular weight fraction. The authors concluded that neither the water-holding capacity nor the oxidative gelation of arabinoxylan could explain their findings, and suggested that other mechanisms are involved in the role of arabinoxylan in breadmaking.

Arabinoxylan-degrading enzyme preparations are used in the baking industry to improve baking performance. Rouau *et al.* (1994) applied such enzyme preparation (Grindamyl S100) and followed its effects during baking of wheat bread. Twelve flour samples were included in the study, and their optimum level of enzyme varied between 50 and 150 ppm. The enzyme addition increased the amount of arabinoxylan solubilised during dough processing, but no relationship was found between arabinoxylan content and optimal level of enzyme. At excessive levels, doughs become slack and sticky. Although more arabinoxylan was solubilised at the excessive level of addition, the apparent intrinsic viscosity of dough water-extractable arabinoxylan was greater at the optimum level of addition. The impact on baking performance was thus related to molecular size, as well as the amount of extractable arabinoxylan.

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28 Cereal β-Glucans: Structure, Properties and Health Claims

Peter J. Wood

28.1 Introduction

The Food and Drug Administration (FDA) of the USA has allowed (Anon. 1997) a health claim that inclusion of oat products in the daily diet may reduce the risk of coronary heart disease. The ruling was based on review of data suggesting that consumption of oats may lower serum cholesterol levels. The claim requires a total daily consumption of 3 g or more of 'β-glucan soluble fiber' from oats. The ruling therefore implies that the main (but possibly not only) active ingredient in oats is the endospermic cell-wall polysaccharide (1→3)(1→4)-β-D-glucan, or β-glucan. Polysaccharides of this structure have only been reported in grasses, or *Graminae*, and in some lichens (e.g. *Cetraria islandica*), and may impart high viscosity in solution. In some circumstances, controlled by details of structure and molecular weight, gel formation is possible. The main sources of food interest are the kernels of oats, barley, wheat and rye.

The mechanism(s) by which oat β-glucan, and other so-called 'soluble fibres', lower serum cholesterol levels has not been clearly established, but viscosity is believed to play a role (Jensen *et al.* 1993). This is certainly the case in the possibly related ability to attenuate postprandial blood glucose and insulin levels (Jenkins *et al.* 1978; Wood *et al.* 1994a).

This chapter will review the physicochemical characteristics of cereal β-glucan, and how these properties might influence physiological effects in people.

28.2 Physicochemical characteristics of cereal β-glucan

28.2.1 *Structure*

Cereal β-glucans are linear polysaccharides which can be viewed as a cellulose chain (~70% 4-O-linked β-D-glucopyranosyl units) interrupted by 3-O-linked β-D-glucopyranosyl units (~30%). The distribution of these two linkages is not random; the (1→3) linkages occur singly, and most of the (1→4) linkages occur in groups of two or three, leading to a structure of predominantly β-(1→3)-linked cellotriosyl and cellotetraosyl units. This view of the structure, worked out many years ago, for oat β-glucan, by analysis of fragments released by enzyme hydrolysis (Parrish *et al.* 1960), has not substantially changed over the years.

Modern methods of analysis have improved our knowledge of details of structure and variations between the cereals. The most powerful of these methods is analysis of fragments released by lichenase, a (1→3)(1→4)-β-D-glucan-4-glucanohydrolase (EC 3.2.1.73) which is highly specific for cleavage of the 4-O-glycosidic linkage of 3-O-substituted glucose residues. Although any chromatographic analysis may be applied (Woodward *et al.* 1983a; Wood *et al.* 1991a), high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection is a particularly powerful method for analysis of the released oligosac-

charide fragments (Wood *et al.* 1994b). The molar ratio (values uncorrected for differences in oligosaccharide response factors) of (1→3)-linked cellotriosyl to (1→3)-linked cellotetraosyl units, which constituted 85–90% by weight of the polysaccharides was lower for oats (2.1–2.4) than it was for barley (2.8–3.3), rye (3.0–3.2) or wheat (3.0–4.5)(Wood *et al.* 1994b; Cui *et al.* 2000). In addition to 3-O-β-cellobiosyl- and cellotriosyl-D-glucose, higher degree of polymerisation (DP) soluble oligosaccharides up to DP 9 are detected on HPAEC. These oligosaccharides contain more than two β-(1→4)-linked glucopyranosyl units, terminated at the reducing end by a β-(1→3)-linked glucose (i.e. cellodextrins terminated at the reducing end by a 3-linked unit). It is noteworthy that an increase in DP 9 material is observed in analysis of oat, barley, rye and wheat β-glucan; this is the DP at which the oligosaccharides become essentially water insoluble. A comparison of all the oligosaccharides released from oat and barley β-glucan is shown in Table 28.1. Molecular modelling suggests that in the intact polymer chain the regions of more than three consecutive β-(1→4)-linked glucopyranosyl units exercise a significant influence on molecular extension (Buliga *et al.* 1986).

Table 28.1 Analysis* of oligosaccharides released by lichenase from oat and barley β-glucan. (Adapted from Wood *et al.* 1994b.)

	% by weight†	
Oligosaccharide DP	Oat	Barley
Water-soluble portion‡		
3	55	62.1
4	36	29.4
5	3.3	3.9
6	2.6	2.2
7	0.6	0.4
8	0.8	0.4
9	1.6	1.5
Water-insoluble portion§		
7	2	1.7
8	13.2	7.7
9	38.9	37
10	10.4	8
11	12.8	12.9
12	7.8	9.3
13	4.2	5.3
14	3.2	4.4
15	4.6	7.9

DP, degree of polymerisation.
* Using high-performance anion exchange chromatography with pulsed amperometric detection, without correction for differences in response factors (which decline with increasing DP).
† Means of triplicate analyses.
‡ Normalised area percentage based on total analysed soluble oligosaccharides.
§ Normalised area percentage based on total insoluble oligosaccharides analysed in precipitates, obtained in 3.9% yield from oats and 2.6% from barley.

The (1→3)-linked cellotriosyl and (1→3)-linked cellotetraosyl units of barley β-glucan are randomly distributed (Staudte *et al.* 1983), but the distribution of the more cellulose-like regions has not been evaluated. There is evidence, however, suggesting chemical heterogeneity of barley β-glucan. Izawa *et al.* (1993) found a higher proportion of β-(1→3) linked cellotriosyl units in less soluble fractions. Izydorczyk *et al.* (1998) reported that fractions requiring alkali for extraction contained a higher proportion of cellulose-like regions. While these latter structures could have profound effects on behaviour in water (Buliga *et al.* 1986), recent data (Böhme & Kulicke 1999a; Cui & Wood 2000) support the conclusions of Izawa *et al.* (1993) that increasing structural regularity, arising from increasing proportions of β-(1→3)-linked cellotriosyl units as in wheat β-glucan and lichenan, reduces solubility, and also increases tendency to gel.

28.2.2 *Molecular weight (MW)*

Notable among earlier studies, before on-line MW sensitive detectors for high-performance size exclusion chromatography (HPSEC) became readily available, Woodward *et al.* (1983b) determined the MW of barley β-glucan using sedimentation and osmometry, while Vårum and colleagues (1988, 1992) used both osmometry and light scattering to evaluate oat β-glucan.

It is evident that the β-glucan used in these studies was not of the highest MW, and while the data provided valuable information on molecular conformation, interpretation of data on real foods requires knowledge of material as solubilised from intact cell walls.

In this respect, HPSEC analysis is particularly useful for cereal β-glucan, since dye-binding of Calcofluor (Wood *et al.* 1991b) allows specific and quantitative detection of β-glucans without interference from other components. Comparisons of different products and extracts may be readily made without purification, but to determine true MW distributions β-glucan standards should be used for calibration of the column. Standards (which are available from Megazyme International, Bray, Ireland) may be evaluated using on-line, low-angle laser light scattering (LALLS), multi-angle laser light scattering (MALLS), or right-angle laser light scattering (RALLS), in conjunction with viscometry.

Recently, Gómez *et al.* (1997) used HPSEC with MALLS and intrinsic viscosity to characterise barley β-glucan (in pure water). These studies demonstrated the formation of aggregates in dilute solution, a phenomenon first reported by Vårum and colleagues (1988, 1992) and also observed in our laboratory (unpublished data). The presence of small numbers of high-molecular weight aggregates may distort MW distribution values.

For this and other reasons, such as the use of different extraction methods, comparisons of data and assessment of published differences in MW distributions of β-glucan from different cereal sources remains difficult. In our own laboratory (Wood *et al.* 1991b; Beer *et al.* 1997a), β-glucan in extracts from various oat cultivars was of higher MW than β-glucan in equivalent barley extracts, both showing maximum (peak) values in the range $2–3 \times 10^6$ g/mol, with the former publication reporting overall higher values. Bhatty (1995) also reported that the MW of oat β-glucan was somewhat greater than that of barley β-glucan. Autio *et al.* (1992) reported that the MW of oat β-glucan was cultivar-dependent, with a highest value of 1.5×10^6, whereas Wood *et al.* (1991b) and Beer *et al.* (1997a) observed little cultivar variation. The values reported in these studies are significantly greater than those reported in studies with purified extracts (Gomez *et al.* 1997; Vårum & Smidsrød 1988).

28.2.3 *Behaviour in solution*

Since viscosity may control the physiological behaviour of β-glucan, it is necessary to consider factors involved in its measurement and particularly, how best to compare different materials. It is common in the literature describing clinical effects of soluble fibres, for reference to be made to a possible importance of viscosity, but often either no data, or insufficient details for interpretation or subsequent comparisons, are provided. Although viscosity is a fundamental characteristic, measurements and comparisons are awkward, since viscosity depends on measuring conditions such as shear rate and temperature. For example, the relative 'viscosities' of two β-glucans of different MW, or of β-glucan and guar, can differ by as much as an order of magnitude depending on the concentration and shear rate of measurement (Wood *et al.* 1990).

The underlying property of interest is the hydrodynamic volume occupancy of the molecule, which depends primarily on: (1) molecular weight distribution; and (2) conformation in solution, in turn controlled by structure. Polysaccharide volume occupancy in solution can rapidly reach the point where significant overlap and entanglement of molecular domains can occur, leading at low concentrations to marked hindrance of water flow. An additional mechanism whereby polysaccharides influence water's ability to flow is through intermolecular associations where an essentially infinite network is formed, so there is no flow, but instead a gel with elastic properties.

Cereal β-glucans exist in solution as random coils and may exhibit gelling behaviours under certain conditions. Doublier and Wood (1995) showed that, for an oat gum containing 80% β-glucan, as expected for random coil behaviour, above about 0.3% concentration (critical concentration c*):

$$\eta_{sp0} \propto (c[\eta])^{3.9}$$

where, η_{sp0} is specific viscosity at zero shear rate, c is concentration, and $[\eta]$ intrinsic viscosity. From this it can be seen that, above 0.3%, viscosity is highly concentration-dependent; a doubling of concentration could produce a 15-fold increase in viscosity. Recently, Böhme and Kulicke (1999a) have suggested that the exponent may be even higher, leading to an even greater sensitivity of viscosity to concentration above the critical (entanglement) concentration.

Since $[\eta] = K(MW)^{\alpha}$ (Mark-Houwink relationship for random coils in solution), viscosity is also highly sensitive to MW (although somewhat less than to concentration).

Doublier and Wood (1995) demonstrated weak gel formation in aqueous solutions of oat β-glucan using dynamic (oscillatory) viscosity measurements. This was only observed in partially depolymerised samples. The lower the MW, the lower the flow viscosity at all shear rates, but there was evidence for weak gel characteristics in the partially hydrolysed samples. This tendency to show gel behaviour was known for partially depolymerised barley β-glucan in beer (Letters 1977), but until recently quantitative measurements had not been made. Morgan and Ofman (1998) used gelation of low-molecular weight barley β-glucan for isolation purposes, similar to the standard method for isolation of the structurally similar, low-molecular weight lichenan from *Cetraria islandica*. Cui and Wood (2000) isolated a wheat β-glucan which was of lower MW than that from oats or barley, and which had an increased proportion of β-(1→3)-linked cellotriosyl units relative to oat and barley β-glucan, thus making it structurally more regular and like lichenan. On storage at 4°C, solutions (2%

w/v) of this β-glucan showed distinctive gelling behaviour in which the storage modulus G' was greater than the loss modulus G" and somewhat insensitive to frequency over the frequency range 0.1–50 Hz (Fig. 28.1). A sharp melting transition was observed at 40°C. No gelation was observed with prolonged storage of 0.5% solution at 4°C. The gelling behaviour of β-glucans has been studied in detail by Böhme and Kulicke (1999a, b), using a selection of barley β-glucans of different MW and oat β-glucan and lichenan. The overall conclusions are that gelation occurs more readily with:

- more regular structure, in effect with increasing proportion of β-(1→3)-linked cellotriosyl units so lichenan > wheat > barley > oats;
- lower molecular weight (lower limit for this not known);
- prolonged solution storage time and lower temperature;
- higher concentration; and
- cycles of shear and 'rest'.

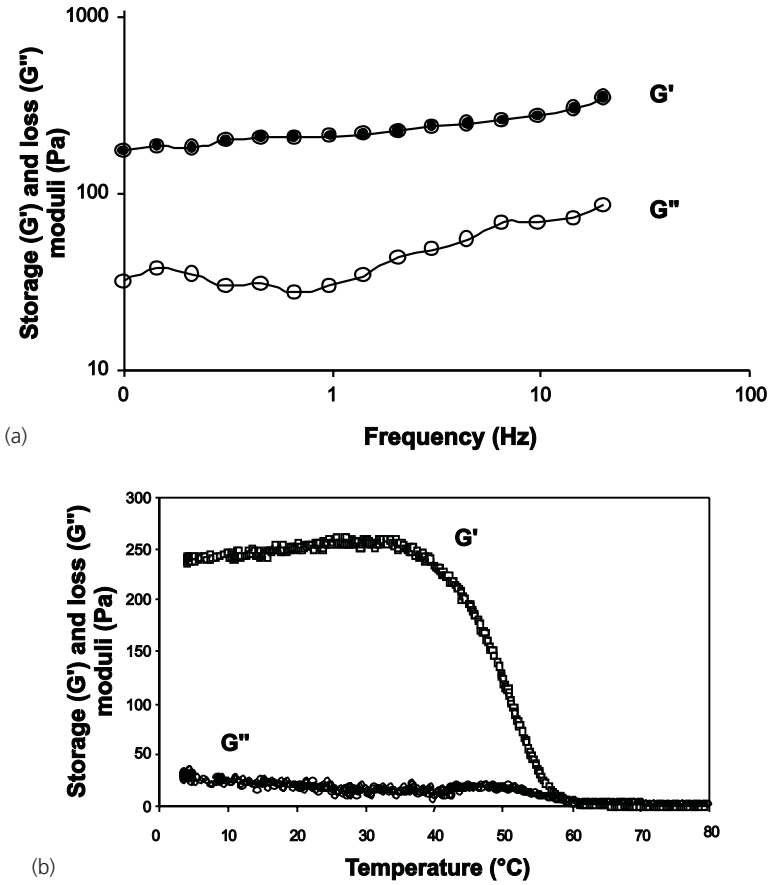

Fig. 28.1 (a) Mechanical spectrum of 2.0% (w/w) wheat β-glucan gel at 4°C. (b) Melting curve of 2.0% (w/w) wheat β-glucan gel at 1 Hz of frequency. Measured with Bohlin CVO Rheometer, cone/plate 4°C. (Adapted from Cui and Wood 2000.)

From the perspective of food functionality, and especially soluble fibre effects and health benefits, neither viscosity nor gelling characteristics come in to play prior to hydration and solubilisation. The term solubility usually refers to extractability under certain defined conditions, in the case of dietary fibre those of a specified analytical methodology. Data from extractions using different conditions are not comparable, and from the perspective of attempting to evaluate how a food might influence gut rheology, physiological extraction conditions should obviously be used.

MW of the β-glucan, structure, cell-wall thickness and entanglement with other cell-wall polymers may all play a role in the susceptibility of cereal β-glucan to extraction with mild reagents and conditions. McCleary (1988) reported ~90% extraction of barley β-glucan with water at 40, 65 and 95°C, whereas Beer *et al.* (1997a) reported ~65% of the total β-glucan in oats and waxy barley and ~50% in non-waxy barley was extracted by water at 90°C. Two additional hot-water extractions and a final dimethyl sulphoxide (DMSO) extraction increased the total β-glucan solubilised to ~80%, but total extraction required sodium hydroxide. Wood *et al.* (1991b) reported that ~45% of barley and 70% of oat β-glucan were extracted by carbonate (pH 10) at 60°C. In order to extract wheat β-glucan, which is insoluble in hot water, Cui *et al.* (2000) used 1 M NaOH at 25°C for 2 h – conditions shown not to degrade the β-glucan. However, Beer *et al.* (1997a) observed loss in MW during extraction of oats and barley in 1.25 M NaOH at 22°C for 16 h. Analytical use of NaOH (Carr *et al.* 1990) and acid (Ahluwahlia & Ellis 1984) for complete extraction leads to depolymerisation.

Processing, cooking and storage may influence both extractability and MW. Using an in-vitro system simulating the human digestion, Beer *et al.* (1997b) showed differences in extractability and MW of β-glucan in oat brans and muffins (Table 28.2). Three oat bran samples showed differences in extractability and MW of β-glucan; baking to muffins increased extractability, but decreased MW. A major difference in extractability between muffins from different recipes was also detected, and perhaps more importantly from the point of view of clinical trials, frozen storage decreased extractability.

28.3 Clinical studies of oat β-glucan

Space prevents a full review of clinical studies of the effects (which may or may not be related

Table 28.2 Percentage extracted and peak molecular weight (MW) of β-glucan solubilised from original brans and muffins by an *in-vitro* digestion method.

Sample	Solubilised β-glucan (% of total)	Peak MW ($\times 10^{-3}$)
Bran A	12.9	1100
Bran B	25.1	1800
Bran C	28.7	1900
Bran A muffin	55	600
Bran B muffin	77.8	780
Bran C muffin	85.3	950
Bran A muffin: 8 weeks frozen storage	27	560
Bran B muffin: 8 weeks frozen storage	39.8	760
Bran C muffin: 8 weeks frozen storage	36.1	900

Source: adapted from Beer *et al.* 1997.

to β-glucan content) of consuming oat products. Reviews of the effects on serum cholesterol may be found in the Federal Register of the US FDA (Anon. 1997, 1998) and in a recent meta-analysis (Brown *et al.* 1999). The lack of a commercially available isolate at affordable cost made studies of a specific β-glucan effect difficult. To overcome this problem, during the 1980s, Agriculture Canada's Food Research Institute, in collaboration with the POS Pilot Plant in Saskatoon, prepared sufficient quantities to allow clinical trials (Wood *et al.* 1989).

28.3.1 *Blood glucose and insulin*

Attenuation or flattening of the postprandial elevation of blood glucose and insulin is of benefit to both healthy and diabetic people. Observational studies (Salmeron *et al.* 1997) suggest that low-glycaemic index (GI) diets, and diets high in cereal fibre, may be associated with decreased risk of development of non insulin-dependent diabetes mellitus (NIDDM).

The pioneering studies of Jenkins *et al.* (1978), which demonstrated that the lowering of postprandial blood glucose and insulin levels by soluble fibres was viscosity-dependent, has been supported by a number of subsequent studies, most commonly with guar gum (Ellis *et al.* 1995) which, like β-glucan, behaves as a random coil in solution.

By varying both dose and MW of oat β-glucan in a drink model, Wood *et al.* (1994a) demonstrated an inverse linear relationship between log[viscosity] of the mixtures consumed and the magnitude of the blood glucose and insulin response to a 50 g oral glucose load. Except for the highest viscosity samples, individual comparisons with the control did not detect significant differences, but the regressions, which showed that viscosity accounted for 79–96% of the modifications in glucose and insulin response, were highly significant. The logarithmic nature of the relationship means that very large changes in viscosity might be required to elicit significant changes in response. Thus, a doubling of viscosity from 1.0 to 2.0 Pa.s (at 30 s^{-1}) was required to reduce the mean peak blood glucose increment from 2.1 to 2.0 mM/L (less than standard error). This highlights the difficulties likely to be encountered in obtaining valid comparisons of materials, even when useful data on viscosity are reported.

As discussed previously, viscosity is controlled, *inter alia*, by molecular weight and concentration in solution. Accordingly, following determination (M.U. Beer & P.J. Wood, unpublished results) of the MW of the β-glucans used in the studies of glycaemic response (Wood *et al.* 1994b), the data were examined to determine if response could be related to these more fundamental and readily determined and compared parameters. The data (Table 28.3) showed that peak blood glucose response, area under the 2-h curve and glycaemic index (not shown) were inversely related to log (concentration × molecular weight).

Similar relationships, as in the drink model above, have been reported using an extruded oat bran product (Tappy *et al.* 1996). In our hands (Braaten *et al.* 1994a), an experimental high β-glucan content (~15% w/w) oat bran was compared with cream of wheat (wheat farina) to which β-glucan isolate was added to the same level as that in oat bran. Both meals, which were similarly textured hot cereal porridge, similarly reduced the postprandial plasma glucose and insulin levels compared with control wheat farina, in both healthy people and subjects with NIDDM. The study showed that both the native cell-wall fibre of oat bran and an isolated oat gum incorporated into a meal, act similarly to lower postprandial plasma glucose and insulin levels. However, the β-glucan, as extracted by an *in-vitro* digestion procedure from the oat bran (~30% of the total) was about twice the MW of the β-glucan in the isolate (which was completely soluble). Thus, the relative contributions to supernatant viscosity (volume occupancy) from MW and concentration would differ in the two cases. Also, the peak blood

Table 28.3 Relationships between blood glucose parameters and $\log_{10}$[viscosity]* or $\log_{10}$[concentration × M_W].†

Variables	a	b	r^2	P‡
Viscosity§				
Peak Δ glucose (mmol/l)	3	–0.31	0.90	<0.0001
AUC glucose (mmol·min/l)	136	–12.1	0.79	<0.0001
Concentration × MW**	aN	bN		
Peak Δ glucose (mmol/l)	7.3	–0.9	0.83	<0.05
AUC glucose (mmol·min/l)	289	–32	0.77	<0.05

* Variable = a + b $\log_{10}$[viscosity]; viscosity, mPa.s at 30 s^{-1}.
† Variable = a' + b' $\log_{10}$[c.M_W]; c, concentration g/100 ml; M_W is weight average molecular weight.
‡ Significance of regression relationship.
§ Data points in regression include control values, viscosity = 1 (water).
** Control (water) values not included in regression.

glucose increments observed with the oat bran and cream of wheat plus β-glucan meals (2.0 and 1.8 mM/L respectively, not significantly different) were in the range of values where sensitivity to viscosity would be difficult to detect without using more subjects.

In a study of two doses of the commercially available β-glucan isolate known as 'Oatrim', Hallfrisch *et al.* (1995) reported lower postprandial blood glucose and insulin levels at two doses, both doses being lower than those showing significant effects in the study of Wood *et al.* (1994a) The study differed from that of Wood *et al.* (1994a) in that responses arose from both an acute effect and long-term adaptation to the β-glucan isolate.

28.3.2 *Cholesterol*

The health claim in the US, which allows a statement that oats may lower risk of heart disease, is predicated on ability to lower serum cholesterol levels, a risk factor for heart disease. In general, cereal consumption seems to be associated with a lower risk of heart disease, to a greater extent than might be expected from any potential cholesterol-lowering effect (Wolk *et al.* 1999). While observational studies indicate that other factors seem to be involved in risk modification, it has generally been observed in clinical studies that 'viscous' soluble fibres such as guar gum (Blake *et al.* 1997) lower serum cholesterol levels. Unlike wheat, oats are a source of viscous soluble fibre, and the epidemiological observations suggest a slightly greater risk reduction for heart disease associated with oats, as opposed to 'cold' cereal consumption.

This supports studies (over 50 in number) on the effect of oat products on serum lipid levels in humans, which generally indicate a lowering of serum cholesterol levels. The results in the literature are variable however, and in the 33 studies selected by the FDA as meeting minimal requirements for consideration, only just over half (17) clearly demonstrated a beneficial cholesterol lowering. A recent meta-analysis (Brown *et al.* 1999) which examined literature on various soluble fibre sources, found a wide variation in reported responses, and summarised the data as indicating that the cholesterol-lowering effect was minimal and likely to be of low significance for modification of risk.

Notwithstanding the controversy over whether or not oat bran has a specific cholesterol-lowering effect, the ability of some polysaccharide isolates to lower serum cholesterol levels is well known (Anderson *et al.* 1990), and in the case of psyllium has led to another allowed FDA claim (Anon. 1998). Consideration of the data presented earlier in this chapter and the following data on the effects of β-glucan isolate provides some leads as to why clinical trials of foods containing soluble fibre may have variable outcomes.

Braaten *et al.* (1994b) fed ~6 g β-glucan per day for 4 weeks to 19 hypercholesterolaemic subjects in a placebo-controlled, cross-over design study. There were no changes in nutrient intake or weights of the subjects during the study. The data, summarised in Table 28.4, showed significant lowering of total and low-density lipoprotein (LDL)-cholesterol over the 4-week period of consumption, with no change in high-density lipoprotein (HDL)-cholesterol. In this study the dose fed was based on the earlier study (Wood *et al.* 1994a) on glycaemic response. The material was well characterised (Wood *et al.* 1989, 1994b) and known to be of high MW, was delivered in a completely soluble drink form, and subjects were instructed to consume the product intimately mixed with the main meals of the day. This ensured that, if viscosity plays a role in the mechanism of cholesterol lowering by modifying luminal viscosity during nutrient absorption, then the effect would be exerted. An additional aspect of this study was that blood lipid measurements were carried out weekly, which addressed the problem of individual blood lipid fluctuations (Thompson & Pocock 1990) by establishing a more stable reference (baseline) value for each subject. In addition to potentially enabling the detection of weaker effects, the procedure facilitated mathematically objective identification of individuals in whom the blood cholesterol was lowered by oat gum. Individual response might have a genetic basis (Hegele *et al.* 1992), and there may be an age/gender effect (Keenan *et al.* 1991). These factors could explain the failure of oat bran to reduce blood cholesterol levels in some studies. In this study, five subjects could be statistically identified as non-responders. The mean LDL-cholesterol lowering in the remaining 14 subjects was 13%.

Table 28.4 Blood cholesterol levels (mmol/l).

	Time (weeks)				
	0	1	2	3	4
Total cholesterol					
Placebo	6.57	6.92	6.82	6.76	6.67
β-Glucan	6.76	6.52	6.54	6.42	6.15
P-value*	0.089	0.022	0.038	0.07	0.001
HDL-cholesterol					
Placebo	1.26	1.34	1.28	1.28	1.29
β-Glucan	1.26	1.30	1.33	1.31	1.27
LDL-cholesterol					
Placebo	4.45	4.75	4.65	4.63	4.54
β-Glucan	4.59	4.43	4.33	4.21	4.16
P-value*		0.051	0.004	0.013	0.003

**P*-values for treatment – placebo comparison. >0.1 if no value shown.
HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Beer *et al.* (1995) have also reported clinical trials using well-characterised (Beer *et al.* 1996) oat β-glucan extracts. While Beer *et al.* observed an increase in HDL-cholesterol, no changes in total or LDL-cholesterol levels were found. However, in this study the subjects were normo-cholesterolaemic, feeding was for just 2 weeks, and there was no washout period, potentially making it difficult to observe any effect. Additionally, the product had a low solubility, which might limit the ability to modify gut viscosity.

Behall *et al.* (1997) reported significant lowering of total and LDL-cholesterol with both 1% and 10% β-glucan levels of Oatrim. Cholesterol levels decreased while establishing subjects on a maintenance diet, which contained 1.3 g per day of soluble β-glucan. In the Oatrim phases, 2.1 g β-glucan per day reduced total cholesterol levels by 9.5% from the maintenance diet phase, and an additional 5% reduction was achieved with 8.7 g β-glucan per day. Subjects lost weight during all phases, but to a greater extent when consuming the Oatrim.

28.4 Conclusions

Oat β-glucan, like other viscous polysaccharides, attenuates blood glucose and insulin levels and reduces serum cholesterol levels in hypercholesterolaemic subjects. The lowering of blood glucose and insulin is viscosity-dependent and thus, also molecular weight- and concentration-dependent.

Lower daily insulin levels are associated with lower serum cholesterol levels (Jenkins *et al.* 1989). A clear relationship between viscosity and serum cholesterol lowering, as found for glycaemic response, is not strongly established, but has been demonstrated in a hamster model (Gallaher *et al.* 1993). In humans, a low-viscosity gum (acacia) did not lower serum cholesterol levels, while a mixture of high-viscosity gums did, but this experimental design was also comparing chemical structure (Jensen *et al.* 1993). In other experiments, much higher doses of gum acacia (arabic) did lower serum cholesterol levels (McClean Ross *et al.* 1983), but this comparison does not distinguish specifically between dose and viscosity, which requires examination of differing molecular weights of the same gum. In a comparison of 'high-' and 'medium-' viscosity guar gum, in which the viscosity assignments were based on commercial description and no measurements were quoted, the 'higher' viscosity elicited a greater reduction in serum cholesterol levels, but the statistics of this were not reported (Superko *et al.* 1988). Recently, a partially depolymerised guar gum was shown to lower serum cholesterol levels (Blake *et al.* 1997), but this product was still of relatively high MW (similar to the MW of oat β-glucan used by Braaten *et al.* 1994b).

Obviously, however, if viscosity does play a role in determining efficacy of products, the factors controlling viscosity which include amount of β-glucan (or other fibre), solubility, molecular weight and structure must be considered when designing experiments, and should be considered in literature evaluation. Amount of fibre is readily determined and well known for most products, but the effects of process and cooking on solubility and molecular weight are less understood. Since the literature does not normally provide the necessary information, detailed comparisons of fibres and evaluations of the level of effect, such as that of Brown *et al.* (1999), are precarious. There are also significant non-product-related reasons for variable results in the literature, such as subject characteristics and mode of delivery of the fibre. Wolever *et al.* (1994) showed that psyllium consumed between meals was less effective (indeed not significantly different from control) than when consumed with the meal.

Future research in this area should focus on obtaining a clearer understanding of soluble fibre properties in real food matrices, establishing the role of viscosity (or otherwise) and establishing the mechanism of action.

[Contribution no. M008 of the Food Research Program.]

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Part 8

Legume-seed Polysaccharides

29 Novel Galactomannans and Galacto-manno-oligosaccharides from Guar

Mariel Brooks, Ross Campbell and Barry V. McCleary

29.1 Introduction

The traditional source of galactomannan is from the seeds of the carob tree, which was cultivated many centuries before the Christian era. Carob is a native of Southern Europe and the Near East, the best quality seeds coming from Sicily, where the trees were probably planted in the sixteenth to seventeenth centuries. Guar emerged as a commercial source of galactomannan in response to the limited supply of locust bean gum (LBG) to the USA, during World War II. The guar plant is native to north-west India and Pakistan, where it has been grown for thousands of years for use as cattle fodder and as a green vegetable. Commercially available galactomannans are derived from the seed endosperms of carob (locust bean, *Ceratonia siliqua*), guar (*Cyamopsis tetragonolobus*) and to a lesser extent, tara (*Caesalpinia spinosa*) (Dea & Morrison 1975; Neukom & Nittner 1980).

Locust-bean and guar galactomannans have similar solution viscosities, but differ markedly in their ease of dissolution and in their interaction properties. The limiting viscosity number (LVN) for guar galactomannan is about14 dl/g (determined in dilute solution), whereas that for LBG is about 11 dl/g (McCleary *et al.* 1976). Guar galactomannan has the ability to hydrate rapidly in cold water to produce highly viscous solutions, whereas LBG requires heating to about 85°C to achieve complete solubilisation. The viscosities of 1% solutions of guar and locust-bean galactomannans, hydrated at 25°C, are 4200 and 100 cps, respectively (Whistler & Hymowitz 1979). The difficulties in the dissolution of LBG are believed to be due to inter-chain associations, which restrict hydration. These associations occur at regions in the mannan backbone which are unsubstituted or lightly substituted with galactose (Morris *et al.* 1977).

LBG interacts strongly with a number of polysaccharides including agar, carrageenans and xanthan, whereas guar gum does not (Dea & Morrison 1975). This is known to be due to the lower D-galactose content of the LBG as well as the 'fine-structure' of distribution of the D-galactosyl residues along the D-mannan backbone (McCleary *et al*. 1985). Cyclical shortages of LBG have resulted in an up to 400% increase in the price of this material from year to year. Consequently, considerable effort has been expended over the past 20 years to find an alternative polysaccharide with similar functional/interaction properties. Potential alternatives include konjac glucomannan (Dea & Morrison 1975) and galactomannans from various other legume seeds, particularly those of the *Cassia* species. Each of these options have their own problems, specifically, the stability of the polysaccharide (e.g. deacetylation of glucomannan under alkaline conditions) or possible toxicity of other seed components.

An alternative approach and the one we decided to adopt is to modify enzymatically guar galactomannan to produce a material similar to LBG (McCleary *et al*. 1981, 1984). Basically, this involved treating guar gum with a specific α-galactosidase enzyme, devoid of

β-mannanase, to reduce the galactose content to a level similar to that in LBG (22%) with no depolymerisation of the mannan main chain.

In this chapter we will describe the development of this range of galactomannan products, from the initial concepts through to the first commercially available range of products, namely Sherex QSG (QSG). QSG is a novel range of polysaccharides with high functionality as gelling agents and as soluble dietary fibres. Novel, depolymerised galactomannan materials produced by combined or sequential treatment of guar gum with α-galactosidase and β-mannanase will also be described.

29.2 Enzymatic hydrolysis of galactomannans

A range of galactomannan-degrading enzymes have been described in literature, including α-galactosidase (Dey 1978), β-mannanase (Emi *et al*. 1972), β-mannosidase (McCleary 1983) and *exo*-β-mannanase (McCleary 1982). In this chapter, the use of α-galactosidase and β-mannanase to produce novel galactomannans and galacto-manno-oligosaccharides will be described.

29.2.1 α-*Galactosidase*

α-Galactosidase catalyses the random cleavage of (1–6)-α–linked D-galactosyl residues from the mannan backbone of galactomannans (McCleary *et al.* 1981, 1984; Dea *et al*. 1986; Bulpin *et al*. 1990). To study the effect of removal of galactose from the mannan backbone on the solution and interaction properties of the galactomannan, it is essential that the α-galactosidase employed is devoid of the chain-splitting enzyme, β-mannanase. α-Galactosidase enzymes vary markedly in their ability to remove galactose from galactomannans. In general, microbial enzymes are not effective in catalysing this reaction. From our studies, two very effective enzymes were identified (McCleary *et al.* 1981, 1983). These are the α-galactosidase enzymes that are synthesised in the endosperm tissue of lucerne and guar seed during seed germination. These enzymes were purified by affinity chromatography on a column of galactosylamine immobilised to *N*-ε-aminocaproyl-Sepharose 4B. This purification effectively removed all of the contaminating β-mannanase.

Enzyme purity and solution properties of galactose-depleted galactomannans

To demonstrate the absence of β-mannanase in α-galactosidase preparations, the action of α-galactosidase on dilute solutions of galactomannan was studied by viscometric and reducing sugar procedures. The action of pure α-galactosidase and pure β-mannanase on LBG in dilute solution (15 ml, 0.1%) is shown in Fig. 29.1. With α-galactosidase (200 mU), it is possible to reduce the galactose content from 23% to 15% with no fall in viscosity. In contrast, as little as 0.24 mU of β-mannanase causes an immediate and significant fall in viscosity (McCleary *et al*. 1981). This is clearly a simple and effective way to check the purity of the α-galactosidase preparation, although excessive treatment of galactomannan with α-galactosidase will eventually cause a viscosity drop. This is not due to mannan chain cleavage, but rather to association of the galactose-depleted mannan chains to form a precipitate

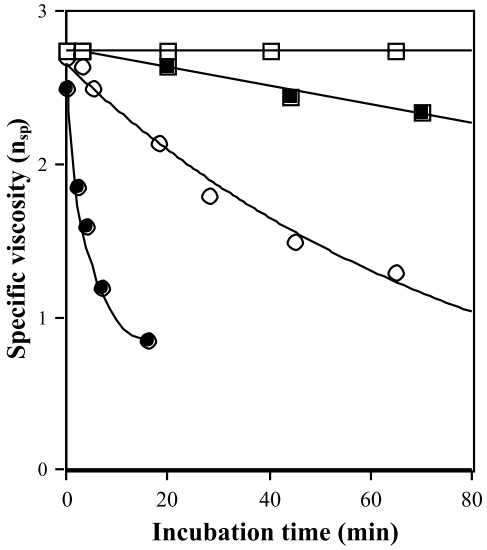

Fig. 29.1 Effect of α-galactosidase and β-mannanase on the solution viscosity of carob galactomannan. Carob galactomannan (15 ml, 0.1%) in 0.1 M sodium acetate buffer (pH 4.5) was incubated at 30°C in an Ubbelohde suspended level viscometer with either α-galactosidase (□) 200 mU (on this substrate) or β-mannanase (●) 24 mU, (○) 2.4 mU or (■) 0.24 mU.

that eventually settles from solution (Fig. 29.2). This precipitation is time-dependent and directly related to the concentration of the galactomannan.

Removal of galactose from guar galactomannan results in galactomannans that have increased limiting viscosity numbers and steeper viscosity curves (Fig. 29.3). However, if the viscosity curves are plotted against the concentration of the 'mannan-backbone' in these polymers, a single curve is obtained independent of the galactose content (McCleary *et al*. 1981). This finding indicates that the solution viscosity of such galactomannans is totally dependent on the nature of the mannan backbone. The galactose side chains play a very important role in determining the ease with which galactomannans can dissolve. However, they do not affect the degree of interaction between galactomannan molecules in dilute solution (assuming, of course, that the galactose content is sufficient to maintain solubility), nor do they have any apparent effect on the conformation of galactomannan molecules in solution.

Interaction properties of galactose-depleted galactomannans

As the D-galactose content of galactomannans is lowered, the degree of self-association of the galactomannan, and the degree of interaction with other polysaccharides such as agar, carrageenan and xanthan increases. At a concentration of 0.4%, guar galactomannan solutions are stable at 20° or 4°C indefinitely. However, α-galactosidase-modified galactomannans with a D-galactose content of 15–20% form gels on storage at 4°C for 15 days. Solutions of galactomannans that contain less than 10% galactose are quite unstable at 30° or 4°C, and an insoluble mannan-type precipitate rapidly forms.

To study the effect of the removal of galactose on the degree of interaction with xanthan and agarose, a range of galactose-depleted guar galactomannan samples were prepared. Guar

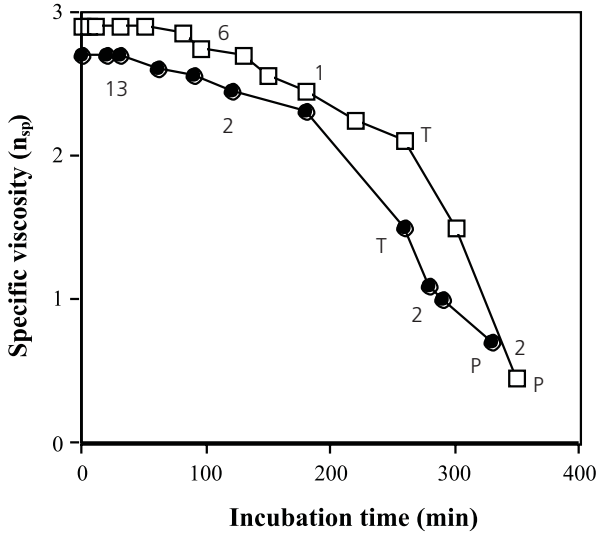

Fig. 29.2 Effect of galactose removal on the solution viscosity and solubility of galactomannans from guar (□) and locust bean (●). Galactomannan solution (17 ml, 0.1%) in 0.1 M sodium acetate buffer (pH 4.5) was incubated with lucerne-seed α-galactosidase A (370 mU) in an Ubbelohde suspended level viscometer at 40°C. Samples were removed for analysis of galactose content. Numbers represent the galactose content of the remaining galactomannan. At point T, the solution was very turbid: at point P, a precipitate had formed.

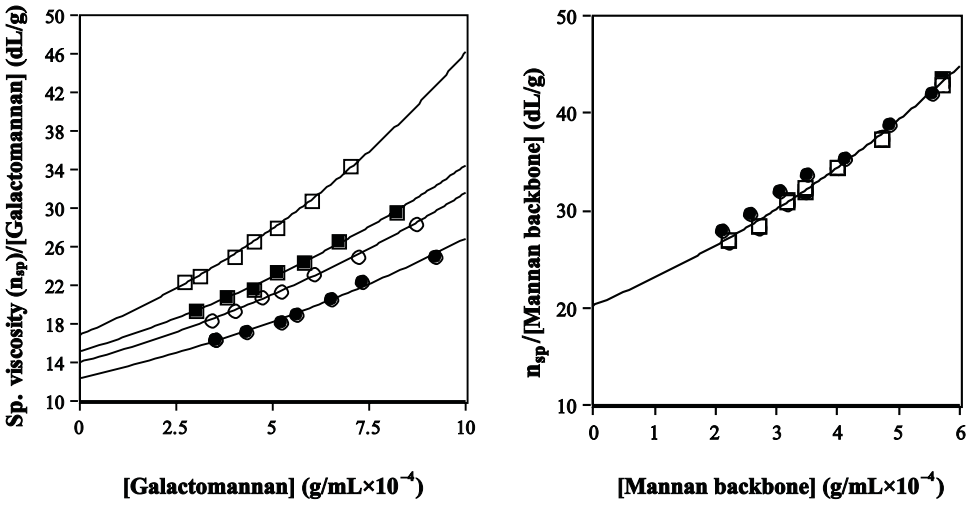

Fig. 29.3 Effect of galactose removal from guar galactomannan on limiting viscosity number. The galactose: mannose ratios of the polysaccharides are 38:62 (●), 32:68 (○), 27:73 (■), and 15:85 (□).

galactomannan was treated with varying levels of pure guar-seed α-galactosidase (devoid of β-mannanase) to produce galactomannan samples with galactose contents of 39.0 (A) (native guar gum), 33.6 (B), 28.7 (C), 24.6 (D), 19.2 (E), 16.4 (F), 13.7 (G) and 10% (H). The behaviour of these materials, and of LBG (23% galactose), in mixed polysaccharide systems was

studied spectroscopically and using destructive and non-destructive rheological techniques (McCleary *et al*. 1984; Dea *et al*. 1986). Some of the properties of these materials are shown in Table 29.1.

On admixture of xanthan (0.5% w/v) with native guar gum (sample A) and the least modified sample (B) (1.0%), no evidence of gel formation on cooling to 20°C was observed. Mixtures of xanthan with samples C to H formed gels under these conditions. The gel formed using sample C was, however, only just self-supporting, and was too weak to permit accurate measurement of yield stress using the Instrom Materials Tester. The yield stress values for the gels formed with the other modified guar samples and with carob gum are shown in Fig. 29.4 and Table 29.2. As the galactose content of the modified guar samples decreased from 24.6% (sample D) to 13.7% (sample G), the yield stress values for the mixed xanthan/galactomannan gel increased markedly. Because of its low galactose content (10%), sample H had only limited solubility. When dissolved alone in water it showed a tendency to partially precipitate on standing at room temperature. This also occurred in mixtures with xanthan, and resulted in the formation of an uncharacteristic turbid mixed gel. The lower value of yield stress for the gel obtained from xanthan and sample H is considered to be due to this limited solubility.

Examination of the same polysaccharide mixtures using a Rheometrics Mechanical Spectrometer gave the storage modulus (G') values shown graphically in Fig. 29.4. The G' values show a similar trend to that of the yield stress. The G' data are however more sensitive, and indicate that although sample B (34% galactose) does not cause xanthan to gel, the two polymers do show a significant increase in rheological interaction over that with guar gum.

29.2.2 β-*Mannanase*

β-Mannanase acts by the random cleavage of the D-mannan chain, producing a series of manno- and galacto-manno-oligosaccharides and giving a rapid viscosity decrease (Dekker

Table 29.1 Properties of locust bean gum (LBG) and D-galactose-depleted guar galactomannan (EMG) samples.

sample	Galactose content of galactomannan samples			Intrinsic viscosity (dl/g)		Degree of hydrolysis by *A.niger* β-mannanase (%)
	Enzymatic	Spectroscopic	GLC	I*	II†	
A (native)	39.0	40.0	38.4	16.0	14.1	5
B	33.6	34.0	32.7	19.3	14.4	14
C	28.7	28.0	26.5	16.6	17.8	20
D	24.6	22.5	21.9	19.6	18.0	23
E	19.2	18.7	18.9	21.8	19.5	29
F	16.4	13.7	14.3	21.4	19.6	30
G	13.7	12.2	11.9	21.2	19.6	31
H	10.0	–‡	9.8	–‡	–‡	32
LBG	23.0	23.0	22.6	13.4	10.9	22

* Determined using a Contraves low-shear viscometer.
† Determined using an Ubbelohde suspended viscometer.
‡ Not determined due to polymer solubility problems.

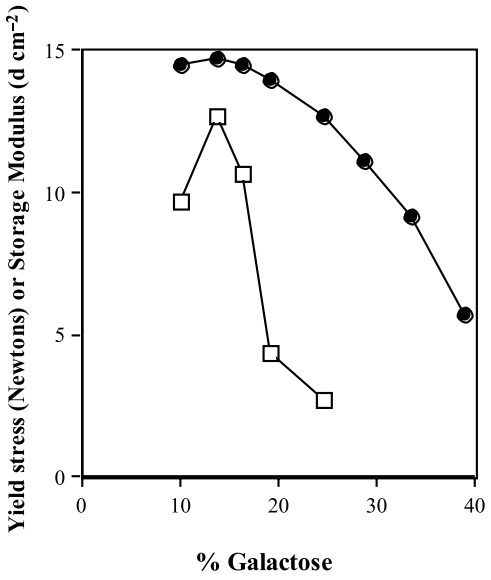

Fig. 29.4 Variations in the yield stress (●) and storage modulus (□) for mixed gels of xanthan (0.5%) and modified galactomannans (1.0%) with galactose content of galactomannan.

Table 29.2 Interaction properties of locust bean gum (LBG) and D-galactose-depleted guar galactomannan (EMG) samples with agarose and xanthan.

Galactomannan sample	Positive contribution to optical rotation change from galactomannan 436 nm* (°)	Average yield stress† (N)	Storage modulus† (d·cm^{-2} × 10^{-3})	Gel melting point† (°C)
A (native guar)	0.003	–‡	0.57	–‡
B	0.007	–‡	1.45	–‡
C	0.008	–‡	2.63	35–36
D	0.015	2.6	4.25	37–39
E	0.018	4.4	5.86	40–41
F	0.030	10.9	6.55	44–46
G	0.040	12.9	6.74	45–47
H	–§	9.8	5.79	47–49
LBG	0.021	3.4	2.80	38–40

* Obtained from the temperature dependence of optical rotation traces for mixtures of agarose (0.05%) and galactomannan (0.1%).
† Obtained from examination of the mixed gels of xanthan (0.5%) and galactomannan (1.0%). The yield stress values are the average of three separate measurements.
‡ Either gels did not form, or were too weak to allow measurement of properties.
§ Not determined due to polymer solubility problems.

& Richards 1976). Hydrolysis of galactomannan by this enzyme is affected by the degree of galactose substitution and the fine structure of distribution of the galactosyl residues along the mannan backbone (McCleary & Matheson 1983). β-Mannanase enzymes from differ-

ent sources vary in their ability to cleave at specific points in the mannan backbone which are highly substituted with galactose (McCleary 1979). Characterisation and quantification of the oligosaccharides produced on hydrolysis of a particular galactomannan by a specific β-mannanase allows the fine structure of distribution of galactosyl residues to be defined (McCleary *et al.* 1985). This fine structure can, in turn, be related to the degree of interaction of the galactomannan with agarose, carrageenan and xanthan.

Guar galactomannan is hydrolysed to an extent of 5% by *Aspergillus niger* β-mannanase. This treatment yields polysaccharide galactomannan material with greatly reduced solution viscosity. Such material is available commercially as 'Sunfibre' from Taiyo Kagaku Co. Ltd, Japan. The possible commercial production of a range of low degree of polymerisation (DP) galacto-manno-oligosaccharides by concurrent or sequential treatment of guar gum with α-galactosidase and β-mannanase has not been reported. Some properties of such materials will be described later in this chapter.

29.3 Properties of industrially produced galactose-depleted guar galactomannans (enzyme-modified guars)

In the first section of this chapter we have shown that, by selective removal of galactose units, it is possible to convert guar gum to a galactomannans with galactose contents and interaction properties similar to that of locust bean gum. This is performed with a specific α-galactosidase selective for polymeric guar (McCleary *et al.* 1981; McCleary 1983). In this section, we will describe the interaction and gel-forming properties of galactose-depleted guar galactomannans obtained from a commercial scale process (commercially available from Quest International under the trade name Sherex QSG).

Three enzyme-modified guar galactomannan (QSG)samples (containing 19, 21 and 25% D-galactose, respectively, as analysed by Dionex ion chromatography) were produced in large quantity. This was facilitated by the availability, in-house, of a purified α-galactosidase essentially devoid of β-mannanase.

29.3.1 *Interaction with κ-carrageenan*

Guar, which does not gel to any significant extent, is transformed by enzyme modification into a polymer, which forms gels with κ-carrageenan (Fig. 29.5). Gel strength is directly dependent on galactose content; enzyme-modified guar containing 25% galactose gels to a much lesser extent than modified guar containing lower amounts of galactose. Interestingly, those samples with galactose contents less than 19%, produce, in admixture with κ-carrageenan, a gelling synergy that is stronger than that available with LBG. The carrageenan/enzyme-modified guar gels were prepared using 0.4% semi-refined κ-carrageenan, 0.4% enzyme-modified guar and 0.2% potassium chloride. The gels were made by dispersing the hydrocolloid/salt mix in deionised water before heating in a boiling water bath for 20 min. The hot solution was allowed to set overnight at room temperature before measurement.

The texture of κ-carrageenan/enzyme-modified guar gels can be varied using modified guars of various galactose content. The influence of the galactose content of enzyme-modified guar samples on the texture of κ-carrageenan/modified guar gels is depicted in Fig. 29.6. From this illustration it can be seen that κ-carrageenan/modified guar (21% galactose) show

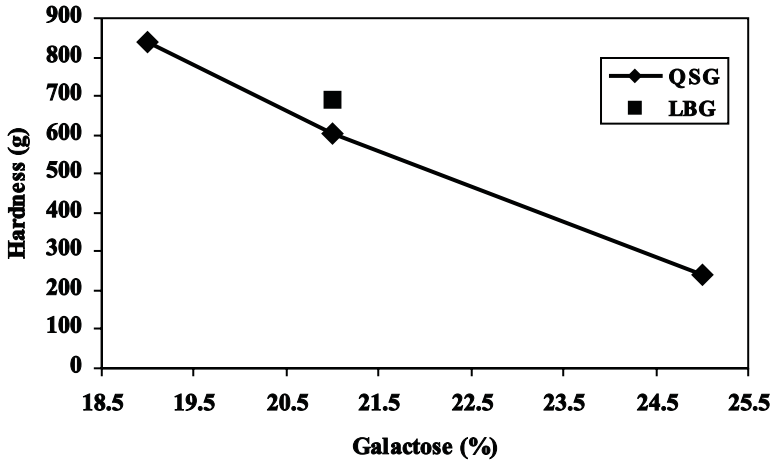

Fig. 29.5 Effect of galactose content of EMG samples on the hardness (g) of mixed κ-carrageenan/EMG gels.

textural properties similar to those of κ-carrageenan/LBG mixtures. With κ-carrageenan/enzyme-modified guar gels in which the modified guar has 19% or 25% galactose, texture properties are quite different to gels with LBG. Thus, admixtures of κ-carrageenan with galactose-depleted guar samples of varying galactose contents offer the opportunity of producing gels with a wide range of textural properties.

Texture profile analysis is an objective method of sensory analysis that attempts to link mouthfeel to a defined measurement. In this test, gels are twice subjected to compression in a reciprocating motion. The aim is to mimic the chewing action of the jaw. Gel texture measurements were performed using a Stable Micro Systems TA-XT2i Texture Analyser (Pons & Fiszman 1996). The texture parameters, including hardness, break distance, cohesiveness, adhesiveness and chewiness, were measured by texture profile analysis methods (0.5 mm/s test speed, 35 mm penetration distance, 1 cm^2 probe, 20°C) where: (1) hardness (g) is defined

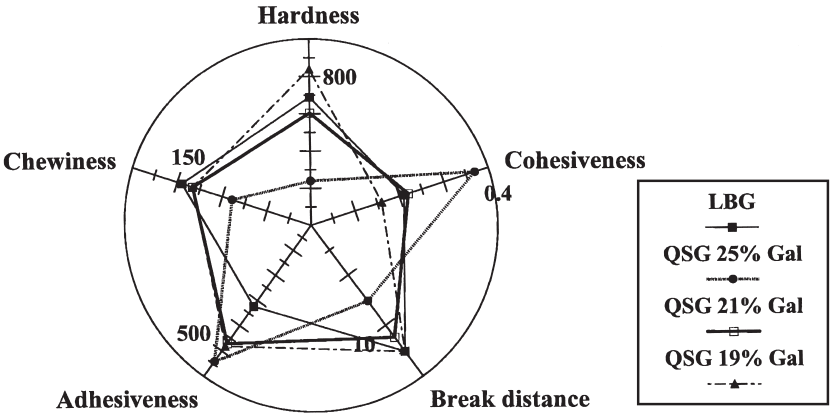

Fig. 29.6 Texture profile analysis of mixed κ-carrageenan/enzyme-modified guar gels. Galactomannans used in the mixtures were LBG and modified guars (QSG) with 25, 21 and 19% galactose.

as the maximum peak force during the first compression cycle; (2) cohesiveness is defined as the ratio of the positive force area during the second compression, to that during the first compression; (3) break distance is the distance moved (in mm) before the gel breaks; (4) adhesiveness is defined as the negative force area for the first bite, and represents the work required to overcome the attractive forces between the surface of a food and the surface of other material with which the food comes in contact; and (5) chewiness is defined as the product of hardness, cohesiveness and stringiness.

Guar gum gives highly viscous aqueous solutions, with viscosity values typically in the region of 5000 cps (1% solution). However, preparations which give viscosities in the region of 7000 cps (1% solution) are also available commercially. Typically, the viscosity of guar gum solutions are much greater than locust bean gum at the same concentration (approximately 3000 cps at 1% w/v). Through careful enzymatic modification of guar gum, it is possible to produce a hydrocolloid that has the gelling characteristics of LBG while retaining the high viscosity of guar gum. Galactose-depleted guar preparations have been prepared which give aqueous viscosities of <100 to 4000 cps.

It would appear that the gel hardness of κ-carrageenan/enzyme-modified guar (21% galactose) mixtures is not directly related to the viscosity (molecular size) of the modified guar (Fig. 29.7). Gel strength remains the same for κ-carrageenan/enzyme-modified guar mixtures over the modified guar viscosity range of 200 to 3000 cps, but drops significantly for modified guar gums of lower viscosity. A major advantage of this property in practical situations is that lower viscosity galactose-depleted guar materials allow easier factory processing of the galactomannans (while retaining full gel strength in interactions with κ-carrageenan).

29.3.2 *Interaction with xanthan*

The gelling synergy between enzyme-modified guar galactomannan and xanthan is illustrated in Fig. 29.8. Gels were made as follows: xanthan (0.5% w/v) was dissolved in hot deionised water, and enzyme-modified guar (0.5%) was separately dispersed in deionised

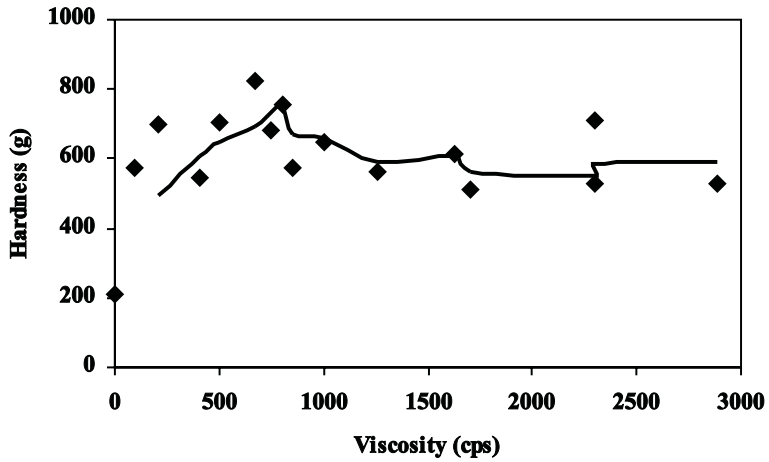

Fig. 29.7 The effect of molecular size (expressed as viscosity in cps) of enzyme-modified guar samples on the hardness of mixed κ-carrageenan/modified guar (QSG) gels.

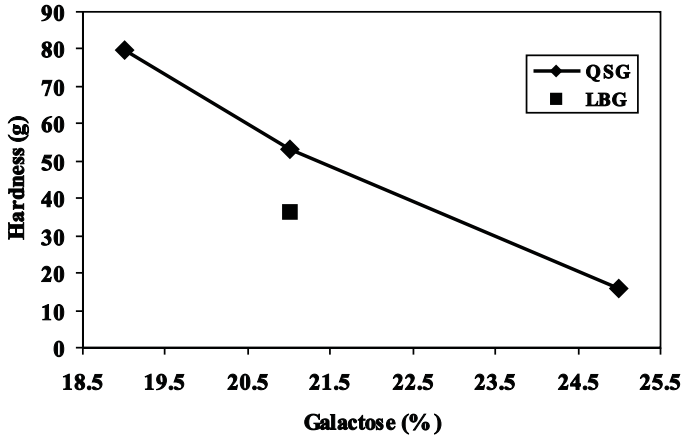

Fig. 29.8 The effect of galactose content of enzyme-modified guar (QSG) samples on the hardness of mixed xanthan/modified guar gels.

water and then heated in a boiling water bath for 20 min. The two hot solutions were combined and thoroughly mixed in a ratio of 1:1, and then poured into gel jars and allowed to stand overnight at room temperature before measurement.

Guar, which does not gel to any significant extent, is transformed into a polymer, which gels with xanthan above and beyond the synergy seen between xanthan and LBG. Interestingly, even at the same galactose content, galactose-depleted guar has stronger synergy with xanthan than does LBG. This synergy is important in applications such as mayonnaise and cream-cheese manufacture. This trend of increased synergy of modified guar with κ-carrageenan and xanthan, as the galactose content of the modified guar decreases, is also apparent in milk systems, and follows the same trend regardless of viscosity and solubility of the modified-galactomannan. It is well known that the maximum synergy of gelation for locust bean galactomannans/κ-carrageenan mixtures is obtained at a polymer ratio of 1:1, and that for LBG/xanthan is obtained at a ratio of 1:3 (Dea & Morrison 1975). This relationship holds true for enzyme-modified guar samples with galactose contents similar to those of LBG.

The effect of galactose content of enzyme-modified guar samples on variations in the texture profiles of xanthan/galactomannans gels can be seen in Fig. 29.9. Admixtures of xanthan and enzyme-modified guar (with 21% galactose) give textural properties close to LBG/xanthan mixtures. When modified guar materials of lower or higher galactose contents (19% or 25%) are used, extremes of gel textural properties are observed. The brittleness (break distance) of xanthan/enzyme-modified guar gels is not affected to any great extent by the galactose content of the modified guar samples (over the range of 19–25%). This observation contrasts with results obtained with κ-carrageenan/enzyme-modified guar gels, which show reduced brittleness with decreasing galactose content of the enzyme-modified guar.

Another property of mixed gel systems containing enzyme-modified guar, which is affected by the galactose content of the modified guar, is the gel melt and set temperatures. The set and melt temperature profiles of xanthan/modified guar gels shown in Fig. 29.10 were performed on a Carri-Med CSL controlled stress rheometer (cone diameter 4.0 cm; angle 2°;

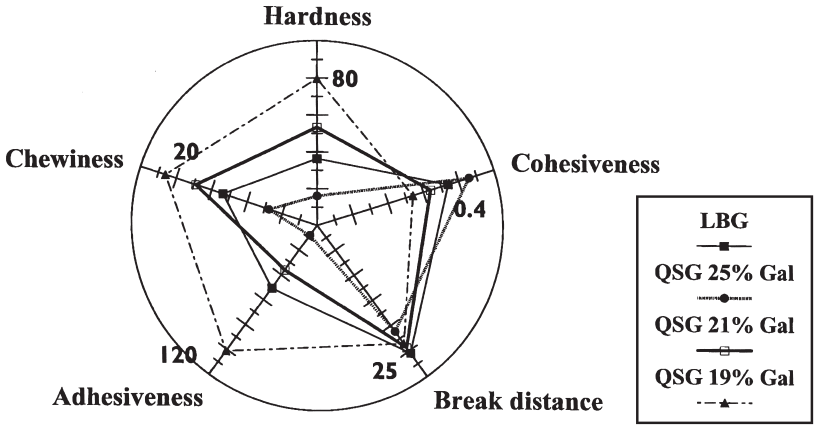

Fig. 29.9 Texture profile analysis of mixed xanthan/enzyme-modified guar gels. Galactomannans used in the mixtures were LBG and modified guars (QSG) with 25, 21 and 19% galactose.

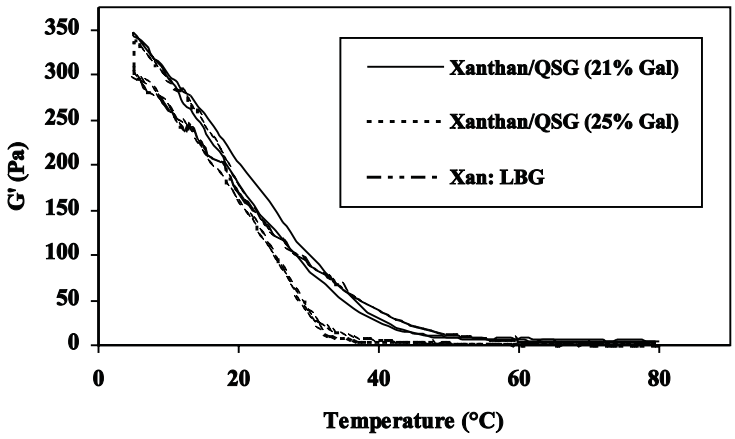

Fig. 29.10 The effect of galactose content of enzyme-modified guar (QSG) samples on the melt/set profiles of xanthan/modified guar gels.

truncation 60 µm; frequency 1 Hz). The cooling-heating cycles were performed at 2°C per minute, between 80° to 5° to 80°C. Mixtures containing high galactose enzyme-modified guar melt at lower temperatures than gels containing the lower galactose enzyme-modified guar. This property is particularly relevant in applications such as dessert jellies, where a melt-in-the-mouth texture like that of gelatin is required. It also has relevance in cold-deposit desserts where the mix is cooled before filling into dessert pots.

29.3.3 *Solubility*

Guar gum is soluble in cold water, whereas dissolution of LBG typically requires heating and agitation. Removing galactose from the mannan backbone of guar has the effect of decreas-

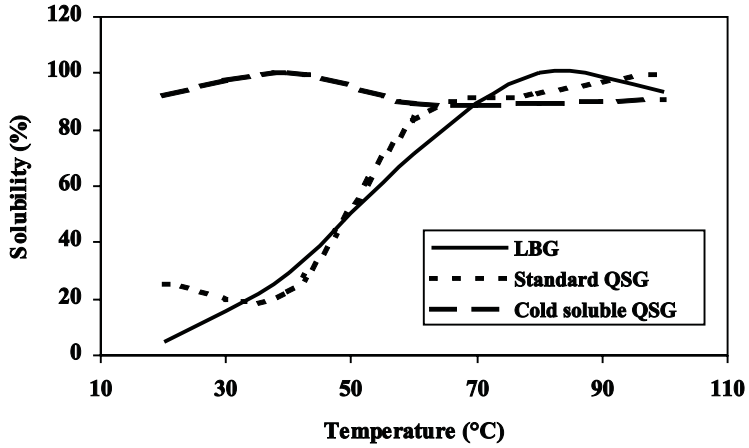

Fig. 29.11 Solubility of LBG and enzyme-modified guar samples.

ing the solubility of the polymer in cold water and giving it properties similar to those of LBG. However, careful control of processing conditions during the enzymatic modification of guar gum make it possible to limit this process and to retain the original cold-water solubility property of the guar gum. In effect, both hot- and cold-water-soluble modified guar materials can be produced. The solubility of cold-water-soluble enzyme-modified guar is compared with that of standard modified guar (same galactose content) and locust bean gum in Fig. 29.11. Solubility is expressed as a percentage of the measured viscosity relative to the maximum viscosity for the material (all viscosity measurements were taken of 1% galactomannan solutions using a Brookfield DV-II viscometer at 20°C and 30 rpm). It is evident that cold-water-soluble enzyme-modified guar has reached maximum solubility at 20°C, whereas standard QSG and LBG both require heating to 85°C to attain maximum solubility. This property finds application in the preparation of cold-mix desserts and of ice creams processed at low temperatures.

29.4 Properties of oligosaccharides produced on hydrolysis of guar gum and enzyme-modified guar samples by β-mannanase

The extent of hydrolysis of galactomannans by β-mannanase is determined by the galactose content of the galactomannan, and the fine structure of distribution of D-galactosyl residues on the linear, 1,4 β-D-mannan backbone (McCleary & Matheson 1983; McCleary *et al.* 1985). Consequently, guar galactomannan, with a D-galactose content of 38%, is hydrolysed to an extent of just 5% by *A. niger* β-mannanase and 2% by guar-seed or *Bacillus subtilis* β-mannanase. Removal of galactose with α-galactosidase dramatically affects the extent to which guar gum is hydrolysed by β-mannanase, as shown in Table 29.1. As the galactose content is reduced, the extent of hydrolysis by *A. niger* β-mannanase increases, and the proportion of low-DP oligosaccharides increases (Table 29.3). The oligosaccharide mixture consists of mannobiose, mannotriose and galacto-manno-oligosaccharides of DP 3 and larger. The structures of these oligosaccharides are predetermined by the specific action pattern

of the β-mannanase that is used to hydrolyse the galactomannan. Also, the proportions of particular oligosaccharides in the hydrolysate are determined by the D-galactose content of the galactomannan or the enzyme-modified guar sample used. It is evident from the marked difference in the oligosaccharide profile for β-mannanase-treated LBG and enzyme-modified guar samples with similar galactose contents (Table 29.3), that the pattern of distribution of D-galactosyl residues in LBG and modified guar are quite different.

The health benefits of dietary fibre oligosaccharides have been recognised, and these materials are likely to gain approval as acceptable dietary fibre supplements. Consequently, a range of new materials is being introduced, including fructo-oligosaccharides, galacto-oligosaccharides and polydextrose. The possibility of producing a parallel range of oligosaccharides through biotechnological approaches should be fully investigated. Here, we have demonstrated the possibility of producing 'tailor-made' oligosaccharides from guar gum. However, similar approaches could also be employed to produce oligosaccharide mixtures through the controlled enzymatic hydrolysis of other agricultural products or by-products such as potato fibre or sugarbeet pulp.

29.5 Conclusions

Enzyme-modified guar samples, like guar gum, can be defined as soluble dietary fibre. Consequently, these materials, as well as having functionality as viscosity modifiers and gelling agents, also have a physiological functionality. The value of guar gum and depolymerised guar gum as soluble dietary fibre is well known. It is now also recognised that short-chain fructo-oligosaccharides and other non-digestible oligosaccharides act as soluble dietary fibre. The potential value of low DP galacto-manno-oligosaccharides has not yet been fully appreciated or evaluated. However, these materials may serve as yet another

Table 29.3 Oligosaccharides produced on hydrolysis of locust bean gum (LBG) and of D-galactose-depleted guar galactomannan (EMG) samples by *Aspergillus niger* β-mannanase.

Oligosaccharide		Galactomannans, galactose contents and amounts of oligosaccharides†						
		Carob	Galactose-depleted guar galactomannans					
DP	Structure*	23%‡	39%	33.6%	28.7%	24.6%	19.2%	13.7%
1	Man	1†	0	0.5	1	1.5	2	2.5
2	Man_2	20	1	4.5	10.5	16.5	20.5	28.5
3	Man_3	13	0.5	5	7.5	12	21.5	37
3	Gal^1Man_2	12	2.5	13	23	27	24	16
4	Gal^1Man_3	7	0.5	5	9	11	13	9
7	$Gal^{3,4}Man_5$	15	1	5	5	6.5	5.5	1.5
>7	–	32	94.5	67	44	25.5	13.5	5.5

DP, degree of polymerisation.

* Man, mannose; Man_2, (1→4)β-D-mannobiose; Man_3, (1→4)β-D-mannotriose; Gal^1Man_2, 6^1-α-D-galactosyl(1→4)β-D-mannobiose; Gal^1Man_3, 6^1-α-D-galactosyl (1→4)β-D-mannotriose; $Gal^{3,4}Man_5$, $6^3 6^4$-di-α-D-galactosyl(1→4)β-D-mannopentaose.

† Amounts of oligosaccharides (wt %).

‡ Galactose contents of galactomannan samples as determined using enzymatic procedure.

source of valuable dietary fibre. By judicious use of α-galactosidase and β-mannanase enzymes it is possible to produce oligosaccharide mixtures with varying molecular size ranges. Furthermore, with intermediate-sized oligosaccharides, removal of galactose produces manno-oligosaccharide mixtures that have a 'creamy' texture. Guar galactomannan is known to be a versatile food ingredient, and through enzymatic modification the true value of this polysaccharide may be realised.

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30 Physiological and Technological Functions of Partially Hydrolysed Guar Gum (Modified Galactomannans)

Lekh Raj Juneja, Senji Sakanaka and Djong-Chi Chu

30.1 Introduction

In recent years, dietary fibre has been recognised as an important nutritional supplement, and consequently the beneficial effects of dietary fibre have received much attention. However, in humans, there are differences in the physiological effects of water-soluble dietary fibre and insoluble dietary fibre (Dreher 1987). Historically, dietary fibre has been defined essentially as natural polysaccharides and lignin. Human digestive enzymes do not decompose dietary fibre, although some water-soluble dietary fibres are utilised by the intestinal bacteria and short-chain fatty acids are produced. Thus, from a physiological point of view, this difference between soluble and insoluble dietary fibre has important connotations.

Some soluble dietary fibres of high molecular weight (and thus high viscosity in aqueous solution) have been shown to affect absorption, or to delay the absorption of toxic substances in the intestine. It is has also been shown that these dietary fibres can prevent the atrophy of the small intestinal villi that are generated as a result of long-term supplementation of liquid food.

One of the most promising fibres is guar gum, derived from the endosperms of the guar bean. The guar plant, *Cyamopsis tetragonolobus* L., has been grown in southern Asia, especially India and Pakistan, since ancient times and used both for human food and animal feed stuffs. Since the 1950s, the seed of the guar plant has been processed into guar gum in ever-increasing amounts, to meet the demand of the modern food industry (Maier *et al*. 1993).

Although guar gum is generally used as a viscosity-building and water-binding agent, this high viscosity limits its use in various food applications. Partially hydrolysed guar gum (PHGG) is a natural, water-soluble dietary fibre that is produced by controlled partial enzymatic hydrolysis of guar galactomannan, the active ingredient in guar gum. Since the metabolic, nutritional and analytical properties of the low-viscosity PHGG correspond to those of guar gum, this is the product of choice for nutritional purposes, i.e. for the fibre-enrichment of processed foods. In this chapter, the chemical properties, functional applications and physiological effects of PHGG (Product name Sunfiber®, Taiyo Kagaku Co., Ltd, Japan) are reviewed (Table 30.1).

Table 30.1 Typical analytical data of partially hydrolysed guar gum (PHGG).

Dietary fibre	81.4% (AOAC method)
Moisture	4.5%
Protein (N×6.25)	0.4%
Ash	1.0%
Viscosity (5%, 5°C)	10.5 mPa·s
pH (5%)	6.4

Functional Food Ingredients

WATER-SOLUBLE DIETARY FIBER

SUNFIBER®

PARTIALLY HYDROLYZED GUAR GUM

Taking care of your health ...

Treatment of constipation

Improvement of glycemic response

Enhancement of mineral absorption

Reduction of the incidence of diarrhea

Improvement on atrophy of terminal ileum villi

Hypocholesterolemic and Hypolipidemic effect

Improvement of intestinal microflora balance (Prebiotic effect)

Meeting the demands for your appetite ...

Masking of unpleasant taste of artificial sweetener

Improvement of texture

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30.2 Physicochemical properties of PHGG

Guar gum is produced by milling the endosperm of guar seed, and the major soluble polysaccharide is galactomannan. Galactomannans consist of a linear backbone of (1→4)- β-linked D-mannopyranosyl units to which single α-D-galactopyranose units are attached to C-6 in various proportions (Stephen 1983). Galactomannans find widespread use as food ingredients for their thickening, gelling and stabilising properties. The ratio of mannose to galactose in the galactomannan of guar gum and PHGG is approximately 38 to 62 (Robinson *et al.* 1982; Englyst & Cummings 1988). A diagrammatic representation of the structure of guar galactomannan and PHGG is shown in Fig. 30.1. The D-galactose residues are distributed irregularly along the D-mannan backbone (McCleary *et al*. 1985).

The main reasons for the discontinuation of enteral nutrition has been the gastrointestinal side effects, particularly diarrhoea. Unfortunately, the factor limiting the use of soluble fibre in enteral formulas is the high viscosity of the formulations at concentrations that are physiologically effective. This has prevented their use in tube feeding. However, advances in fibre and formula technology now permit the addition of PHGG to enteral nutrition.

Several enzymes hydrolyse galactomannan. Guar gum is partially depolymerised by *endo*-β-D-mannanase by selectively cleaving the mannan backbone (McCleary 1979; Balascio *et al.* 1981; McCleary & Matheson, 1983). A manufacturing scheme for PHGG is shown in Fig. 30.2. The molecular weight of PHGG components range from 1000 to 100 000 Da, and the average molecular weight is about 20 000 Da (Fig. 30.3). PHGG gives a low-viscosity, clear aqueous solution. The viscosity of 1% aqueous solution of commercial food-grade guar gum ranges from 2000 to 3000 cps, while that of a 5% solution of PHGG is less than 12 mPa.s (Takahashi 1990) (Fig. 30.4). Until recently, the factor limiting the use of guar gum at physiologically effective concentrations in liquid products has been the high viscosity of guar gum in solution. To resolve this problem, we have produced a purified, partially hydrolysed form of guar gum with a significantly reduced solution viscosity (PHGG). PHGG is stable even at low pH values, and is soluble at all of the pH values commonly found in foods (Chudzikowski 1971; Edwards *et al.* 1987).

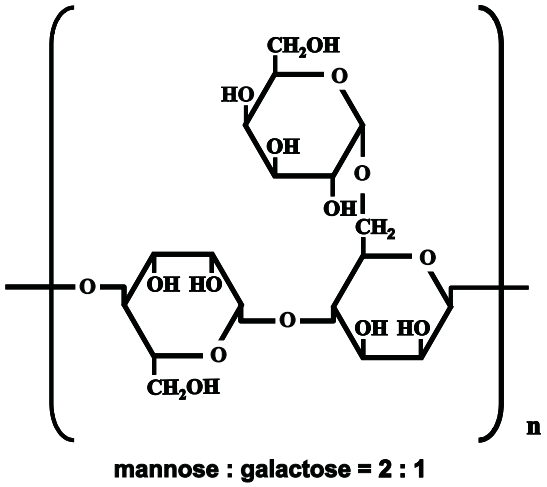
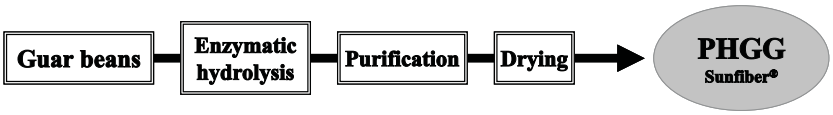


Fig. 30.1 Chemical structure of partially hydrolysed guar gum (PHGG).



PHGG is produced from plant seeds (guar beans) by enzymatic hydrolysis and purification.

Fig. 30.2 Manufacturing process of PHGG.

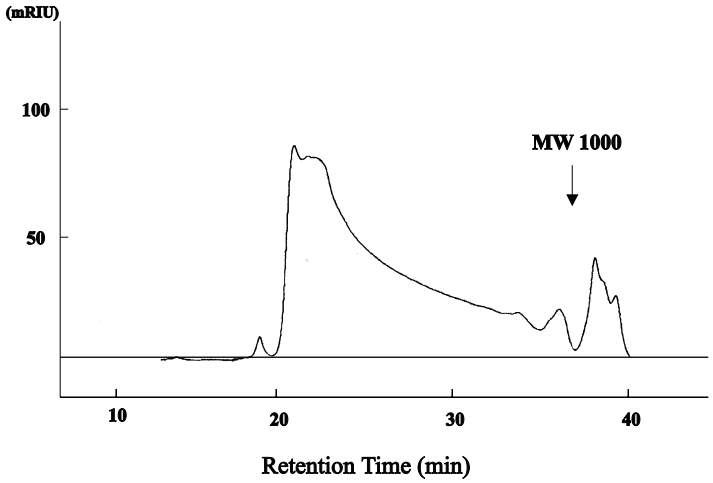


Fig. 30.3 Gel filtration pattern of PHGG. Column: YMC DIOL-120 (500 mm × 8.0 mm); mobile phase, water; flow rate, 0.5 ml/min; detector; RI.

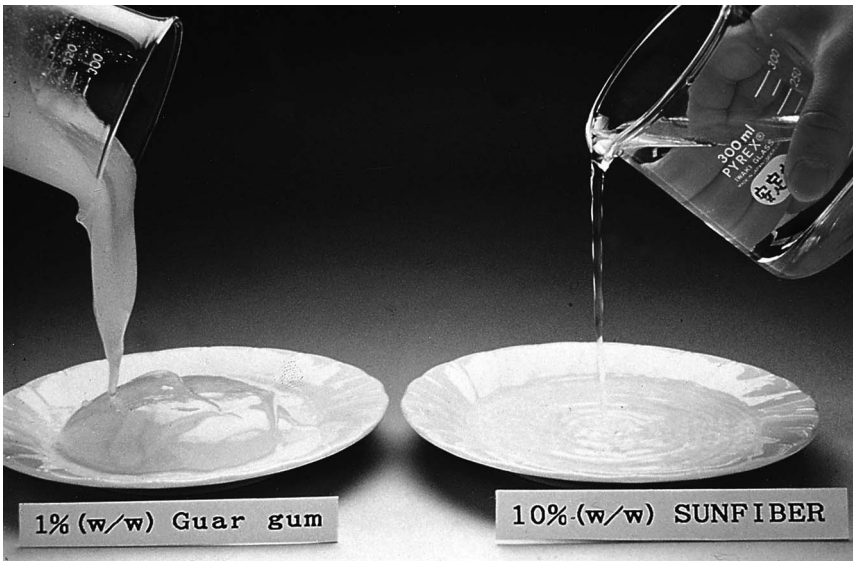


Fig. 30.4 Comparison of viscosity between 1% guar gum solution (left) and 10% PHGG solution (right).

The specific chemical and physical properties of PHGG make it possible to improve the quality of food items. For example, PHGG can, improve the processing of cereals by increasing flowability, provide body and mellow flavour to most beverages, stabilise the colloid system of dry and liquid meal replacements, mellow the tartness and firm the texture of yoghurt, stabilise the foam system of shakes, improve the suspension of particulate matter in soups and dressings and give good eating qualities to baked goods (Greenberg & Sellman, 1998).

30.3 Nutritional and health benefits of PHGG

30.3.1 *Intestinal degradation of PHGG, and benefits on the intestinal microflora*

While PHGG cannot be digested by mammalian small-intestinal enzymes, it is readily broken down in the large intestine by extracellular and membrane-bound glycolytic enzymes of the intestinal microflora (Balascio *et al.* 1981; Tomlin *et al.* 1986, 1989). The first step in the metabolism of ingested guar gum is partial hydrolysis by bacterial *endo*-β-mannanase. The reaction products are galactomannans of shorter chain length, similar to those present in PHGG. Several studies have been performed on the *in-vitro* fermentation of PHGG and guar gum (Tomlin *et al.* 1986; McBurney & Thompson 1987, 1989; Wyatt *et al.* 1988; Adiotomre *et al.* 1990; Gibson *et al.* 1990). These studies have consistently demonstrated that galactomannan is readily fermented by the faecal microflora (particularly anaerobic bacteria) (Salyers *et al.* 1977). The effect of PHGG intake (7 g per volunteer, three times daily for 14 days) on the faecal microflora, bacterial metabolites and pH was investigated in our laboratories using nine healthy human volunteers (Okubo *et al.* 1994). The *Bifidobacterium* sp. count and the percentage of this species in the total count increased significantly during the PHGG intake period (Fig. 30.5). Among the acid-forming bacteria, *Lactobacillus* sp. also increased. The faecal pH and faecal bacterial metabolites, such as β-glucuronidase activity, putrefactive products and ammonia content were significantly decreased by PHGG intake. During the weeks after PHGG intake had ceased, the bacterial counts and their biological manifestations appeared to return to the former state. Clearly, the intake of PHGG resulted in improvements

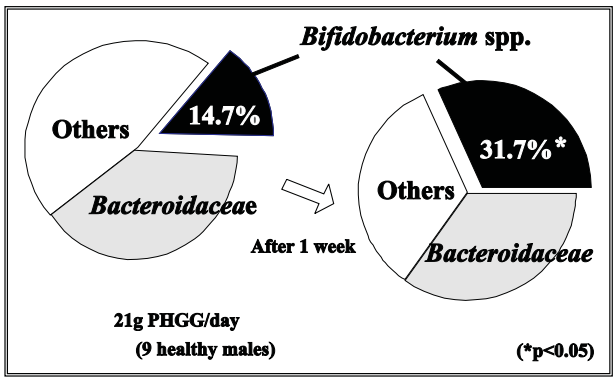


Fig. 30.5 Effect of PHGG on improvement of the intestinal microflora in humans. The percentage composition was presented as % of the bacterium versus total bacterial cell counts. Significant difference: $*P < 0.05$.

in the human intestinal microflora. It is well known that lactic acid-forming bacteria have beneficial effects on human health through the production of amino acids, by aiding defence activity against infection, by pathogen inhibition, and through immunopotentiation.

30.3.2 *Improvement of gastrointestinal distress by PHGG*

The consumption of some types of dietary fibres, at recommended levels, is generally not associated with any significant risk for human health. However, it is well known that the ingestion of excessive amounts of non-absorbed, fermentable carbohydrates may cause laxation and gastrointestinal distress. Information on the tolerance of PHGG or guar gum is available from numerous clinical trials in which the beneficial effects on lipid and carbohydrate metabolism were investigated (Cummings *et al.* 1978; McIvor *et al.* 1985; Tuomilehto *et al.* 1988; Behall *et al.* 1989; Todd *et al.* 1990; Kawatra *et al.* 1991). It has also been shown that the diarrhoea induced by long-term consumption of a liquid diet was associated with a reduction in absorptive capacity and absorptive area due to the loss of brush-border enzymes and mucosal cells (Johnson *et al.* 1975; Blackburn *et al.* 1984; Hosoda *et al.* 1989).

The effects of two liquid diets with or without PHGG on intestinal function and microflora of rats were investigated. Male Wistar rats, allocated into five groups: standard rat chow (MF), low-residue diet (LRD) and elemental diet (ED) with or without 1.5% PHGG at the dose of 60–70 kcal daily for 2 weeks. Atrophy of the terminal ileum villi was detected in the LRD and ED groups. Supplementation with PHGG, however, improved the observed atrophy of the terminal ileum ($P < 0.05$). The specific activities of diamine oxidase and alkaline phosphatase in mucosal scrapings of the lower ileum of rats in the LRD and ED groups was significantly lower than those in the MF group. However, in rats supplemented with PHGG those activities increased significantly. The addition of PHGG to the liquid diet of long-term enterally fed patients appeared to improve the gastrointestinal tolerance and bowel control (Takahashi *et al.* 1995).

The main reasons for discontinuation of enteral nutrition has been the gastrointestinal side effects, particularly diarrhoea. The factor limiting the use of soluble fibre, such as guar gum, in enteral formulas has been the high viscosity of the formulation that prevents their use in tube feeding. It was of immediate interest to whether PHGG would retain the beneficial physiological properties of guar gum and could be safely administered to patients in the form of clinical nutrition products. Studies were established to determine whether surgical and medical patients enterally fed a formula enriched with PHGG showed less diarrhoea than patients fed a standard formula without PHGG. Basically, the effects of feeding a standard diet compared with the same diet supplemented with 20 g of PHGG per 1000 ml was studied using a randomised, prospective, double-blind trial with 100 patients. The results indicated that supplementation is beneficial in reducing the incidence of diarrhoea in enteral nutrition patients (Homann *et al*. 1994) (Fig. 30.6). In separate studies it has also been shown that PHGG affects the prolongation of colon transit time (Meier *et al.* 1993; Takahashi *et al.* 1993). The effects of PHGG on mean transit time, stool weight, faecal pH and serum lipids were studied in 11 healthy men. A daily dose of 21 g (corresponding to 15 g total dietary fibre according to AOAC method 985.29) was consumed for 18 days, in liquid formula diets. The PHGG had no negative effect on large bowel function, and there were no complaints about gastrointestinal side effects (Meier *et al.* 1993). In another study, involving 12 healthy human volunteers consuming an average daily dose of 21 g of PHGG for 7 days, the effect on intestinal transit time was investigated with a liquid enteral formula diet. There was no difference in

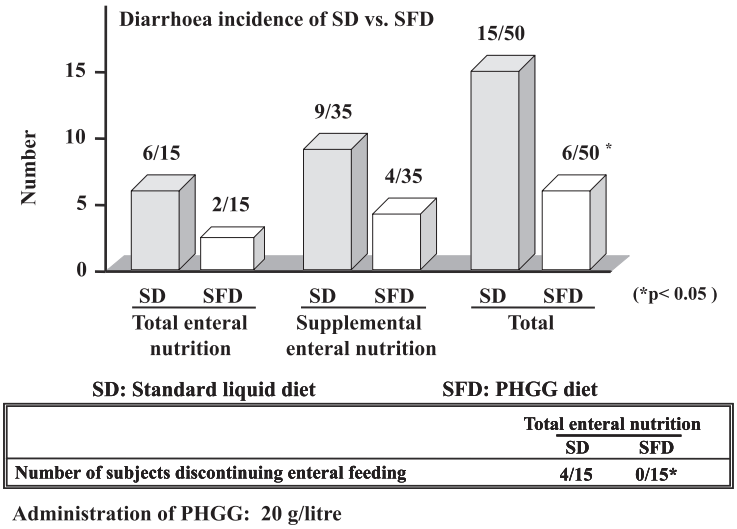


Fig. 30.6 Reduction effect of PHGG on incidence of diarrhoea. Patients were assigned randomly to receive either a standard liquid diet (SD) or the same diet supplemented (SFD) with 20 g of PHGG per litre. Both diets were lactose-free, containing 52% of the energy as carbohydrate, 14% as proteins and 34% as fat. Significant difference: $*P < 0.05$.

stool frequency or consistency (with and without PHGG fortification), and neither diarrhoea nor constipation was observed in the group with the PHGG. In another study (Takahashi *et al.* 1993), PHGG was well tolerated when given with beverages after each meal for a period of 4 weeks. None of the healthy volunteers complained about diarrhoea or unacceptable gastrointestinal symptoms.

30.3.3 *Improvement of constipation by PHGG*

Constipation was one of the first symptoms to be classified as a fibre deficiency disorder. Dietary fibre has been shown to improve faecal bulk and consistency and increase intestinal motility. Cummings *et al.* (1978) reported that daily intake of 20 g of guar gum increased the faecal weight of healthy volunteers by up to 20%. In rats fed diets supplemented with 2.5% and 5% PHGG, it was found that the faecal output was significantly increased.

The influence of PHGG on constipation in women has been investigated by administering PHGG as a beverage (11 g/day) for 3 weeks. Defecation frequency, faecal pH, weight and moisture, and bacterial flora content of the faeces were investigated and compared with the control periods. The defecation frequency increased from 0.46 ± 0.05 per day to 0.63 ± 0.05 per day. Faecal moisture significantly increased from 69.1% to 73.8% by ingestion of PHGG (Takahashi *et al.* 1993, 1994a) (Fig. 30.7). Faecal moisture content also increased, consistent with a lowering of faecal pH ($r = -0.478$). Compared with the control period, the frequency of *Lactobacillus* spp. occurrence in faeces significantly increased ($P < 0.05$). All of these results clearly indicate that PHGG softens and improves the output of faeces. The effect of PHGG on faecal output was also demonstrated in another study involving volunteers ingesting PHGG at a dose of 36 g/day for 4 weeks (Shankardass *et al.* 1990).

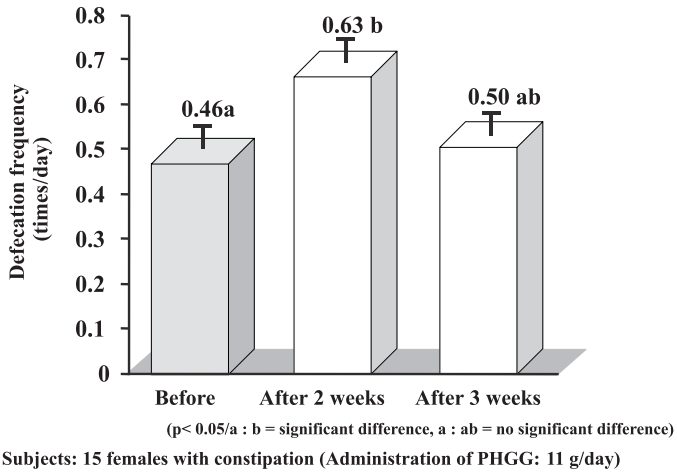


Fig. 30.7 Improvement by PHGG of defaecation in females suffering from constipation (values are mean ± SE). The different superscripts indicate significant differences between each week of PHGG treatment ($P < 0.05$).

Constipation is frequently found in chronic care patients who are fed exclusively on enteral formula or low-residue diets. Dietary fibre has been reported to regulate faecal transit time by reducing both constipation and diarrhoea. A clinical study has shown that PHGG was effective in preventing constipation in the institutionalised elderly women and men who took laxatives on a regular basis. It was concluded that PHGG should be considered as a replacement for laxatives for elderly women and men who find traditional fibre supplements unpalatable or for people who need fibre added to their tube-feeding formula (Patrick *et al.* 1998).

30.3.4 *Effect of PHGG on mineral absorption*

High-fibre diets have been shown to reduce significantly the balance of calcium, magnesium, zinc and copper (Kelsay *et al.* 1979a, b). It has also been reported that guar gum decreases the utilisation efficacy of protein and lipid (Poksay & Schneeman 1983; Vahouny *et al.* 1987). This effect may be explained by a high adhesive effect of high-viscosity guar gum which leads to interference with the digestion and absorption of nutrients (Ikegami *et al.* 1990).

Our research groups have revealed that PHGG and its metabolites increase the bioavailability of ferrous iron. Ferrous ion levels in haemoglobin, serum and liver in rats were unchanged when rats were fed iron-deficient diets with PHGG, while those in the control group fed the same diets without PHGG were significantly decreased. In an iron balance test for 3 days, administration of PHGG caused an increase in iron adsorption in intestinal villi (Takahashi *et al.* 1994c) (Fig. 30.8). It was suggested that PHGG might be effective in improving ferrous iron utilisation in subjects suffering from iron deficiency as a result of blood loss with menses, urinary iron loss with intravascular haemolysis, pregnancy and/or inadequate dietary intake or malabsorption of iron. It has also been reported that intestinal calcium absorption lowered by partial nephrectomy was fully restored by means of feeding guar gum hydrolysate (Hara *et al.* 1996). A possible mechanism for the increase in the caecocolonic mineral absorption associated with feeding PHGG may be attributed to gut

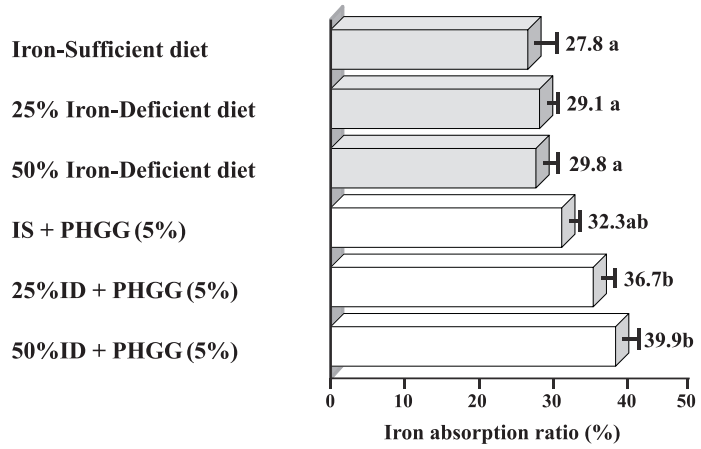


Fig. 30.8 Enhancement of iron absorption by PHGG (values are mean ± SE). The different superscripts indicate significant differences between treatments in the test groups ($P < 0.05$). Iron absorption ratio (%) = iron absorption × 100/iron intake.

acidification resulting from caecal fermentation of dietary fibre, consequently producing an increase in ionic calcium levels in the large intestine. Another possibility is that the addition of PHGG in liquid diets reduces the effect of ileum villi shrinkage in rat intestine (Takahashi *et al.* 1995) (Fig. 30.9).

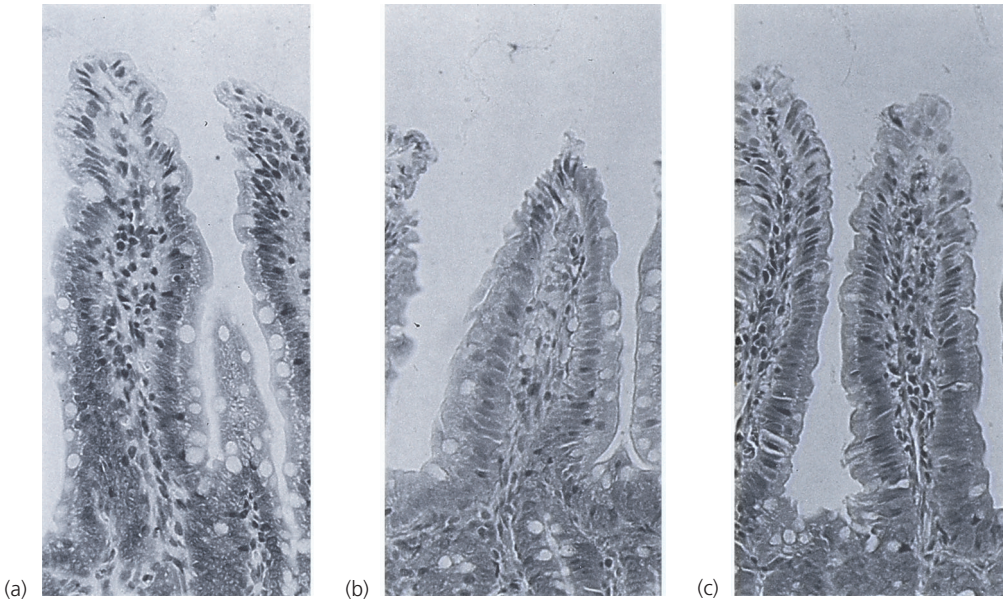


Fig. 30.9 The effect of PHGG on ileal villi of rats fed a liquid diet: (a) conventional rat feed; (b) low-residue rat feed; (c) low-residue rat feed + PHGG (1.5%).

30.3.5 *Effects of PHGG on blood lipid and glucose levels*

Effects on blood lipids

The hypolipidaemic and blood cholesterol-lowering effects of PHGG were examined in a feeding study in rats and in a study with human volunteers (Aro *et al.* 1981; Anderson & Bryant 1986). It was found that PHGG was effective in reducing serum triacylglycerol as well as cholesterol levels in the rats fed high-fat diets employing lard or palm oil as the dietary fat (25% in the diets) (Ide *et al.* 1991). In the rat study, it was found that both PHGG and guar gum showed both cholesterol- and triglyceride-lowering effects. It was thus suggested that PHGG might affect bile acid secretion, leading in turn to a cholesterol-lowering effect. In the human volunteer study, the administration of three portions each of 12 g PHGG per day reduced the total serum cholesterol, while levels of other serum lipids remained unaffected (Takahashi *et al.* 1993).

These studies led to the suggestion that PHGG can be used as a hypocholesterolaemic agent (Figs. 30.10 and 30.11).

Effects on blood glucose

In experiments with humans and rats, PHGG was shown to have a blood glucose-lowering effect (Wolever *et al.* 1979; Yamatotaya *et al.* 1993; Tsuda *et al.* 1998). In a recent study using liquid meals in non-insulin-dependent diabetes patients, the known plasma glucose- and insulin-lowering effects of guar gum were also observed with the PHGG. This indicates that the effect is not mediated exclusively through viscosity increases resulting in delayed gastric emptying (Golay *et al.* 1995). The use of PHGG (the low-viscosity form of guar gum) thus permits the preparation of liquid products suitable for oral supplementation, or

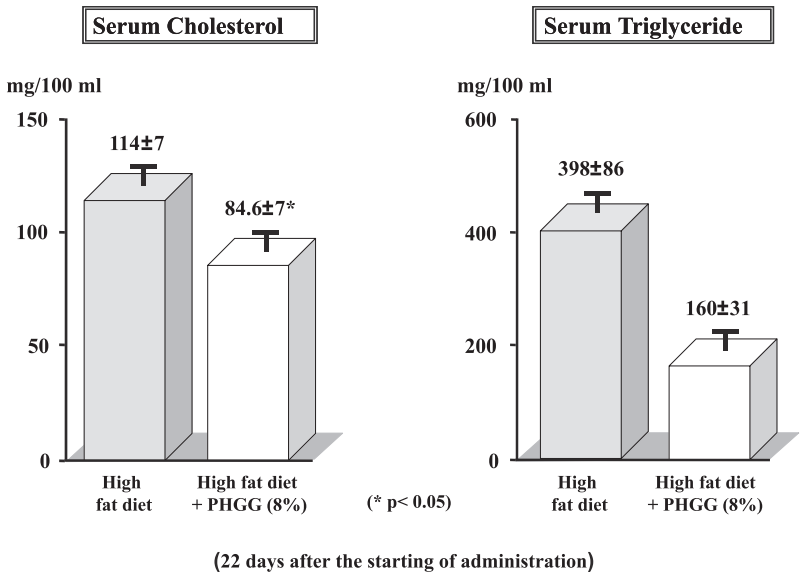


Fig. 30.10 The hypolipidaemic effect of PHGG in rats (values are mean ± SE). Significant difference: $*P < 0.05$.

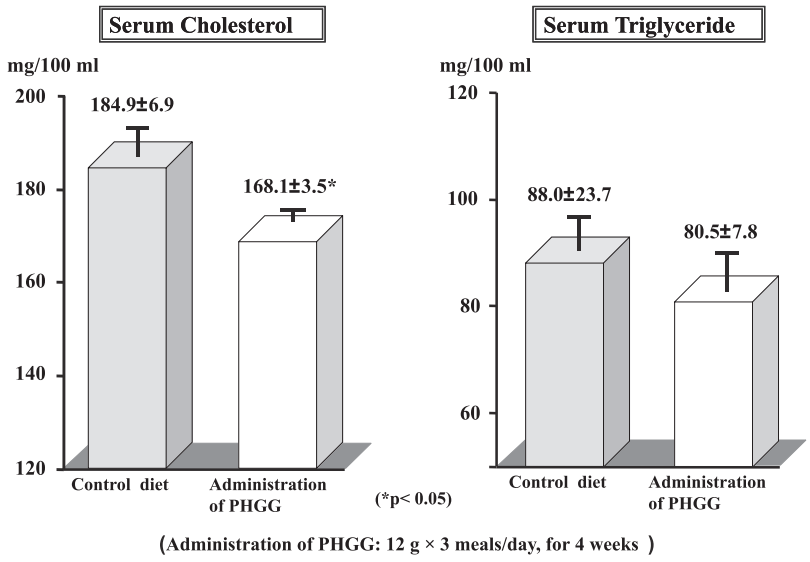


Fig. 30.11 The hypolipidaemic effect of PHGG in human volunteers (n = 8) (values are mean ± SE). Significant difference: $^*P < 0.05$.

application via a gastrostomy tube. In another study, the effects of PHGG on the elevation of blood glucose and insulin secretion after sucrose intake were investigated using healthy human volunteers. When 30 g sucrose was taken, peak levels of both blood glucose and insulin were seen 30 min after ingestion. When 5 g of PHGG was taken simultaneously with the 30 g of sucrose, the blood glucose level at 60 min after ingestion was significantly lower ($P < 0.05$) than when sucrose was taken alone. The level of serum insulin was also lower than that of the control. These data show that even though the viscosity of PHGG is dramatically lower than that of intact guar gum, it has the same reducing effect on blood glucose level (following sucrose intake) (Tsuda *et al.* 1998).

30.3.6 Prevention of Salmonella enteritidis *infection*

Recently, serious contamination by *Salmonella* sp. both in and on eggs has been reported in Europe, the US (Center for Disease Control 1996; Wilson *et al*. 1998) and in Japan (Konuma *et al.* 1995). The infection of *S. enteritidis* (SE) in poultry is one of the most serious problems in the poultry industry due to the related outbreaks of food poisoning (Hennessy *et al.* 1996; Dodhia *et al.* 1998; Vought & Tatini 1998). It has been reported that some types of carbohydrates, yeast and especially the mannose residues are effective in preventing the *Salmonella* sp. colonisation (Oyofo *et al.* 1989; Line *et al.* 1998). Consequently, we have studied the preventive effects of PHGG on the colonisation of SE in young and laying hens (Ishihara *et al*. 2000). Feed supplemented with 0.025, 0.05 and 0.1% PHGG was examined in orally SE-infected young hens (Table 30.2). It was found that the administration of PHGG decreased the incidence of SE in organs, increased the excretion of SE in faeces, and decreased the agglutinating antibody titre to SE in serum. Feed supplemented with 0.025% PHGG was the most effective. Also, with feed supplemented with 0.025% PHGG the numbers of

Table 30.2 Effect of partially hydrolysed guar gum (PHGG) administration to young hens on the incidence of *Salmonella enteritidis* (SE) infection in various organs.*

Group/organ	Days after infection					Frequency of SE in organs (%)
	0	3	7	14	21	
Group A (control)						
Internal organs	0/25	0/25	0/25	0/25	0/25	
Intestine	0/25	0/25	0/25	0/25	0/25	0
Whole bird	0/5	0/5	0/5	0/5	0/5	
Group B (SE-infected)						
Internal organs	0/25	1/25	8/25	0/25	0/25	
Intestine	0/20	13/20	11/20	9/20	5/20	26.7
Whole bird	0/5	4/5	4/5	3/5	3/5	
Group C-1 (0.025% PHGG)						
Internal organs	0/25	0/25	0/25	0/25	0/25	
Intestine	0/20	4/20	6/20	0/20	0/20	5.6**
Whole bird	0/5	2/5	3/5	0/5	0/5	
Group C-2 (0.05% PHGG)						
Internal organs	0/25	1/25	0/25	0/25	1/25	14.4*
Intestine	0/20	8/20	10/20	3/20	3/20	
Whole bird	0/5	3/5	4/5	2/5	2/5	
Group C-3 (0.1% PHGG)						
Internal organs	0/25	3/25	4/25	3/25	0/25	
Intestine	0/20	8/20	8/20	3/20	3/20	17.8
Whole bird	0/5	3/5	4/5	2/5	2/5	

*Values shown as positive samples/total samples observed.
Internal organs/tissues included liver, bile, spleen, heart and ovary.
Intestine included duodenum, small intestine, large intestine and caecum.
*,**, significant at 5% and 1% levels compared with group B, respectively.

Bifidobacterium sp. and *Lactobacillus* sp. (the most numerous useful intestinal bacteria in the caecum of the young hen) were increased.

The effect of excretion of SE via the faeces was also studied using laying hens (Ishihara *et al*. 2000). The incidence of SE on the surface of the eggshell, and in egg white and egg yolk was reduced when the feed of laying hens was supplemented with 0.025% PHGG. Thus, administration of PHGG may prevent the colonisation of SE in both young and laying hens – an effect consistent with an improved balance of intestinal microflora that has also been seen in rats and humans.

The results of all of these studies indicate that PHGG, in addition to having the nutritional properties of dietary fibre, also has beneficial effects on a number of physiological functions.

30.4 Safety aspects and applications

The safety of PHGG can be largely established from the safety data for guar gum. The safety

of guar gum has been assessed by JECFA in 1975 and by the EC Scientific Committee for Foods (SCF) in 1978 (WHO 1975; SCF 1978). In the USA, guar gum has been considered GRAS (Generally Recognised as Safe) in numerous food applications, since 1974 (Code of Federal Regulations 1974). A low-viscosity galactomannan (PHGG) must therefore also be considered as safe. A history of safe use has been established in Japan where the product has been used as a dietary fibre in various foods since 1987.

The toxicity of PHGG has been tested with male and female Sprague-Dawley strain rats at dose levels of 0, 0.5 and 2.5 g/kg of body weight per day for 28 days. The results showed that PHGG was well tolerated, and that neither food consumption nor body weight gain were influenced by the treatment. Urinalysis, haematological examination and blood chemical analysis did not reveal any abnormalities that could be attributed to the treatment (Takahashi *et al.* 1994b). In another study, dietary levels of PHGG up to 10% were tolerated without any signs of toxicity in a subchronic (13-week) feeding study in rats.

The mutagenicity of PHGG was examined in a microbial reverse mutation assay with *Salmonella typhimurium* TA100 and TA98 strains. Concentrations of up to 5 mg/plate had no adverse effect on reverse mutation rates (Takahashi *et al.* 1994b). Administration of PHGG for 4 weeks to adult men at a dose of 36 g/day caused no adverse side effects (Takahashi *et al.* 1993), while in another study daily intake of 20–40 g/day of PHGG was found to be well tolerated and to cause no adverse side effects (Meier *et al.* 1993; Takahashi *et al.* 1993).

In most developed countries, the estimated total dietary fibre intake by humans varies between 10 and 25 g/day. A WHO study group has recommended a daily intake of ~37 g total dietary fibre (Takahashi *et al.* 1994b). Based on these recommendations, the American Diabetes Association has recommended a fibre intake of 40–50 g/day (ADA 1991), while in Japan a desired intake of 20–30 g/day (based on an average intake of 2000–2500 kcal/day) has been recommended.

30.5 Conclusions

Partially hydrolysed guar gum is dietary fibre that has many of the properties associated with dietary fibre. PHGG ingestion results in an increase in faecal bulk and frequency, and softer stools in persons with constipation. Faecal pH is lowered, inducing the growth of *Lactobacillus* sp., and there is an increase in beneficial *Bifidobacterium* sp., in the large intestine. The absorption of minerals is not negatively affected, while the absorption of iron is improved. Furthermore, the ingestion of PHGG lowers serum cholesterol and triglyceride levels in the rat, and serum cholesterol levels in humans. PHGG is tasteless, colourless and produces clear, low-viscosity solutions at 5% concentration – properties which make it an excellent source of dietary fibre for many food applications. PHGG is a very versatile dietary fibre; it shows interesting physiological effects while still exerting the functional and nutritional effects of a dietary fibre. PHGG can be fully integrated into food materials without altering the rheology, taste or appearance of the product (Fig. 30.12). PHGG, marketed as Benefibre® in the USA, has self-affirmation on GRAS status as standard grade partially hydrolysed guar gum (Angels 1995; Rulis 1995). PHGG, under the trade name Sunfiber®, is now used in a number of beverages, food products and medicinal foods world-wide, and is recognised as a safe, natural and functional dietary fibre.

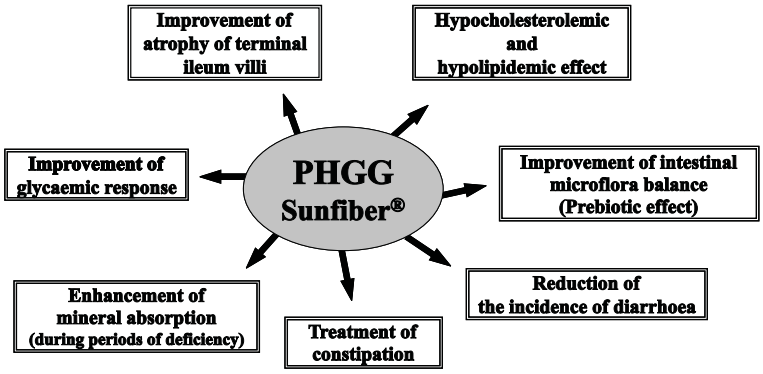


Fig. 30.12 Functional effects of PHGG.

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31 Dietary Fibres of Lupins and Other Grain Legumes

Hano Peter Pfoertner and Jürgen Fischer

31.1 Grain legumes in human nutrition

In human nutrition, plant seeds – which represent more than 70% of all raw materials – are the most important agriculture product. They are either consumed directly in the diet, or are processed. Seeds form the basis of flour production for bread, pasta and tortillas. Drinks such as beer and coffee are also made from seeds, and seeds are of major interest in the production of food ingredients such as oil, protein and starch. Grain legumes – which are of special interest because they are rich in protein – belong to the family of Leguminosae, whose members include peas, various beans, soy, chickpea, peanuts and lupins. The protein content of seeds in general is >20%, and may reach up to 40% of the dry weight. For comparison, cereals contain usually between 7 and 15% protein.

The above-mentioned grain legumes are non-endospermic, dicotyledenous plants in which the cotyledons are developed as the plant storage tissue of the seed. The stored reserves supply the growing embryo with nitrogen, carbon and energy during germination. A protective seed coat, which is about 6–12% of the seed's weight, covers the cotyledons and embryo.

As well as storing nitrogen as protein, grain legumes also accumulate carbon as reserves. Oil crops such as soybean and peanuts use oil as reserve substances. Peas, lentils and certain beans accumulate starch as the major carbon source. In the case of lupin, carbohydrates and oil can be found in the cotyledons. A special feature of lupins is the storage of high amounts of non-cellulose, non-starch polysaccharides (NSP) in the thickened cell walls of the cotyledons. Starch exists in lupins as a temporary deposit, formed during seed development, and is present in mature seeds only in small amounts or not at all. A comparison of the main components of lupin, pea and soy is shown in Table 31.1.

As well as the major reserve components, legumes also contain a range of minor components, some of which have anti-nutritional properties. A comparison is given in Table 31.2.

Table 31.1 Main components of grain legumes (values in % of dry matter).

	Lupin	Pea	Soybean
Protein	36–48	20–25	31–36
Lipid	4–12	1–2	16–21
Available carbohydrates	5–8	44–54	5–6
Crude fibre	15–18	16–18	3–6
Mineral	3–4	3–4	4–5

Table 31.2 Potential anti-nutritional factors in grain legumes.

	Lupin	Pea	Soybean
Trypsin inhibitor (mg/g)	0.18	1.25	17.90
Phytate (%)	0.44	1–2	1.59
Saponins (%)	1.4	0.06	1.9
Plant phenolics (%)	0.29	0.29	0.57
Oligosaccharides (%)	4.6	7.5	5.7
Lectins	–ve	–ve	+ve

Source: Johnson & Gray 1993; Shahidi 1990.

Lupins differ from soy in that they have low trypsin inhibitory activity and negligible haemagglutinin (lectin) activity, which makes them edible without prior cooking.

Lupins – sometimes better known as a garden flower – have been cultivated for thousands of years. Various species were used as food over 3000 years ago in the Mediterranean, and over 6000 years ago in the highlands of South America. A factor limiting their use has been the alkaloid content, which gives a bitter taste and some toxicity. Processing steps such as soaking in running water, have been employed to remove most of the alkaloids. The discovery of low-alkaloid mutants in the 1920s renewed the interest in lupins. Nowadays, the so-called sweet lupin is an alternative to soybean. Four species are of interest: the white lupin (*Lupin albus*); the yellow lupin (*L. luteus*); the pearl lupin (*L. mutabilis*); and the Australian or narrow-leafed lupin *(L. angustifolius*).

The use of varieties containing less than 500 mg/kg of alkaloids has been shown to cause no health problems in the direct consumption of seeds (Petterson 1998). The so-called bitter varieties of lupin, which have an alkaloid content of up to 4%, can be used without problems in industry. Using patented separation steps, it is possible to eliminate the alkaloids and to isolate protein, phospholipids, oil and dietary fibre in high purity.

31.2 Dietary fibres of grain legumes

Organic components of plants which cannot be degraded and adsorbed in the human intestine are, by definition, dietary fibres. Most components are part of the plant cell walls, which consist of a complex matrix of cellulose, hemicellulose, pectin and lignin. Depending on cell age, location or cellular function, the architecture as well as the nature of the components differ largely (Selvendran & Robertson 1994).

Legume seeds consist of two main tissues, the seed coat (hull) and the cotyledons. Consequently, we need to distinguish between dietary fibres derived from seed coats (outer fibre) and dietary fibre of cotyledon cells (inner fibre). A major difference between inner and outer dietary fibre is the relative content of cellulosic and non-cellulosic polysaccharides. Generally, outer fibre consists mainly of cellulose, insoluble hemicelluloses and varying levels of lignin, which strengthen the secondary cell walls. The hull fibres are comparable with fibres which can be isolated from cereal bran. Hulls are naturally rich in dietary fibres and therefore directly usable as commercial fibre. The dietary fibre content of cotyledons is lower and is poor in cellulose. The major component of cotyledonary fibres are NSP. The composition of the carbohydrates of the seed coat and the cotyledons of a lupin variety is shown in Table 31.3. The dietary fibres in lupin cotyledons are mainly non-structural polysaccharides

Table 31.3 Carbohydrate composition (%) of *Lupin angustifolius*, cultivar Gungurru (Petterson 1998).

	Hull	Cotyledon
Dietary fibre	88	29*
Cellulose	49	1.7
Lignin	1.5	0.8
Oligosaccharides	0.4	7.7
Sucrose	1.0	3.8

*Mainly non-starch polysaccharides (NSP)

with a rhamnogalacturonan backbone with galactose and arabinose containing side chains. This complex chain of sugars and uronic acid (which is mostly insoluble) is able to bind water to about eight times their weight – a property which is of physiological, as well as of technological interest (Fischer 2000). It can be expected that lupin cotyledon fibres will have a lowering effect on the blood cholesterol level in humans. Such an effect has been demonstrated in animals (Evans 1994). The nutritional importance of the more insoluble cellulosic fibres of the hulls is mainly as a faecal bulking agent, with consequent positive effects on both constipation and diverticulosis.

For the commercial production of dietary fibre isolates or concentrates, the seed coats and cotyledons can both be used as raw material. Outer fibre is produced by milling the hulls (seed coat) derived from dehulling the seeds, for example for the production of flour. 'Edible bran' is often used as a synonym for the outer fibre (c.f. cereal bran, also manufactured as a by-product of milling). Such outer fibres are available in different particle sizes, ranging from fine flakes to microfine powder (Herbafood Ingredients 2000).

In contrast, the inner fibre can only be produced in combination with an extraction process of the seeds (cotyledons), in which oil, starch and protein are also produced. Depending on the refining conditions, commercially available fibres (concentrates or isolates) contain varying levels of protein (lupin) or starch (pea). These non-fibre components can have a major influence on the technological function of the commercial dietary fibres. In the case of pea cotyledon fibre, which has a starch content of around 30%, heating above the gelation point of the starch can lead to gelled structures. The typical compositions of currently available dietary fibre from lupin, pea and soy are shown in Table 31.4.

Table 31.4 Typical composition of commercial dietary fibres.

	Lupin		Pea		Soy
	Seed coat	Cotyledon	Seed coat	Cotyledon	Seed coat
Total dietary fibre	80	80	89	60	75
Soluble dietary fibre	8	8	7	ND	10
Protein	14	15	5	7	8
Lipid	2	0.5	1.5	0.5	2
Available carbohydrates	1	3	2	30	6
Mineral	4	2	4	2.5	5

ND, not determined.

31.3 Properties of dietary fibres of grain legumes

The use of commercial dietary fibre as an ingredient in food or as a nutritional supplement depends on several factors, including:

- the origin of the raw material (edible or not?);
- the physiological properties of the fibres (prebiotic, cholesterol-lowering, constipation-reducing);
- its organoleptic properties;
- its particle size and haptic (mouthfeel) impression in food;
- its interaction with other food ingredients such as water or oil;
- the minor components and their functionality (e.g. starch, protein); and
- price and availability.

For the development of fibre-enriched products, or simply for use as a low-calorie ingredient, the first considerations are the sensory behaviour in the final product. For most applications it is of interest that known organoleptic properties such as colour and taste remain unchanged. Some basic characteristics of commercial fibres are given in Table 31.5. The sensory properties of a fibre can be varied by milling and sieving steps, this being especially true for liquid products where the size and hardness of particles is important. Generally, cellulosic fibres (made from hulls) have a sandy character, while cotyledonary fibres are smooth. High and stable water binding properties are preferable for products in which syneresis is a problem.

31.4 Application of dietary fibres in food systems

The food industry of today has available a range of commercial dietary fibres with very specific functions, thanks to a developing production technology. The behaviour of different commercially available fibres has been studied at the Technical University of Berlin (Niemann *et al.* 1999), where as a model system a soup was used. One target was the replacement of starch as thickening agent. In this work it was found that all of the commercial fibres tested were able to increase the product's viscosity. Differences existed between the shear and heat stability properties during storage, as well as in the soaking properties. Dietary fibre of pea cotyledons was found to be preferable when good cold or hot soaking properties and long-term high shear stability are necessary. The cotyledon fibre of lupin is best used if low

Table 31.5 Chemical–physical properties.

	Colour	Taste	Water-Binding capacity (ml/g)	Oil-binding capacity
Lupin cotyledon	White	Nearly neutral	8–10	1.5–2.0
Lupin hulls	Creamy-light tan	Neutral-nutty	7–8	1.6–1.7
Pea cotyledon	White	Neutral-beany	9–11	ND
Pea hulls	Creamy white	Neutral	4–5	1.8–2.0
Soybean hulls	Light tan	Neutral-nutty	3–5	1.4–1.7

ND, not determined.

swelling, hot soaking and also excellent shear stress stability is required. In contrast, fibres made from wheat stems should be used only for foods that are to be consumed soon after production. A problem with the cellulosic wheat stem fibres is that, initially, they have high water-binding properties, but these properties are not stable during processing or on storage. This occurs because the major component, cellulose bundles, have only a low water retention capacity.

Dietary fibres also find use in products rich in protein and fat, for example meat emulsions for sausages, minced meat products and vegetable or milky spreads. Here, three main properties are of interest:

(1) Function as low caloric filler (replacement of fat).
(2) Neutral sensoric behaviour (colour, taste, texture).
(3) Yield (economic and environmental aspects).

To study these properties we have tested the effect of dietary fibres in a burger preparation that was baked in an oven. The yield was determined by measuring the cooking loss of fat and meat juice. In this application it is important to have a balance between a high water-binding capacity and good oil-binding potential. The best results were obtained when these two functions were related to different chemical compounds of the dietary fibre preparation. This was shown to occur in the case of lupin cotyledon fibres: the high water-binding capacity resulted from the NSP and, in addition, the complex bound proteins led to an improved retention of fat. For these reasons, a yield enhancement and juicy product can be obtained by reducing the cooking loss of liquid phase and oil. The loss of meat juice and fat during the manufacture of a baked chopped meat product is shown in Fig. 31.1. None of the fibres used influenced either the flavour or colour of the meat products. The use of fibres leads to a somewhat firmer bite and, depending on the cooking loss, an increase in juiciness.

In addition, a very interesting application field of fibres is the improvement of the nutritional quality of convenience products. For example, the combination of texturising properties, derived from the insoluble polysaccharides, and the high and stable water-binding and good oil-binding properties, make lupin fibres an ideal choice for baking stable fillings or

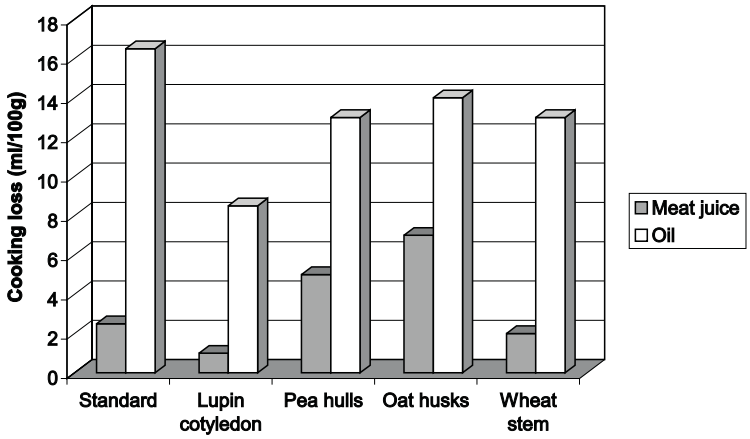

Fig. 31.1 Water and oil retention in a baked burger preparation (500 g meat at 200°C for 50 min).

Fig. 31.2 Baking stability of a protein-stabilised oil-in-water emulsion (50/50): (a) o/w emulsion with 2% lupin cotyledon fibre, 1% guar, 2% soy protein iodate and 1% salt; (b) o/w emulsion with 1% guar, 2% soy protein iodate and 1% salt. Each is shown after 1, 10 and 30 minutes.

toppings. The improvement of baking stability by adding lupin fibres is shown in Fig. 31.2. Oil in water emulsions (o/w: 50/50) were prepared with protein as emulsifying agent and guar gum to increase the viscosity. An enhanced form stability results in the case of added fibre due to the fibrous network formation.

In summary, different dietary fibres made from legume seeds are clearly suitable for a wide range of applications. In this respect, the producers of food or neutraceuticals should choose fibres based on their physiological, physicochemical and technological properties.

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Part 9

Pectins

32 Pectins, their Origin, Structure and Functions

Jean-François Thibault and Marie-Christine Ralet

32.1 Introduction

Pectins are complex polysaccharides that are constituents of plant cell walls. As they are not metabolised during passage through the human upper digestive tract, they belong to the dietary fibre class. Pectins form part of our diet as constituents of food products from vegetables and fruits consumed in their traditional presentation, as ingredients in food products supplemented in pectin-rich dietary fibre, or as additives in gelled food manufactured products. Besides fruits and vegetables, which contain around 5–10% of pectins and 10–32% of total dietary fibre on a dry weight basis (Cho *et al.* 1997a), some by-products of the food industry can be considered as good sources of pectins and dietary fibres. Sugarbeet pulp, the abundant by-product from the sugar industry, or apple pomace and citrus peels from the fruit juice industry, contain around 20–35% of pectins and 72–88% of total dietary fibre on a dry weight basis. The physicochemical properties of the pectins, either within the cell-wall matrix or after extraction, are important from both functional and nutritional points of view. Indeed, they have a physiological role as dietary fibre along the human digestive tract and, in food, also exhibit a variety of functional properties, mainly related to texture. As isolated molecules, pectins exhibit physicochemical properties such as viscosity, gelation and ion-exchange in close relation with their chemical structural characteristics (Voragen *et al*. 1995). Pectin-rich cell walls exhibit peculiar hydration, ion-exchange and rheological properties. Here again, the physicochemical properties are closely linked to the chemical structure, but the physical structure (surface area, particle size, pore size) of the pectin-rich plant material has also to be taken into account. In this chapter, the physicochemical and functional properties of isolated pectic molecules used as gelling agents, and of pectin-rich cell walls or dietary fibre, will be discussed.

32.2 Industrial pectins

32.2.1 *Current raw materials*

Raw materials which currently find widespread industrial use are the dried press cake of apple juice manufacture (dry apple pomace, which contains around 10–15% of extractable pectins), and the wet or dried peels and rags obtained after the extraction of citrus juice (wet or dry citrus pomace, which contains around 20–30% of extractable pectins). Other interesting sources considered for pectin production are sugarbeet pulp, sunflower heads, potato fibre, onion skins, tobacco leaves, wastes from the processing of tropical fruits and more particularly papaya, mango, coffee and cocoa (Voragen *et al*. 1995). These sources are not only interesting with respect to the quality and quantity of pectin that can be extracted from them, but also to the availability of this material in logistically favoured places. For this reason, sug-

arbeet pulp in particular, has always attracted the attention of pectin manufacturers (Thibault *et al*. 2000).

32.2.2 *Extraction of pectin*

Pectins are industrially extracted from citrus peels and apple pomace by hot, acidified water. The extraction conditions (pH, temperature, time) must be optimised in order to provide good yields of material that also has the desired gelling capacity and degree of methylation (DM). The extraction conditions are in the range of pH 1.5 to 3.0, 60–100°C and 0.5–6 h. The separation of the viscous extract from the strongly swollen and partly disintegrated plant material can be achieved by a combination of centrifugation and filtration, but this still remains a key problem in pectin manufacture. Pectins are recovered from the clarified extracts by alcoholic (isopropanol) precipitation. The precipitate is then washed to remove contaminants of heavy metals, acid, sugars, polyphenols, pigments and other alcohol-soluble materials.

Pectin suspended in alcohol is in a very suitable form for further modification. Through acid treatment in isopropanol, they can be saponified to a desired DM under conditions of low pH and temperatures not exceeding 50°C. This treatment can yield highly methoxylated (HM) pectins with DM values in the range 55–75% or lowly methoxylated (LM) pectins with DM in the range 20–45%. LM pectins can also be obtained by treatment with ammonia in the alcoholic suspensions. Under these conditions, methoxyl groups are partly saponified and partly replaced by amide groups, giving amidated pectins (May 1990). Regulations require that the degree of amidation does not exceed 25%. HM, LM and amidated pectins are then standardised to a given gelling power. Each of these pectins differ in their gelling conditions and applications.

32.2.3 *Gelling properties and applications*

The solubility of pectins in aqueous media depends on multiple parameters, the main ones being the counterion nature, the ionic strength and the pH. Solubility is indeed favoured by the dissociation of carboxyl groups, which leads to molecule individualisation through electrostatic repulsion. Once pectins are solubilised, they may gel through specific inter-chain associations.

HM pectins

Jam manufacture is the main use of industrially extracted pectins, taking benefit of the ability of HM pectins to form gels with high amounts of sugar (>60%) and acid. Junction zone formation is made possible through the 'smooth' regions of pectins. The high sugar concentration creates conditions of low water activity which promote chain–chain interactions rather than chain–solvent interactions (Rees 1972). The acid ensures that the carboxyl groups are not dissociated, thus diminishing electrostatic chain repulsion. To avoid turbidity, syneresis and precipitation, there must be junction zone terminating structural elements in the pectin chain. Rhamnose and 'hairy' regions are supposed to play this role. The junction zones are stabilised by hydrogen bonds between non-dissociated carboxyl and secondary alcohol groups (Morris *et al*. 1980) and by hydrophobic interactions between methoxyl groups (Oakenfull & Scott 1984).

The analytical parameter that allows prediction of gelling behaviour and particularly of gel setting time is the DM. This fact has led to a further subdivision of HM pectins based on the setting time or temperature (Table 32.1), with DM ranging from 77 (for ultra-rapid-set pectins; URS) to 58 (for slow-set pectins; SS). Setting times vary from 1–3 min to more than 1 h, and the pH necessary to achieve gelation decreases from 3.4 to 2.6 (for URS and SS pectins, respectively). It is the art of the jam manufacturer and confectioner to choose the pH conditions and the correct type of pectin to achieve the desired setting times or setting temperatures. For example, the production of marmalades requires URS pectins in order to ensure a uniform distribution of fruits in the jelly, while the production of jams from very acid fruits such as blackcurrant, requires the use of SS pectins to avoid pre-gel formation.

LM pectins

Pectins with a low methoxyl content (DM <50%) can gel in the presence of divalent cations (calcium for food purposes). To achieve gelation, the pH range required is between 2.5 and 6, and sugar addition is not necessary (compare this with HM pectins). Formation of junction zones between the 'smooth' regions of different chains leads to gelation (Morris *et al*. 1978) (Fig. 32.1). Because of the electrostatic nature of the bonds, pectin gels are very sensitive to structural parameters which can modify the environment of the carboxyl groups, such as the nature and the amounts of substituents along the polygalacturonic acid backbone. Thus, the gel-forming ability increases with decreasing DM; furthermore, LM pectins with a block-wise distribution of free carboxyl groups are very sensitive to low calcium level (Thibault & Rinaudo 1985).

Amidation increases or improves the gelling ability of LM pectins: amidated pectins need less calcium to gel and are less prone to precipitation by high calcium level (May 1990). Amide groups were shown to be distributed in a blockwise pattern along the pectic chain (Racape *et al*. 1989), and it has been suggested (Voragen *et al*. 1995) that these blocks can promote association through hydrogen bonding (Fig. 32.1).

The applications of LM pectins are directly based on their peculiar gelling properties. As no sugar is needed for gelation, they are widely used in 'low-calorie gelled products'. The large pH zone for gelation makes them useful in food products which have to be gelled in non-acidic conditions, for example to preserve aromas. As calcium is necessary to achieve gelation, LM pectins – particularly amidated ones – are well adapted for gelled milk products in

Table 32.1 High-methoxylated (HM) pectins sub-classifications, degree of methylation (DM), setting time, pH and applications.

	HM pectins			
	Ultra rapid	Rapid	Medium	Slow
DM (%)	74–77	71–74	66–69	58–65
Setting time (min)	1–3	3.7	15–25	30–120
pH	3.1–3.4	3.0–3.3	2.8–3.1	2.6–2.9
Applications	Jams with whole fruits 'Dundee' jellies	'Classical' jams	Acid jams and jellies (raspberry)	Acid to very acid jams and jellies (black currant)

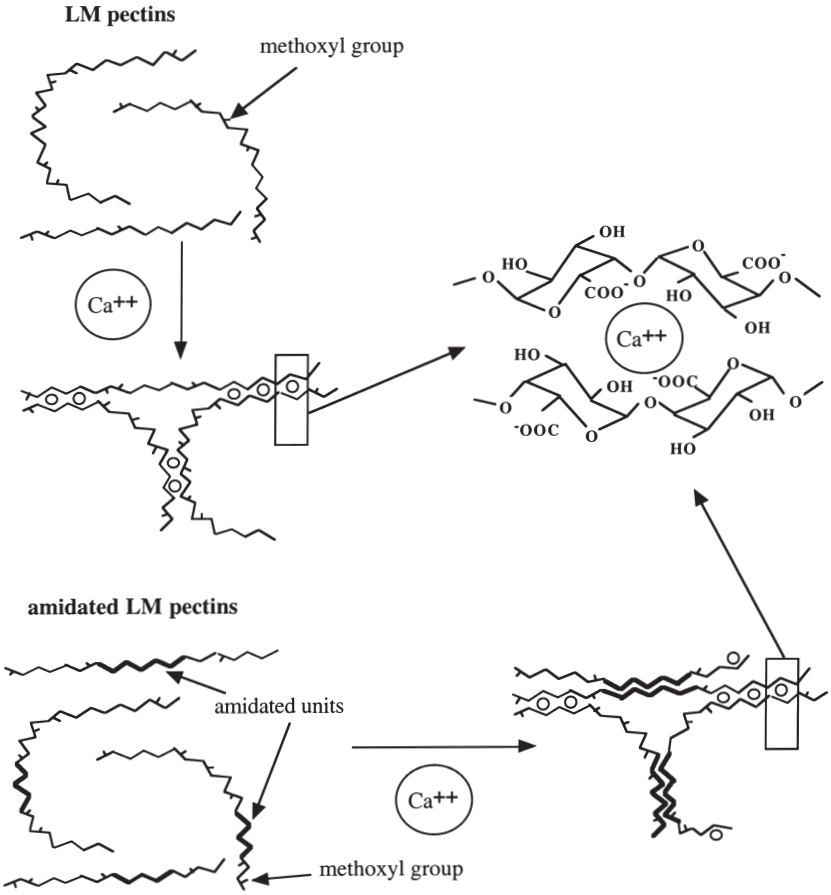

Fig. 32.1 Gelation mechanisms of LM and amidated LM pectins.

which interactions with caseins may also take place. Finally, these pectins also find applications in the production of fruit bases for yoghurt.

32.3 Pectin-rich cell walls as dietary fibre

Both the nutritional value and physicochemical properties of dietary fibres are important in the potential development of a wide range of fibre-enriched products. Fibre-enriched ingredients can be used only if they can be easily incorporated in the formula and if the final product has good sensory characteristics, regardless of the nutritional benefits of the fibres (Thebaudin *et al*. 1997). The physical characteristics of the fibre, as well as the hydration, ion-exchange and adsorption capacities are of importance for their optimal utilisation in foods (Thibault *et al*. 1992, 1994, 2000).

32.3.1 *Physical characteristics*

The three-dimensional network that forms the structure of the cell walls imparts a number of physical characteristics. Surface area, particle size and pore size are probably key parameters.

Surface area values are very scarce and contradictory, ranging from 0.2–0.5 m^2/g for citrus and apple commercial fibres (Robertson *et al*. 2000) to >30 m^2/g for carrot cell walls (Chesson 1998). Such variations may be ascribed to the production process of commercial fibres from the cell walls.

The interest in mean particle size and its distribution in fibre and digesta arises from the recognition that this physical characteristic has an important role both in food processing (Thebaudin *et al*. 1997) and in controlling a number of events that occur in the digestive tract (e.g. transit time, fermentation, faecal excretion) (Guillon *et al*. 1998). Decreasing particle size increases the external surface area, and so increases the area exposed to bacteria during fermentation. The impact of decreasing particle size on fermentation is most marked for lignocellulosic materials such as cereal brans, but has also been demonstrated for pectin-rich fibres (Cloutour 1995; Guillon *et al*. 1998) (Fig. 32.2). Most of the available data on particle size distribution is for cereal dietary fibres, but limited data are now available for pectin-rich fibres (Stephen & Cummings 1979; Robertson 1988; Robertson *et al.* 1992, 2000; Cloutour 1995; Guillon *et al*. 1998). Particle size measurements can be made on hydrated fibres, which makes the measurements more relevant in a nutritional context, for example to characterise masticated material, digesta or faeces.

As well as particle size and surface area, pore size is also a crucial parameter for an understanding of the fermentative degradation of dietary fibres (Guillon *et al*. 1998). Macroporosity and microporosity measurements may help to determine if enzymes or bacte-

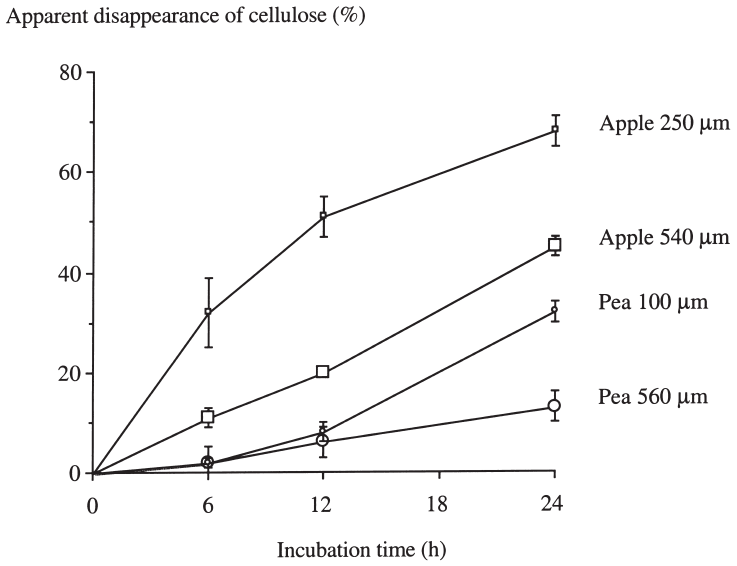

Fig. 32.2 Fermentability of pea and apple fibres of different mean particle sizes. (Data from Cloutour 1995.)

ria are able to penetrate the network and degrade polysaccharides inside the cell-wall matrix, or if a sole surface attack has to be considered. Considering radii of pores between 0.5 to 5 nm, it is likely that enzymes may partly penetrate the network and degrade polysaccharides within the cell-wall structure (Chesson 1998). The importance of porosity – and especially macroporosity – in the control of sugarbeet fibre fermentability has been demonstrated (Guillon *et al*. 1998).

32.3.2 *Hydration properties*

It is crucial to consider fibre-associated water when examining fibre as an ingredient in a commercial formulation, or when investigating the effect of fibre along the gut. This explains the large amount of published data available on this subject. Pectin-rich fibres generally exhibit higher hydration properties than do other fibres, due to the high hydrophilicity of pectic substances. Numerous terms have been used to define hydration properties. Within the recent 'Profibre' EU concerted action group, clear definitions and standards for measurements of hydration properties were major considerations (Robertson *et al*. 2000). Three definitions arose from 'Profibre':

(1) Swelling, which is the volume occupied by a known weight of fibre under the conditions used.
(2) Water retention capacity, which is the amount of water retained by a known weight of fibre under the conditions used (centrifugation for example).
(3) Water absorption, which is the kinetics of water movement under defined conditions (Baumann method, dialysis) (Baumann 1967).

The use of different methods to measure the hydration properties of fibres results in different values being obtained (Rasper 1979; Auffret *et al*. 1994). However, values also depend strongly on intrinsic parameters (chemical structure and physical structure of the fibre components) (Cadden 1987; Auffret *et al*. 1993), on physicochemical environment, and on the 'history' of the fibre, especially the drying process. The influence of the method used, of the fibre origin and of processing is illustrated in Table 32.2.

The hydration properties of dietary fibres determine their optimal usage levels in foods, as a desirable 'texture' must be obtained (Thebaudin *et al*. 1997). High water retention capacities of pectin-rich dietary fibres can be exploited, notably in bakery products. For example, Wang and Thomas (1989) reported that the substitution of flour with citrus fibres, apple concentrates or apple flakes in cakes, muffins and cookies had a positive sensory effect. Hydration properties of fibres also have a deep impact on their fermentability. Higher water-binding capacities were correlated to higher extents of bacterial degradation of sugarbeet fibres (Guillon *et al*. 1998) (Fig. 32.3).

32.3.3 *Cation-exchange capacity*

Because dietary fibres have been shown to bind nutritionally important ions, and thus could have an effect on electrolyte metabolism and mineral absorption, special attention has been drawn to their ion-exchange capacity. Pectin-rich fibres are important in this respect. Due to their high content of negative charges (galacturonic acids), they are believed to bind calcium ions strongly.

Table 32.2 Hydration properties of some pectin-rich dietary fibres.

	Mean particle size	Swelling (ml/g)	Water retention capacity (g water/g dry fibre)
Apple pomace*		7.4	5.4
Fresh apple†			
Alcohol-insoluble solids		48.4	14.2
Citrus peels*		10.4	10.7
Sugarbeet pulp‡	540 µm	21.5	12.6
	385 µm	21.4	12.0
	205 µm	15.9	9.2

* Robertson *et al.* (2000)
† Renard & Thibault (1991)
‡ Auffret *et al.* (1994).

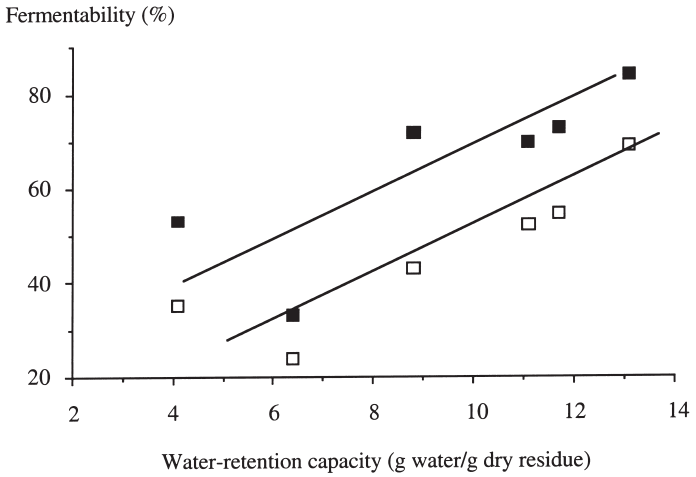

Fig. 32.3 Correlation between hydration properties and fermentability of sugarbeet fibres. (Data from Guillon *et al*. 1998.)

Pectin-rich fibres behave as weak monofunctional cation-exchange resins. There usually is a good agreement between the experimental cation-exchange capacity (number of ionic groups per gram of fibre product determined by pH- or conductimetric titrations), and the theoretical value which can be calculated from the amount of galacturonic acid in the fibre and the degree of methylation of pectins.

The values of CEC for pectin-rich fibres are higher than those found for cereal fibres. CEC values ranging between 0.4 and 0.6 mEq OH^-/g are commonly found (Renard & Thibault 1991; Ralet *et al*. 1991, 1993; Thibault *et al*. 2000). It must be borne in mind that these values always reflect the maximum CEC value, as CEC determinations are conducted on the acidic form of fibres. The binding of ions depends not only on the CEC, but also on the environment

of the fibre (a low ionic strength and a neutral pH being favourable for a strong binding) and on the chemical or structural changes of the ionic groups (along the digestive tract for example). At the present time, there is no clear suggestion that dietary fibres endanger mineral and trace element status in the long term. Although CEC studies of dietary fibre show clear evidence of binding, extrapolation of these effects *in vivo* has not been consistently demonstrated (Cho *et al*. 1997b).

32.3.4 *Adsorption of organic molecules*

Dietary fibres can retain oil (<2 g/g; Thibault *et al*. 1992), a property which is exploited in foods (cooked meat products) to enhance the retention of fat that is normally lost during cooking (Thebaudin *et al*. 1997). Of particular importance is the adsorption of bile acids. Although a hydrophobic mechanism for binding of bile acids has been strongly suggested, especially for lignocellulosic material, other mechanisms such as weak hydrogen bond binding or ion-binding through calcium bridges have also been suggested (Cho *et al*. 1997b). The increased faecal bile acid excretion level *in vivo* was related to the plasma cholesterol-lowering effect of certain dietary fibre sources (Anderson *et al*. 1984), but more recent studies (Story & Furumoto 1990; Gelissen *et al*. 1994) suggest that the bile acid-binding property of fibres may be only one of several factors implicated in the serum cholesterol-lowering effect.

32.4 Conclusions

To date, cereal brans have been the major source of dietary fibre. However, the use of other agro-industrial by-products as potential sources of dietary fibre is attractive from both a functional and a nutritional point of view. Pectin-rich dietary fibres indeed have some special properties. The physicochemical properties of pectin within the fibre matrix relates primarily to hydration, viscosity and gelation, and cation-exchange capacity. The chemical structure of pectic molecules explains some of these properties, and cation-exchange capacity, for example, can be directly related to the amount of non-esterified galacturonic acid in pectins. However, the physical structure of the pectin-rich fibres has also a marked role on the physicochemical properties. Surface area and particle size of fibre materials have been shown to have a deep impact on hydration properties. Moreover, the chemical environment and the 'processing history' of the fibres have also to be considered. Physicochemical properties of pectin-rich fibres, and especially their high hydration properties, are related to peculiar functional and technological applications. Furthermore, these properties can be related to some nutritional benefits, mainly connected with fermentability.

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33 Chemistry and Enzymology of Pectins

Fons Voragen, Gerrit Beldman and Henk Schols

33.1 Introduction

Pectins are important components of plant cell walls, and contribute to many of the functions that cell walls perform in plant tissues. These include physiological aspects related to growth, the determination of cell size and shape, the integrity and rigidity of tissues, ion transport, water holding, and defence mechanisms against infections by plant pathogens and wounding (Basic *et al.* 1988).

On growing, ripening and storage of fruits and vegetables, the changes in the amount and nature of the pectins present determine to a large extent the quality attributes of fresh fruits and vegetables (e.g. ripeness, texture), their processing characteristics in the manufacture of foods (vegetable canning, milling, pressing, filtration, clarification, concentration and thermal treatments of juices, nectars, purees, preserves), and the extractability (bioavailability) of important constituents of plant raw materials such as sugar, oil, proteins, colorants, antioxidants and vitamins (Voragen *et al.* 1995).

Pectins are extracted from suitable plant material (from by-products such as citrus peel and apple pomace), and are used in the food industry as a natural ingredient due to their ability to form gels at low concentrations (jam, jelly and marmalade and confectionery industry), and to increase the viscosity of liquid foods. Pectins are also widely applied as stabilisers in acid milk products and as fat mimetics (Voragen *et al.* 1995).

Pectins also command the interest of nutritionists as dietary fibre in general, and more specifically for their potential to lower blood cholesterol levels. They also affect glucose metabolism in that they lower the glucose response curve. Pectic substances are claimed to have pharmaceutical activities such as anti-diarrhoea, detoxicant, regulation and protection of the gastrointestinal tract, immune stimulation, anti-metastatic, anti-ulcer and anti-nephrotic (Endress 1991; Yamada 2000).

The dominant structural feature of pectin is a linear chain of α-(1→4)-linked D-galactosyluronic acid residues in which varying proportions of the acid groups are present as methyl esters. They are generally associated with neutral polysaccharides (arabinans, arabinogalactans and galactans). These neutral polysaccharides are attached to rhamnogalacturonan regions which have a backbone of alternating α-(1→2)-linked L-rhamnosyl and α-(1→4)-linked D-galactosyluronic acid residues (rhamnogalacturonan I). Further structural elements of pectins are xylogalacturonan, apiogalacturonan and rhamnogalacturonan II (Albersheim *et al.* 1996; Schols & Voragen 1996). The term protopectin is often used to designate the native pectin fractions in cell walls that cannot be extracted by non-degradative methods (Sakai 1992), but is quite unspecific.

Pectins occur in most dicotelydenous plant foods, particularly in the middle lamella and primary cell walls. Their extraction, fractionation and analysis can be very complicated, depending on the information that is desired, for example insolubility/solubility or gelling in

aqueous systems. Pectins may differ in their galacturonic acid and neutral sugar content, glycosidic linkage composition, degree of methyl esterification and acetylation, amide content and molecular weight (distribution). These parameters may influence the stability as a function of pH, temperature and other matrix factors, the formation of supramolecular structures, and the degradability by enzymes. High-methyl esterified pectins are very unstable in systems with pH values over 5 where they will depolymerise rapidly when the system is heated. Removal of methyl esters and acetyl groups may already occur at neutral pH (Voragen *et al.* 1995).

Pectins can also be degraded and modified by various enzymes, which occur in many higher plants and are produced by many microorganisms. The significance of native pectic substances in food technology and nutrition can be properly evaluated only if the activity of these enzymes is taken into account. Two families of enzymes active on pectins can be distinguished: (1) those active towards homogalacturonans; and (2) those active towards rhamnogalacturonans. Both groups can be subdivided into esterases (methyl and acetyl), hydrolases and lyases. Of the latter two groups, *endo* and *exo* enzymes are known (Pilnik & Voragen 1991; Beldman *et al.* 1996). Several species of the colonic flora are able to degrade pectic substances (Van Laere *et al.* 2000).

33.2 Chemical structure of pectins

The pectic substances are probably the most complex class of plant cell-wall polysaccharides. In essence they comprise two families of covalently interlinked acidic polymers (Voragen *et al.* 1995):

(1) Galacturonans, being pectin segments with exclusively α-(1,4)-linked D-galactosyluronic acid residues in the backbone, such as the linear homogalacturonan, and the substituted xylogalacturonan and rhamnogalacturonan type II (RG-II); the latter having a galacturonan backbone heavily branched with side chains containing (rare) sugars such as apiose, rhamnose and KDO (3-deoxy-D-manno-octulosonic acid).
(2) Rhamnogalacturonan type I with a backbone of alternating α – (1→2)-linked L-rhamnosyl and α-(1→4)-linked D-galactosyluronic acid residues ramified with different types of neutral oligo- and polysaccharides (e.g. arabinans, galactans and arabinogalactans), predominantly attached to O-4 of the rhamnosyl residues.

From results of our studies, the concept was postulated that pectins have an intramolecular distribution in which the neutral sugars are concentrated in blocks of more highly substituted rhamnogalacturonan regions ('hairy'), separated by unsubstituted ('smooth') regions containing almost exclusively D-galactosyluronic residues (Fig. 33.1).

A portion of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II can be solubilised from plant cell walls by treatment of the walls with pure *endo*-polygalacturonase, even though this enzyme only cleaves homogalacturonan chains in non-or low methylesterified sites of the backbone (McNeil *et al.* 1984). More complete solubilisation of the pectic polysaccharides as present in plant cell walls can be obtained by using a mixture of *endo*-polygalacturonase and pectin methylesterase (or pectinlyase), cellobiohydrolase and *endo*-glucanase (Vincken *et al.* 1997). Under these conditions the homogalacturonan will be degraded to mono-, di- and small oligogalacturonides. Xylogalacturonans are released

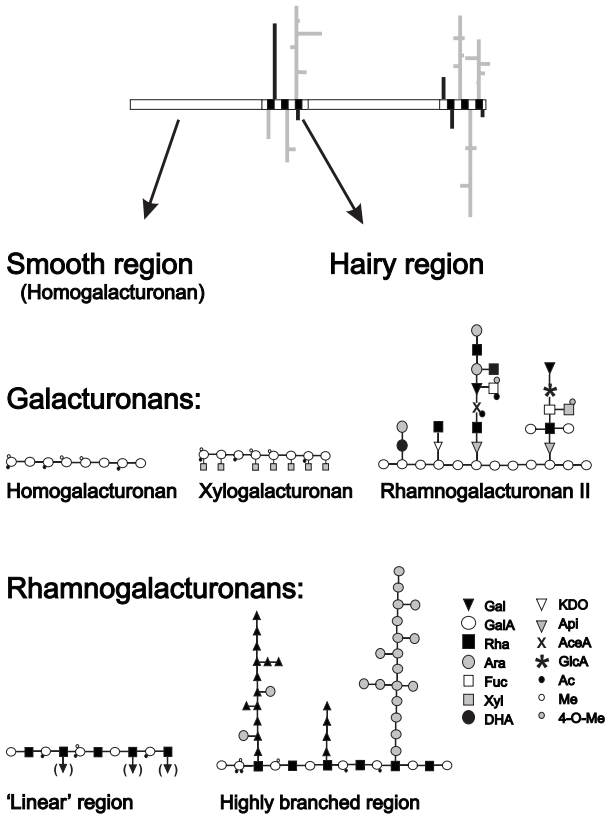

Fig. 33.1 Structural elements of pectin. Occurrence, amount and chemical fine structure of the individual segments may vary significant depending on origin of the pectin. (From Schols and Voragen 1996; Oosterveld 1997; Huisman 2000.)

from ramified rhamnogalacturonan fractions by rhamnogalacturan hydrolase or rhamnogalacturonan lyase (Schols & Voragen 1996). Rhamnogalacturonan I and II are also solubilised in 'polymeric form'. Together, these complex pectic segments form the so-called 'hairy regions' (see Fig. 33.1) to emphasise the contrast to unbranched homogalacturonan ('smooth regions').

Pectins consisting almost exclusively of *homogalacturonans* are renowned for their ability to form gels, a property widely utilised in the food industry and in all likelihood a property that determines some of the functions of pectin in primary cell walls (Voragen *et al.* 1995). This type of pectin is manufactured industrially by acid extraction of dry apple pomace or wet (blanched) or dried peels and rags after the extraction of citrus juice (lime, lemon, orange). Typically, the predominant sugar residue of these pectins is galactosyluronic acid (>75%), but some neutral sugars are also present (e.g. arabinose, galactose, rhamnose and xylose) in varying amounts, depending on the origin of the pectin (Voragen *et al.* 1995). These neutral sugars are apparently remnants from the hairy regions which survive the acid extraction conditions. Due to the ready availability of large quantities of sugarbeet pulp, this source has always attracted the interest of pectin manufacturers and scientists.

An important feature of both homogalacturonans and rhamnogalacturonans, is the partial O-substitution of the galactosyluronic acid residues at O-6 with a methanol and/or at O-2 and/or O-3 with an acetyl group. The degree of methyl esterification (DM) is defined as the number of moles of methyl esters per 100 moles of galacturonic acid residues. Pectins are called high methoxyl (HM-) pectins when the value for DM is 50 or higher; when DM is lower than 50 they are called low methoxyl (LM-) pectins. For native apple pectins, a random distribution of the methyl esters groups over the galacturonan backbone was found. For commercially extracted pectins with tailored DM, the distribution depends on the raw material and the extraction and de-esterification conditions. HM-pectins have the ability to form gels with sugar and acid – so-called low-water-activity gels or sugar-acid-pectin gels. Such a gel is considered as a three-dimensional network of pectin molecules in which the solvent (water) with the co-solutes (sugar and acid) is immobilised, resulting in a system resisting deformation and showing a stress–strain relationship for small deformations. The build-up of the three-dimensional network is based on the formation of junction zones in which there are chain associations stabilised by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by hydrophobic interactions between methyl esters. LM-pectins can gel in the presence of divalent cations (usually calcium for food applications). In these systems, gelation is due to the formation of intermolecular junction zones between homogalacturonic (smooth) regions of different chains. The structure of such a junction zone is generally ascribed to the so-called 'egg-box' binding process, but this mechanism is still controversial. The gel-forming ability of LM-pectins increases with decreasing DM. LM-pectins with a blockwise distribution of free carboxyl groups are very sensitive to low calcium levels (Voragen *et al.* 1995).

Acetyl groups are generally present in rhamnogalacturonans and only present in very low amounts in homogalacturonans from, for example apple and citrus, but are present in much higher amounts in homogalacturonans from sugarbeet and potato. The presence of acetyl groups prevents gel formation with Ca^{2+}ions, but gives the pectins emulsion-stabilising properties. The degree of acetylation (DA) is defined as moles acetic acid per 100 moles of galactosyluronic acid residues, and in this way the DA may exceed 100%. In this definition, it is assumed that only the hydroxyl groups of galactosyluronic acid residues and not other sugar residues present in the pectin are acetylated. Some characteristics of pectins as obtained from various sources (Voragen *et al.* 1995) are shown in Table 33.1. Commercial LM-pectins can also be amidated, the degree of amidation (DAm) being defined similar to the DM. Amidation increases or improves the gelling ability of LM-pectins in that they need less calcium to gel and are less prone to precipitation at high calcium levels (Voragen *et al.* 1995).

33.3 Structural elements of pectin

33.3.1 *Xylogalacturonan*

Xylogalacturonan is a relatively recently discovered subunit of pectic substances. Xylogalacturonan is a linearly branched homogalacturonan in which xylopyranosyl residues are β-(1,3)-linked to part of the galactosyluronic acid residues in the galacturonan backbone. Different populations of xylogalacturonan have been isolated that have ratios of xylosyl residues to galactosyluronic acid residues ranging from 0.4–0.9, while the proportion of methylesterified galactosyluronic acid also ranges from 40–90%. Xylogalacturonan has been found especially in selected tissues of some plants, where it may have a specialised function associ-

Table 33.1 Characteristics for pectins extracted from various sources.

Origin	Extraction	GalA (%)	DM (%)	DA (%)
Mango	Acidic	54	68	4
Sunflower	Acidic	81	17	3
Sugarbeet	0.05 M HCl; 85°C	65	62	35
Carrot	0.05 M HCl; 85°C	61	63	13
Grape	Oxalate pH 4.5; 20°C	63	69	2
Sunflower	Oxalate	83	27	10
Peach	HCl to pH 2; 80°C	90	79	4
Siberian apricot	EDTA	64	57	8
Cythere plum	0.05 M HCl; 85°C	65	65	16
Potato	HCl, pH 2; 80°C	40	53	15

DA, degree of acetylation; DM, degree of methyl esterification; GalA, galacturonic acid.
Source: Voragen *et al*. 1995.

ated with storage tissues of reproductive organs, as the cell walls of peas, soybeans, kidney beans, apple fruit, pear fruit, onions, cotton seed and watermelon (Weightman *et al*. 1994; Schols & Voragen 1996).

33.3.2 *Apiogalacturonan*

Apiogalacturonan is also a linearly branched homogalacturonan to which mono- and di-apiosyl side chains are attached. Apiogalacturonan has been found in the cell walls of the aquatic monocots duckweed (*Lemna* spp.) and sea grass (*Zosteracea)* (Hart & Kindel 1970; Ovodov 1975)

33.3.3 *Rhamnogalacturonan II*

Rhamnogalacturonan II has been found in the cell walls of many tissues of edible plants such as apple (juice), pear, kiwi, grape (wine), beet fibre, carrot, tomato, onion, pea, radish and ginseng leaves (Pellerin *et al.* 1996; Doco *et al.* 1997). In apple, tomato and carrot, contents of 400 mg, 200 mg and 800 mg/kg fresh weight have been estimated, respectively, while for white wine values of 30–50 mg/l are reported (Pellerin *et al.* 1996). The structure of rhamnogalacturonan II (RG-II) is highly conserved, as apparently identical structures have been obtained from the cell walls of a large variety of sources. The backbone of RG-II contains nine (1,4)-linked α-D-galactosyluronic acid residues to which four structurally distinct oligoglycosyl side chains are attached to O-2 or O-3 of four of the backbone residues in a well-defined manner. These side chains contain rhamnose and several rare characteristic glycosyl residues such as apiose, 3-*O*-methyl-l-fucose, 2-*O*-methyl-d-xylose, aceric acid (3-*C*-carboxy-5-deoxy-l-xylose), KDO (3-deoxy-D-manno-octulosonic acid) and DHA (3-deoxy-D-lyxo-heptulosaric acid). RG-II is a low-molecular weight (~4.8 kDa) complex polysaccharide segment present in primary walls predominantly as a dimer that is covalently cross-linked by borate diesters (Pellerin *et al.* 1996). Through covalent cross-linking of rhamnogalacturonan II units from two pectin molecules, interconnection of pectic polysaccharides is achieved, thus improving the integrity of the pectin network. Due to their low molecular weight they may be absent in fruit juices when these have been subjected to an ultrafiltration treatment,

depending on the molecular weight cut-off of the membranes used. The borate diester form of RG-II has the ability to form complexes with selected divalent (e.g. Pb^{2+}, Ba^{2+}, Sr^{2+}) and trivalent (e.g. La^{3+}) cations, and is therefore believed to account for the low level of free Pb^{2+} in wine. Pb^{2+} complexed in this way is not absorbed through the gastrointestinal tract, and may therefore reduce the risk of chronic saturnism (chronic lead intoxication). The RG-II diester isolated from the medical herb Panax Ginseng has been reported to enhance the expression of macrophage Fc receptor (Yamada 2000).

33.3.4 *Rhamnogalacturonan I*

Rhamnogalacturonan I ideally has a backbone composed of alternating α-(1,2)-linked L-rhamnosyl and α-(1,4)-linked D-galacturonosyluronic acid residues. This backbone may contain as many as 300 rhamnosyl and 300 galactosyluronic acid residues. Part of the galactosyluronic acid residues may be methyl esterified and/or acetylated (McNeil *et al.* 1984). Also, rhamnose-rich pectin fragments with a rhamnose: galacturonic acid ratio varying between 0.1 and 1 (and thus deviating from a strictly alternating rhamnosyl-galactosyluronic acid sequence) occur quite generally (Schols & Voragen 1996). Depending on the cell wall material, ~20% to ~80% of the 1,2-linked L-rhamnosyl residues are branched at O-4, with side chains which can vary in size from a single glycosyl residue to 50 or more glycosyl residues and which are composed of D-galactosyl, L-arabinosyl and small amounts of L-fucosyl residues. This indicates that rhamnogalacturonan I (RG-I) possess many different side chains. From degradation studies of rhamnogalacturonan I with rhamnogalacturonase and rhamnogalacturonan lyase it was concluded that the distribution of the side chains along the rhamnogalacturonan backbone was blockwise. The reaction mixtures always contained polymeric, highly ramified fragments and typical oligomeric rhamnogalacturonan fragments in which part of the rhamnosyl residues were substituted at O-4 with one galactosyl residue. This was a common feature found in the cell walls of apple, pear, citrus, carrot, onion, potato, onion, leek, soybean and sugarbeet (Schols *et al.* 1994; Schols & Voragen 1996).

D-Galactose and L-arabinose are the major sugars present in the side chains of RG-I predominantly attached to O-4 of the rhamnosyl residues. Their structures can be characterised as arabinans and arabinogalactans, with chain lengths that can vary from 1 to over 50 glycosyl residues.

33.3.5 *Arabinans*

Arabinans are branched polysaccharides with a backbone of 1,5-linked α-L-arabinofuranosyl residues with other α-L-arabinofuranosyl units attached to about one-third of the backbone residues by 1,3 and/or 1,2 bonds (Beldman *et al.* 1997 and references herein). The structure of some of these arabinans can be accommodated in a comb-like model. For others regions with more ramified arrangements of arabinofuranosyl residues, some degree of multiple branching must be included (Aspinall & Fanous 1984). Arabinans in free form or attached to pectin structures have been described for apples, sugarbeet, rapeseed, apricots, tomatoes, carrots, cabbage, parsnip, mung bean, soybean, cow peas, azuki beans, peanuts, mustard seed, grape juice, apple juice, onions, pears and angelica (see references in Beldman *et al.* 1997).

33.3.6 *Arabinogalactans*

Arabinogalactans occur in two structurally different forms:

(1) Arabinogalactan type I. This is a 1,4-linked linear chain of β-D-galactopyranosyl residues connected in general to O-4 of the rhamnosyl residues with up to 25% α-L-arabinofuranosyl residues 1,5-linked in short side chains (Van de Vis 1994). Branching at O-6 with single galactosyl residues has also been found. Recently, two uncommon features were reported for arabinogalactan type I present in soybeans. One was the presence of an arabinopyranose residue at the non-reducing terminus of a galactan sequence, while the presence also of sequences of (1,4)-linked galactose residues interspersed with one internal (1,5)-linked arabinofuranose residue (Huisman 2000) has not been reported previously. Pectins with arabinogalactans type I attached have been found in citrus, potato, soybean, lupin, apple, onion, kiwi, tomato and cabbage (see references in Voragen *et al.* 1995).

(2) Arabinogalactan type II. This is a highly branched polysaccharide with ramified chains of β-D-galactopyranose residues joined by 1,3 and 1,6 linkages. The 1,3 linkages predominate in the interior chains, and the 1,6 linkages occur mainly in the exterior chains. The exterior chains are mainly terminated with L-arabinofuranosyl residues and to some extent with L-arabinopyranosyl residues. Pectins with type II arabinogalactans attached were shown to be present in apples, rapeseed, lemon, beet, grape and cabbage (see references in Voragen *et al.* 1995). Type I and type II arabinogalactans can also be isolated from primary cell walls unattached to other cell-wall components. Type II arabinogalactan is also the quantitatively predominant component of arabinogalactan proteins (more often referred to as AGPs) and as such not a structural component of the primary cell wall (Basic *et al*. 1988). The occurrence and amounts of some structural elements for some different types of plant material are shown in Table 33.2.

33.3.7 *Ferulated pectins*

Pectins isolated from sugarbeet and spinach were found to contain ferulic acid. It is at-

Table 33.2 Occurrence and proportion of the various structural elements of pectin in apple, sugarbeet and soy.

	Soybean meal	Sugarbeet pulp	Apple
Total polysaccharide (w/w % dm)	16	67	20
Pectic substances (% of total PS)	59	40	42
Structural element (% of pectic substances)			
Homogalacturonan	0	29	36
Xylogalacturonan	21	<1	4
Rhamnogalacturonan II	4	4	10
Rhamnogalacturonan backbone	15	8	4
Arabinan	60	46	27
Arabinogalactan I		12	20
Arabinogalactan II	0	0	+

tached to the O-2 position of α-(1→5)-linked-L-arabinofuranosyl residues in the arabinan side chains, and to the O-6 position of galactosyl residues in β-(1→4)-linked galactans (Fry 1982; Rombouts & Thibault 1986). In sugarbeet pulp pectin, 50–55% of the feruloyl groups are linked to arabinose, and approximately 45–50% to galactose residues. Ferulic acid dehydrodimers account for approximately 22% of the total ferulates in sugarbeet (Fig. 33.2; Oosterveld 1997). By oxidative coupling using hydrogen peroxide/peroxidase or ammonium persulphate, ferulated pectins can be cross-linked, resulting in the formation of a gel. In this process the amount of ferulic acid decreases, while an increase in ferulate dehydrodimers by a factor of about five can be observed (particularly the 8-5 and 8-O-4 dehydrodimers).

33.4 Enzymes

The enzymes active on pectins comprise a diverse group. In view of the complexity of the substrate, the multitude of different enzymes can be easily envisaged. These enzymes occur in many higher plants and are produced by many organisms (Pilnik & Voragen 1991; Benen *et al.* 1999; Roy *et al.* 1999). The significance of native pectic substances in food technology and in nutrition can be properly evaluated only if the activity of these enzymes is taken into account. They can produce important textural changes and changes in content, chemical structure, solubility and other physical properties in fruits and vegetables during ripening, storage and processing, as a result of depolymerisation, de-esterification and solubilisation (Voragen *et al.* 1995). Microbial pectic enzymes serve functions in plant pathology, in fermented foods and in fermentation processes in the colon, but are also produced industrially as processing aids for the food and feed industry, e.g. in raw fruit juices to facilitate clarification, in fruit pulps (apples, grapes, berries) to improve juice and colour yield, or biomass conversion. In these processes the nature and content of pectin in the product may change drastically.

Pectic enzymes are classified according to their mode of attack on the pectin substrate. Two families of enzymes can be distinguished: homogalacturonan-degrading enzymes (Fig. 33.3) and rhamnogalacturonan-degrading enzymes (Fig. 33.4). Protopectinases are also mentioned in the literature (Sakai 1992). Enzymes with this name are designated as those which are able to degrade native pectin fractions in cell walls that cannot otherwise be extracted by non-degradative methods, and resulting in the release of soluble, polymeric pectic

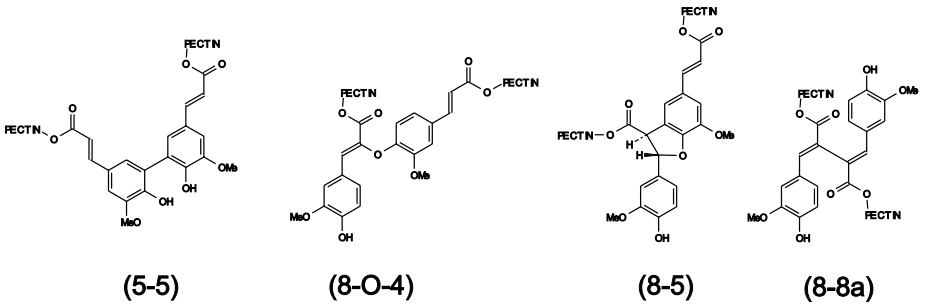

Fig. 33.2 Examples of cross-links formed by oxidative coupling of feruloylated pectins. (From Oosterveld 1997.)

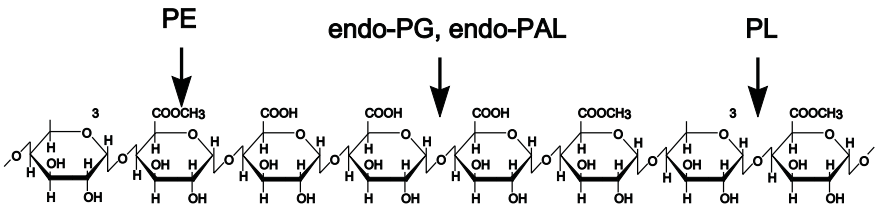

Fig. 33.3 Homogalacturonan fragment of pectin and points of attack of pectic enzymes.

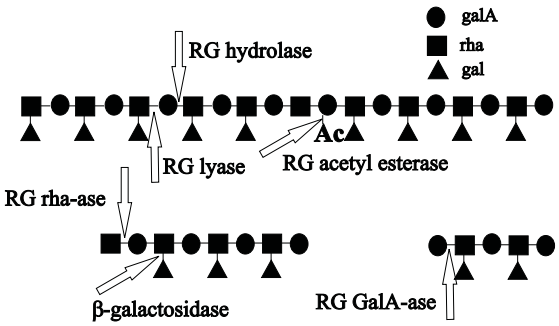

Fig. 33.4 Degradation of rhamnogalacturonans (RG) and oligomers thereof by RG-specific enzymes. galA, galacturonic acid; rha, rhamnose; gal, galactose; Ac, acetyl group. (From Beldman *et al*. 1996; Mutter 1997.)

material. Several protopectinases have been studied in detail, and it transpired that most of them belonged to the polygalacturonases, pectin or pectate lyases, rhamnogalacturonases, arabinanases or galactanases.

33.4.1 *Homogalacturonan-modifying enzymes*

Pectin methylesterases

Pectin methylesterases, or pectin pectylhydrolase (PE; EC 3.1.1.11) split off methyl esters and convert high-esterified pectins to low-esterified pectins. PE occurs in many higher plants, but particularly in tomatoes, citrus, potatoes, apple, banana, berries, cherries, currents, grapes, mango, papaya, passion fruit, peach, pear, plum, beans, carrots, cauliflower cucumber, leek, onions, pea and radish; PE is also produced by many fungi and bacteria (Pilnik & Voragen 1991). Plant PEs have a pH optimum around 7 and act along the galacturonan chain, creating blocks of free carboxyl groups. These blocks are extremely sensitive towards complex formation, and precipitation with Ca^{2+}ions. Fungal PEs have a pH optimum around 4–5. These saponify methyl esters along the galacturonan chain in a more or less random fashion.

Pectin acetylesterase

This enzyme splits off acetyl groups from homogalacturonan regions, making them calcium-

reactive so that calcium pectate gels may be formed. Deacetylation also enhances PE activity, and vice versa (Voragen *et al.* 1995).

Polygalacturonases

Polygalacturonases (poly-α-1,4-D-galacturonide glycanohydrolase; EC 3.2.1.15 and 3.2.1.67) hydrolyse LM-pectins or pectic acid preferentially, because these enzymes can split glycosidic linkages adjacent to free carboxyl groups (Pilnik & Voragen 1991; Benen *et al.* 1999). Polygalacturonases can be divided into enzymes that degrade their substrate by an *endo*- attack (*endo*-polygalacturonase) and those that act from the non-reducing end, removing mono- or digalacturonic acid (*exo*-polygalacturonase). Polygalacturonases can be further differentiated by their ability to tolerate varying numbers of methyl-esterified galactosyluronic acid residues in their active site. Polygalacturonases are produced by fungi and certain bacteria, and also occur in higher plants such as apple (*exo* only), apricot, avocado, banana, berries, cherries, grapefruit (*exo* only), grapes, mango, papaya, passion fruit, peach, pear, carrots (*exo* only), cucumber, potato (*exo* only), radish and tomato. *endo*-Polygalacturonases with their strong depolymerising action are of particular technological importance. By down-regulating their activity through anti-sense technology, the firmness of tomatoes can be influenced (Carrington *et al.* 1993). The pH optimum of polygalacturonases from plant and food grade microorganisms is around 4–5.

Pectate lyases

Pectate lyases (poly-α-1,4-D-galacturonide lyase; EC 4.2.2.2 and 4.2.2.9) split glycosidic linkages adjacent to free carboxyl groups by a *trans*-elimination mechanism (Pilnik & Voragen 1991; Roy *et al.* 1999). This group of enzymes also contains *endo*- and *exo*-enzymes. The preferential substrates for *endo*-pectate lyase are LM-pectins rather than pectic acid because these enzymes also tolerate varying numbers of methyl-esterified galactosyluronic acid residues in their active site. Pectate lyase have an absolute requirement for Ca^{2+} ions and are predominantly produced by bacteria; they have little significance in fruit and vegetable processing because of their high pH optimum (>8). Based on the presence of cDNA encoding putative pectate lyases these activities have also been implied in the ripening of strawberries and bananas (Dominguez-Puigjaner *et al.* 1997; Medina-Escobar *et al.* 1997). *endo*-Pectate lyases have been identified as key enzymes in pectin degradation in the human colon (Dongovski & Lorenz 1998).

Pectin lyases

Pectin lyases (poly-α-1,4-D-methoxygalacturonide lyase; EC 4.2.2.10) split glycosidic linkages between methyl-esterified galacturonide residues by a *trans*-elimination reaction and therefore have a preference for HM-pectins (Pilnik & Voragen 1991). Pectin lyases are only produced by microorganisms, predominantly fungi. Their pH optima are around 6 on almost fully methyl-esterified pectins. On pectins with a lower degree of methyl esterification and with a random distribution of the methyl ester groups, lower pH optima were observed. In the presence of divalent cations such as Ca^{2+}, Sr^{2+} and Ba^{2+}, a new pH optimum is found at about 8.5.

Pectin lyases are common components of commercial pectinases, and are quite effective in solubilising HM-pectins, particularly in combination with cellobiohydrolase (CBH) and *endo*-xyloglucanase (Vincken *et al.* 1997).

33.4.2 *Rhamnogalacturonan-modifying enzymes*

These enzymes were only discovered during the past decade. They comprise two different types of depolymerising enzymes: one is a hydrolase splitting the α-galactopyranosyluronic acid-(1→2)-α-rhamnopyranosyl linkages in rhamnogalacturonan (RG hydrolase), while the other type is a lyase splitting the α-rhamnopyranosyl-(1→4)-α-galactopyranosyluronic acid linkage by *trans*-elimination (RG-lyase). In addition, two other enzymes were found specific for rhamnogalacturonan fragments: a rhamnogalacturonan rhamnohydrolase releasing rhamnosyl groups from their non-reducing end, and a rhamnogalacturonan galacturonohydrolase releasing galacturonosyl groups from the non-reducing end (Beldman *et al.* 1996; Mutter 1997). A specific β-galactosidase is able to release single-unit galactosyl residues from the rhamnogalacturonan backbone. A rhamnogalacturonan acetyl esterase was identified as an accessory enzyme for RG hydrolase and RG lyase. The enzyme specifically removes acetyl groups at both O-2 and O-3 from galactosyluronic acid residues in the rhamnogalacturonan backbone. De-acetylation is an essential first step in the degradation of rhamnogalacturonans by RG hydrolase or RG lyase. All these enzymes were obtained from an *Aspergillus aculeatus* preparation, but they have also been found in *Aspergillus niger*, *Irpex lacteus*, *Trametes sanguinea* and *Botrytis cenera* (Mutter 1997 and references therein). There is growing evidence that rhamnogalacturonan hydrolase also occurs in tomatoes (Gross *et al.* 1995). All RG degrading enzymes have a pH optimum around 5–6.

Rhamnogalacturonan modifying enzymes are of technological importance due to their contribution to the solubilisation and fragmentation of the *hairy* regions of pectins and thus facilitating fruit juice extraction and fruit juice ultrafiltration.

Recently, another new type of enzyme, *xylogalacturonan hydrolase*, has been isolated from *Aspergillus tubigensis* active on xylogalacturonan in an *endo* fashion (Van der Vlught-Bergmans *et al.* 2000).

33.4.3 *Arabinan-degrading enzymes*

As different types of side chains, rich in arabinose and/or galactose, are important constituents of pectins, enzymes that are active towards these polymers will also be discussed. The following classes of enzymes able to release monomeric and/or oligomeric arabinose being active towards arabinans and arabinogalactans can be distinguished: α-L-arabinofuranosidase (EC 3.2.1.55), only active towards oligomeric arabinans and *p*-nitrophenyl-α-L-arabinofuranoside; α-L-arabinofuranosidase active towards polymeric and oligomeric arabinans; *exo*-α-L-arabinanase active towards polymeric and oligomeric arabinans, but not active on *p*-nitrophenyl-α-L-arabinofuranoside, and: *endo*-1→5-α-L-arabinanase (EC 3.2.1.99) and β-L-arabinopyranosidase. These arabinan-degrading enzymes are produced by microorganisms. Arabinofuranosidase active towards polymeric and oligomeric arabinan (fragments) have also been found in carrots, soybean and spinach (Beldman *et al.* 1997 and references herein).

33.4.4 *Galactan-degrading enzymes*

Galactan-degrading enzymes comprise *endo*- and *exo*-1→4-β-D-galactanases, and β-galactosidase able to release β-1→6-linked-D-galactosyl side groups and β-1→4-linked-D-galactosyl residues at the non-reducing end of a galactan backbone. Galactanases active on type II arabinogalactans comprise *endo*- and *exo*-1→3-β-D-galactanases and *endo*-1→6-β-D-galactanases. All these enzymes are produced by a variety of microorganisms, and β-galactosidase and *exo*-1→4-β-D-galactanases have also been found in plants.

Activity of all the enzymes described above results in increased solubilisation of pectins from the cell walls and their further fragmentation, changing their nature from polymeric dietary fibre to oligomeric dietary fibre.

33.5 Analysis of pectins

33.5.1 *Pectin as an ingredient*

The most important chemical characteristics of pectin preparations used as ingredient in food manufacture are the galacturonic acid and neutral sugar content and composition, the degree of methyl esterification (DM), acetyl esterification (DA) and amidation (DAm), and the protein- and ash content. For some pectins (beet) the feruloyl content is also of relevance. Other important characteristics are the molecular weight (Mw) and gelling power (for details, see Voragen *et al.* 1995 and references therein). For a better understanding of pectin interactions with divalent cations or with proteins, extensive research in recent years has been devoted to establishing the distribution of methyl ester groups along the homogalacturonan backbone. The availability of pure pectin depolymerising enzymes (*endo*-PG and *endo*-PL), with known substrate specificity and mode of action, enables us to produce characteristic oligouronides whose structures and relative amounts reflect structural differences of the pectin under investigation. The developments in high-performance anion-exchange chromatography (HPAEC) method at pH 5, together with Matrix-assisted laser desorption/ionisation Time of flight Mass Spectrometry (Maldi T of MS) enabled us to separate and identify the various non- and partially methyl-esterified galacturonic acid oligomers produced after *endo*-PG degradation of pectin (Daas *et al.* 1998). A schematic representation of the degradation of structurally different pectins is shown in Fig. 33.5. The absolute amount of non-esterified mono-, di- and tri-galacturonic acid produced after extended *endo*-PG degradation, related to the total number of non-esterified galacturonic acid residues, is an indicator of the occurrence of sequences of non-esterified galacturonic acid residues (so-called blocks) in pectin, and is introduced as 'Degree of Blockiness' (Daas *et al.* 1999, 2000a). From random methyl-esterified pectins, *endo*-PG liberated the lowest amounts of non-esterified galacturonic acid, even when the DM was relatively low. Information on the length and distribution of blocks of non-esterified galacturonic acid residues along the backbone can be derived from the ratio between non-methyl-esterified oligogalacturonides and (partially) methyl-esterified oligomers (Daas *et al.* 2000 a, b). Computational techniques commonly employed in the determination of the sequence similarity of DNA and proteins were used to discriminate the various types of distributions found and to construct a distance tree and to classify the various pectin preparations (Daas *et al.* 2000a).

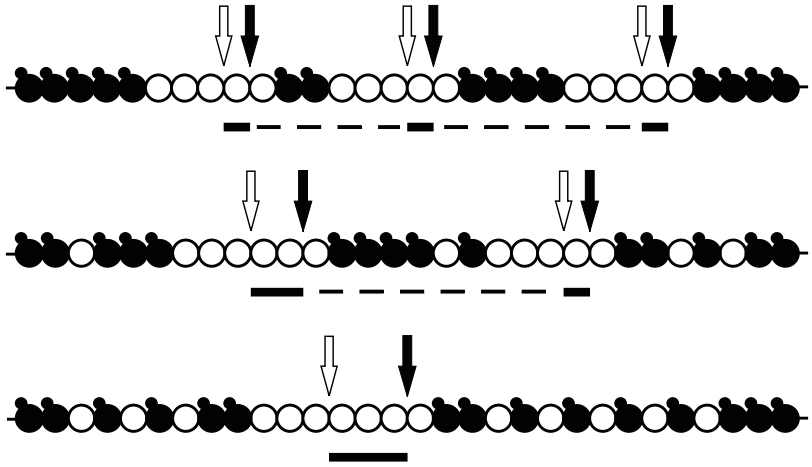

Fig. 33.5 Schematic representation of endo-PG action on three DM 50 pectins with different methyl ester distributions. Methyl- and non-esterified GalA are represented by black and open circles. White and black arrows indicate the first and second linkage split, respectively. The non- and methyl-esterified GalA molecules released are indicated with solid and dashed underscores. It is assumed that endo-PG needs four adjacent non-esterified GalA residues to act. (From Daas *et al*. 2000a.)

33.5.2 *Pectin as a constituent of dietary fibre*

In the analysis of dietary fibre, pectins are analysed in soluble and insoluble fractions as galacturonic acid by colorimetric or HPLC measurement after solubilisation and hydrolysis to galacturonic acid with sulphuric acid or by decarboxylation (Southgate 1995). The fruits and vegetables which are especially rich in pectins have dietary fibre contents in the range of 1–2% (Englyst *et al.* 1988; Southgate 1995). In order to increase our intake in fibre it is therefore preferable to add products concentrated in fibres. Numerous studies have been performed on sugarbeet pulp, apple pomace and citrus peels, which are the raw materials for pectin extraction. Pea hull fibre has also been studied in some detail. Table 33.3 shows the composition of such products. Their high content in galacturonic acid (20–26% for sugarbeet, apple pomace or citrus peels and ~13% for pea hulls) indicates a high content of pectin. The pectins are in general highly methyl-esterified. These fibres have a much higher water-holding capacity than do cereal fibres, this being due to the presence of highly hydrophilic substances, particularly pectins in fruit and vegetable fibres. These properties may have important physiological effects (Voragen *et al.* 1995; Thibault *et al.*, in Chapter 32, this book).

33.5.3 *Pectins as constituents of plant cell walls*

For the purpose of following pectic changes during growth, ripening, storage and processing, and for conducting structural studies on pectins from plant cell walls, various fractional extraction procedures have been developed. These procedures usually start with a clean-up step of the pectin source to inactivate endogenous enzymes and to remove interfering compounds such as sugars, amino acids, organic acids, starch, proteins, nucleic acids, polyphenols, etc. From the remaining purified cell-wall material, pectin fractions anchored in various ways in

Table 33.3 Chemical composition (w/w, percentage) of some pectin-rich dietary fibres.

Component	Sugarbeet pulp	Apple pomace	Citrus peels	Pea hulls
Rhamnose + Fucose	1.1	1.5	1.3	0.9
Arabinose	17.3	8.0	6.4	4.2
Xylose	1.5	5.5	2.4	14.6
Mannose	1.5	1.8	2.2	1.0
Galactose	4.3	5.0	3.2	1.2
Glc	21.7	27.9	19.6	45.1
Galacturonic acid	18.9	25.2	26.0	12.7
MeOH	2.3	2.2	ND	0.5
AcOH	3.6	2.0	ND	1.0
'Proteins'	8.0	5.7	ND	3.8
'Lignin'	1.8	ND	ND	ND
Ash	8.4	2.0	ND	1.7

ND, not determined.
Source: Voragen *et al*. (1995).

the cell-wall 'archi-structure' are sequentially extracted under conditions which correspond to the removal of specific types of linkages (Selvendran & Ryden 1990; Voragen *et al.* 1995; Fig. 33.6). The extracts obtained are then fractionated to homogeneity using size-exclusion and/or anion-exchange chromatography. For structure elucidation of such homogeneous fractions, sugar and glycosidic linkage composition and anomeric configuration of sugar residues are established. As illustrated above, strategies for establishing the fine structure further include fragmentation with pure, well-defined and highly specific enzymes, or with less-specific chemical reactions and fractionation to homogeneity of the fragments in the digests (Lau *et al.* 1988; Voragen *et al.* 1993). These fragments often fit within the analytical range of advanced NMR and mass spectroscopic techniques, and these techniques allow the establishment of the absolute structure, including the presence and distribution of substituents. With the information obtained from such approaches, the tentative structures of the structural elements of pectic substances, as described in Fig. 33.1 and Table 33.2 have been elucidated.

33.5.4 *Distribution of pectins in plant tissues*

The *in-vitro* data from chemical extraction and structure elucidation studies can be complemented by mapping the distribution of pectic epitopes in plant tissue using monoclonal antibodies (mAbs). Several anti-pectin antibodies are now available (Fig. 33.7) and are well described by Willats *et al.* (2000). PAM1 and 2F4 respond against homogalacturonans, the former recognising 30 contiguous de-esterified galacturonosyl uronic acid residues, and the latter Ca^{2+}-linked dimers of homogalacturonans. JIM5 and JIM7 both recognise homogalacturonans with a certain extent of methyl esterification; JIM5 shows increasing binding up to a level of 40% esterification, and JIM7 binds over a range of DM from about 30 to 80%. They tend to label the entire width of cell walls (Knox *et al.* 1990). CCRC-M2 labels the RG-I backbone, while LM 5 and LM 6 recognise pectic side chains, galactans and arabinans, respectively. Recently, an mAb specific for pea xylogalacturonans was also described (Willats *et al.* 2000). Using antibodies, Bush and McCann (1999) were able to characterise

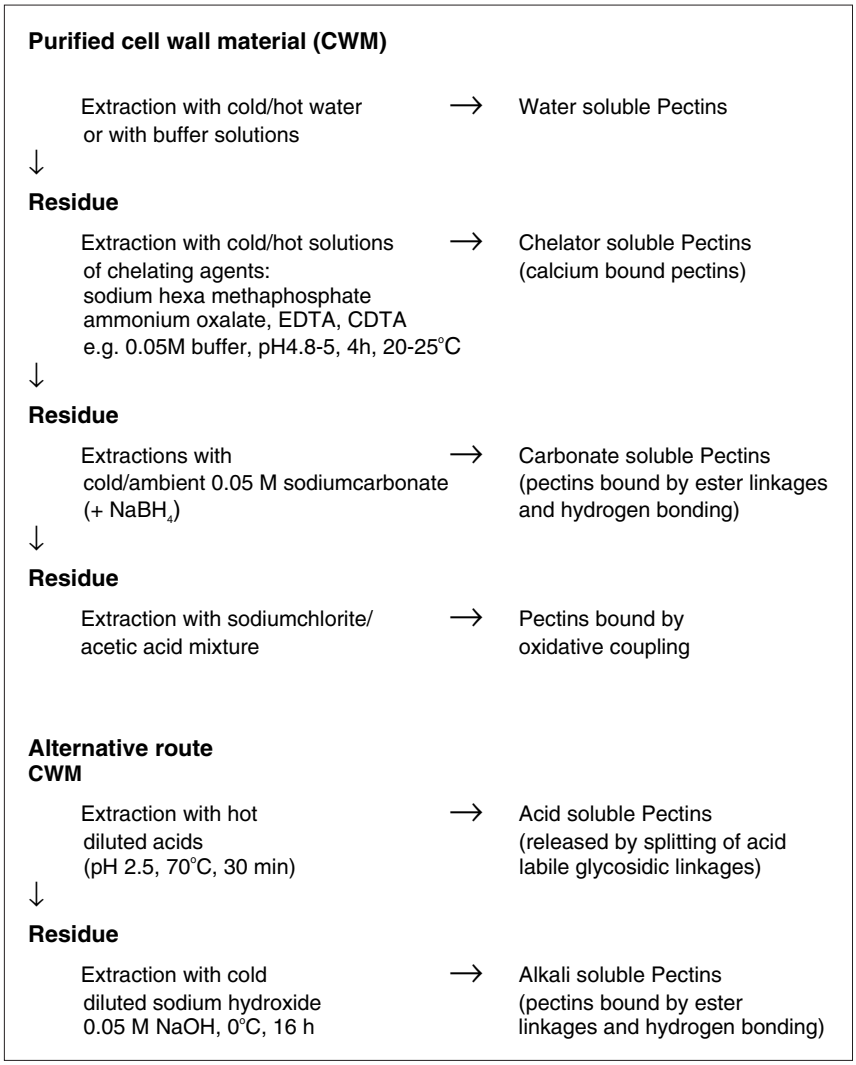

Fig. 33.6 Scheme for laboratory extraction of pectins. (From Voragen *et al*. 1995.)

the distribution of the various structural elements of pectic substances in the cell walls of potato tuber tissues.

33.5.5 *Pectins having prebiotic functions*

Commercial pectins (homogalacturonans) are fermented in the colon with formation of short-chain fatty acids. It was shown that that non-methyl-esterified pectins were more rapidly fermented than methyl-esterified pectins (Dongowski & Lorenz 1998). Evidence was presented indicating that pectate-lyases play an important role in the depolymerisation of the pectins. The authors concluded that unsaturated oligogalacturonic acids might be involved

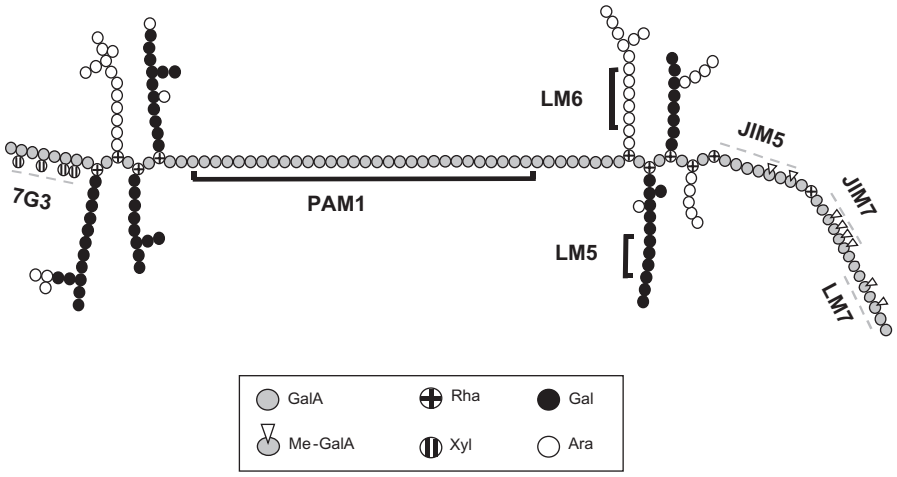

Fig. 33.7 Monoclonal antibodies recognising pectic epitopes. (See also Willets *et al*. 2000.)

in many different physiological processes of which the mechanism and importance is not yet understood (Dongowski & Lorenz 1998).

In a recent study on the *in-vitro* fermentation of various plant cell wall-derived poly- and oligosaccharides by selected bacterial strains isolated from human and porcine faeces, it was also found that homogalacturonan-degrading enzymes are predominantly produced by *Clostridia* spp. (Table 33.4; Van Laere 2000; Van Laere *et al.* 2000). Among others, the substrates used represented structural elements of pectic substances and included an arabinogalactan (type I) -enriched preparation (AGPS) from soy and (arabino)galacto-oligosaccharides (AGOS), an arabinan-enriched preparation (APS) from sugarbeet and arabino-oligosaccharides (AOS), a rhamnogalacturonan-enriched preparation (RGAPS) from apple and rhamnogalacturono-oligosaccharides (RGAOS), and galacturono-oligosaccharides (GAOS). After anaerobic fermentation for 48 h, the amount of residual soluble polysaccharide or oligosaccharides was measured with high-performance size exclusion (HPSEC) or anion exchange chromatography (HPAEC). The degree of fermentation of the polymers was estimated by HPSEC from the shift in the molecular weight, or from the reduced amount of polymer left in the supernatant after fermentation. The presence of monomeric and oligomeric material after fermentation of the polymer was analysed by HPAEC. The fermentation of the oligosaccharides was monitored by HPAEC from the shifts in the elution patterns. Results are summarised in Table 33.4. It can be seen that within the group of *Bacteroides*, the individual species tested fermented all or most of the substrates to some extent, showing that *Bacteroides* spp. have a wide variety of glycanases and glycosidases. The rhamnogalacturono-oligosaccharides were the only type of oligosaccharides that were selectively fermented by them.

Clostridium spp. were also able to ferment most of the substrates to some extent, although they showed low activity toward the highly branched rhamnogalacturonans. The fermentation capabilities of intestinal *Clostridium* spp. are not often reported, as these bacteria are

Table 33.4 Fermentative degradation of various polysaccharides and oligosaccharides by several bacteria as determined with HPSEC or HPAEC.

		Arabinogalactan		Arabinan		(rhamno)galacturonan		
Bacteria	Origin	AGPS	AGOS	APS	AOS	RGAPS	RGAOS	GAOS
	Blank							
Bifido breve	ATCC 15700	±	+	–	±	–	–	ND
Bi. longum	ATCC 15707	±	+	±	+	–	–	–
Bi. infantis	ATCC 15697	–	+	–	–	–	–	–
Bi. adolescentis	ATCC 15703	±	+	±	±	–	–	–
Clostridia beijerinckii	Human faeces	–	ND	±	±	–	–	–
C. clostridiiforme	Human faeces	±	±	±	+	–	–	+
C. ramosum	Human faeces	–	+	±	–	±	–	+
C. sporogenes	Human faeces	–	–	–	–	–	–	–
C. sartagoformum	Human faeces	±	–	–	±	–	–	–
C. perfringens	Human faeces	–	–	–	–	–	–	–
Bacteroides vulgatus	ATCC 8482	±	±	+	±	±	+	–
B. ovatus	ATCC 8483	±	+	–	–	+	+	+
B. thetaiotaomicron	ATCC 29741	±	+	+	–	±	–	–
Lactobacillus casei	Yakult	–	±	–	–	–	–	–
L. acidophilus	Swine faeces	±	+	–	–	–	–	–
L. fermentum	Swine faeces	±	+	–	–	–	–	–
E. coli	Human faeces	±	–	–	±	–	–	–
Klyveromyces pneumoniae	Human faeces	±	+	–	–	–	–	–

AGPS, arabinogalactan-enriched polysaccharide fraction; AGOS, (arabino)galacto-oligosaccharides; APS, arabinan-enriched polysaccharide fraction; AOS, arabino-oligosaccharides; RGPS, rhamnogalacturonan-enriched polysaccharide fraction; RGAOS, rhamnogalacturono-oligosaccharides; GAOS, galacturono-oligosaccharides; AXWPS, arabinoxylan polysaccharide from wheat flour; AXSPS, glucuronoarabinoxylan polysaccharide from sorghum; AXOS, arabinoxylo-oligosaccharides; XOS, xylo-oligosaccharides; FOS, fructo-oligosaccharides; +, complete degradation; ±, partial degradation; –, no degradation; ND, not detected.
Source: Van Laere *et al*. (2000).

normally not dominant in the colon. Their saccharolytic activity should however not be underestimated. It was also shown that individual species belonging to *Bifidobacteria* are able to ferment (arabino)galacto-oligosaccharides and arabino-oligosaccharides, but are not able to utilise rhamno- and galacturono-oligosaccharides. The *Lactobacillus* spp. only fermented polymeric and oligomeric type I arabinogalactans. These results do not allow predictions to be made about the fermentation *in vivo*, since this will depend on various factors such as the availability of other substrates, growth factors, intestinal pH, actual number of bacteria, and the interactions between the different species of bacteria present. They do show that the selected bacterial strains behave quite differently to the various structural elements of pectins. Thus, depending on their composition and structure, the fermentability and utilisation of pectins can differ greatly (Van Laere 2000).

33.6 Concluding remarks

Pectins form a very complex group of heteropolysaccharides comprising principally two families of covalently interlinked acidic polymers: (1) linear homogalacturonans; and (2) highly ramified rhamnogalacturonans. To understand the important effects of pectins on the quality of fresh and processed fruit and vegetable products, on the processing of plant produce, on the use of pectin as ingredient in food manufacture, and on their functions as a dietary fibre component and their effect on health, a detailed knowledge of their chemical structures is required. This includes a knowledge of their structure as part of plant cell walls or in extracted form, of the distribution of the structural elements in the cell walls of plant tissues and the chemical, physical or biochemical changes that they undergo during ripening or processing. The importance of this knowledge has not been sufficiently realised in nutrition science. Analysis methods must therefore be adapted to allow measurement and evaluation of these specific functions.

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Part 10

Resistant Starch

34 Resistant Starch: Plant Breeding, Applications Development and Commercial Use

Ian L. Brown, Ken J. McNaught, David Andrews and Tatsuya Morita

34.1 Introduction

Starch, due to its relative abundance in plants, is the primary source of energy in the human diet (Guilbot & Mercier 1985). However, the importance of starch to other areas of nutrition and health has only become apparent during the past two decades. Meta-analysis has suggested that the consumption of low levels of dietary starch is associated with detrimental physiological conditions, such as colorectal cancer (Cassidy *et al*. 1994). One important reason for the health benefits that appear to be derived from starch consumption stems from the ability of a portion of dietary starch to resist enzymatic digestion in the small intestine (Englyst *et al*. 1992) and make a positive impact on the health of the large bowel. The term 'resistant starch' (RS) has been used to describe these starches (Englyst *et al*. 1992), which act like dietary fibre. There are at least four subtypes of RS (Table 34.1) (Brown *et al*. 1995) which reflect the different ways in which starch may escape digestion in the upper regions of the gastrointestinal tract.

Resistant starch is a component already found in starchy foods consumed by people around the world (Crawford 1987; Ranhotra *et al*. 1999). It has been estimated that an individual's daily intake of RS in European countries is 3–6 g (Dysseler & Hoffem 1994), while in Australia it is approximately 5–7 g (Baghurst *et al*. 1996). This estimation indicates that about 5–10% of ingested starch may normally resist digestion and enter the colon (Baghurst *et al*. 1996). However, it has been suggested that approximately 20 g of RS would be required

Table 34.1 Nutritional classification of starch.

Type of starch	Example of occurrence	Probable digestion in the small intestine
Rapidly digestible	Freshly cooked starchy food	Rapid
Slowly digestible	Most raw cereals	Slowly, but completely
Resistant starch		
RS_1 Physically inaccessible	Partly milled grains and seeds	Resistant
RS_2 Resistant granules	Raw potato, green banana and High-amylose maize starch	Resistant
RS_3 Retrograded starch	Cooked and cooled starchy foods, bread and cornflakes	Resistant
RS_4 Chemically modified starch	Starch ethers, starch esters and crossbonded starches.	Resistant

in humans to obtain positive physiological benefits (Baghurst *et al*. 1996). The limitation in utilizing RS as an ingredient in foods has stemmed from the difficulty in finding sources that perform functionally in foods and that retain their physiological effects after food processing. The efforts of the initial research groups in this area culminated in the EURESTA (European Flair-Concerted Action on the 'Physiological implication of the consumption of resistant starch in man' from 1990 to 1994) programme in Europe. This programme involved 36 groups from 10 countries, and provided direction and research data in the areas of: (1) the definition and analysis of RS; (2) the technology of RS production; (3) the physiological effects of RS in the upper and lower gastrointestinal tract; and (4) the energy contribution of RS. The work undertaken in this programme provided a valuable reference for research that was being undertaken in Australia.

34.2 Resistant starch research in Australia

During the past decade, a detailed research and development programme has been underway in Australia involving Starch Australasia Limited and six research organisations, including the CSIRO Division of Health Sciences and Nutrition, to learn more about the physiological effects of starches and to find ways of utilizing these positive effects in foods to improve public health. The first stage of the programme was the identification of suitable sources of RS for investigation. The systematic examination of the dietary fibre and resistant starch content of existing and experimental Australian maize varieties (Brown 1993) identified that, as the amylose content of maize starch increases, so does the resistance of the starch granules to amylase digestion (Figs. 34.1 and 34.2). Maize varieties homozygous for the amylose extender or 'ae' gene, with amylose levels between 50% and 80%, have been commercially available since the 1950s (Zuber & Darrah 1987). These so-called 'amylomaize' starches have very high gelatinisation temperatures (154–171°C) (Doublier & Chopin 1989) compared with the other types of RS2 starches (normally <70°C) (Snyder 1984), which means that the RS can be preserved when the amylomaize starches are used in prepared foods. When classical maize hybrids were produced with more than 80% amylose, the starch granules proved to be even more resistant to amylolysis, when measured as resistant starch and dietary fibre. The level of dietary fibre and RS in the +80% amylomaize starches was of suf-

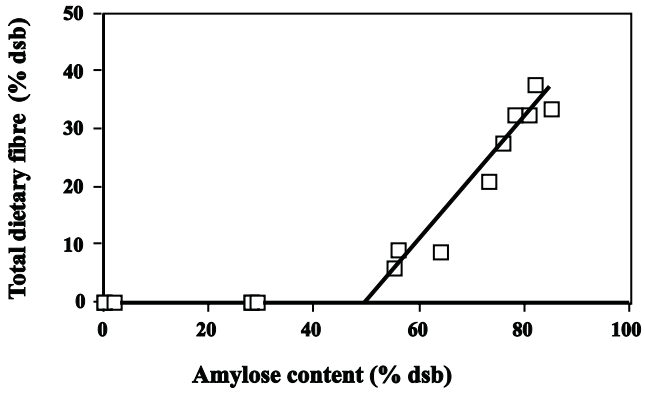

Fig. 34.1 Dietary fibre content of high-amylose maize starch.

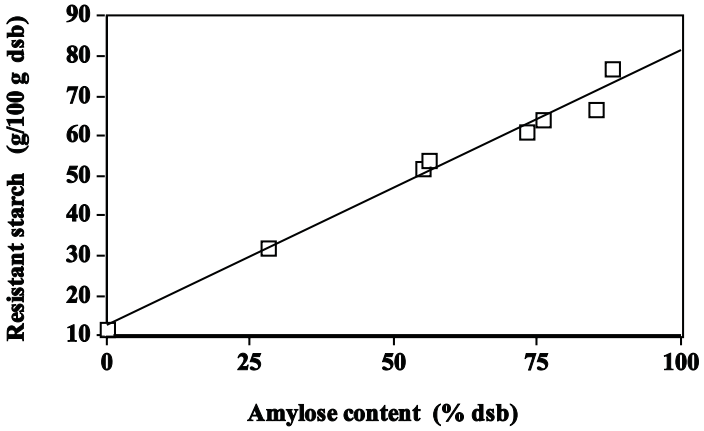

Fig. 34.2 Resistant starch content of high-amylose maize starch.

ficient magnitude to warrant its use as a source of dietary fibre and RS in foods (Brown *et al*. 1995).

The starch obtained from a selected +80% amylose amylomaize variety, which had good agronomic and yield properties, was clinically evaluated and assessed for its contribution to food functionality (Brown *et al*. 1995). This starch was commercially released in 1993 under the trademark Hi-maize™. It was the first commercially available resistant starch ingredient.

The dietary fibre level of the amylomaize starch was determined using the AOAC enzymatic-gravimetric technique (AOAC Method 985.29), as this method is generally accepted for nutrition labelling purposes in most countries. Originally, Hi-maize™ was released with a dietary fibre content of 30% on a dry solids basis, but the range of resistant starch ingredients has now expanded to include variants containing in excess of 60% dietary fibre. Since 1993 a number of other resistant starch ingredients have become commercially available, including RS_3 non-granular recrystallised starch-derived material, such as Novelose® and Crystalean®, and more recently, enhanced dietary fibre granular starches (RS_2) have been made available in Australia, Japan, Europe and the USA.

Although there are internationally accepted methods for determining dietary fibre in food there is currently no universally accepted method for determining the RS content of foods, despite the work undertaken before (Berry 1986) and during EURESTA (Champ 1992; Englyst *et al*. 1992). The difficulty in developing a method arises from the potential for ingredient preparation, food processing and cooking conditions, food storage and an individual's method of food consumption to influence how much RS is physiologically available (Annison & Topping 1994). A number of methods have been suggested that attempt to mimic the physiological availability of starch (Muir & O'Dea 1993a). These include the use of chewing (Muir & O'Dea 1992) to include the effect of mastication on starch digestibility. It is hoped that a simple, reproducible method can be agreed upon in the near future.

34.3 Physiological effects of resistant starch

The nutritional research undertaken to examine the physiological effects of RS by researchers around the world has been summarised in numerous reviews (Muir *et al*. 1993; Annison & Topping 1994; Baghurst *et al*. 1996; Topping & Clifton 2000). This work has identified the potential of RS to have a positive impact on public health. During the past decade, studies conducted in Australia have complemented research that has been undertaken in other countries, and has highlighted a broad range of potentially beneficial physiological properties for RS (Table 34.2).

The physiological effects arising from the consumption of RS occur in the small intestine, through the reduction of both the postprandial glycaemic and insulinaemic responses. This is advantageous for people with impaired insulin sensitivity (Byrnes *et al*. 1995; Noakes *et al*. 1996). Liversey (1994) has indicated that the energy value of completely fermentable RS is estimated to be 8 kJ (2 kcal) per gram compared with 17.5 kJ (4.2 kcal) per gram for completely digested starch. The partial digestibility of commercial RS preparations, containing either 32% RS or 40% RS, has been calculated to be 2.8 and 2.4 kcal/g, respectively (Ranhotra *et al*. 1999). This indicates that RS can reduce the calorific value of foods.

In the large bowel, resistant starch is predominantly fermented by the resident microflora to provide elevated levels of short-chain fatty acids (Phillips *et al*. 1995; Noakes *et al*. 1996; Topping *et al*. 1997), in particular butyrate, and other products which are believed to contribute to bowel health. There is also a marked reduction in the levels of some cytotoxic compounds, such as secondary bile acids (Noakes *et al*. 1996; Topping *et al*. 1997), in the large bowel. It has become evident that the type of resistant starch can also have a significant effect on the site, rate and products of bacterial fermentation within the large bowel (Bird *et al*. 1999).

Considerable interest has been generated from the observation that resistant starch can selectively encourage the growth of both indigenous (Wang *et al*. 1999a) and introduced probiotic bacteria (Brown *et al*. 1998b), in a variety of ways, including the rate of bacterial growth and the type and quantity of fermentation products produced.

Some new applications of resistant starch are beginning to emerge, including its use in an Australian registered bulk laxative pharmaceutical product, and as an adjuvant in

Table 34.2 Nutritional properties of resistant starch.

- Source of dietary fibre
- Lowers glucose response
- Lowers insulin response
- Lowers available calories
- Promotes bowel health
- Escapes digestion in the small intestine
- Fermentable substrate for some bowel microflora, including *Bifidobacterium*.
- Acts as a functional prebiotic for some probiotic microorganisms
- Increases the production of short-chain fatty acids in the large bowel, including acetate, propionate and particularly butyrate
- Increases faecal output, and reduces faecal transit time
- Lowers the levels of secondary bile acids in the large bowel
- Source of dietary fibre for coeliac sufferers

oral rehydration therapy for the treatment of bacterially induced diarrhoea, such as cholera (Ramakrishna *et al*. 2000).

As our knowledge of the effects that individual materials arriving in the large bowel can have on our health increases, it is important to note the differences in their functional and physiological performance. Hayakawa (1998) has suggested a simplified comparison of the relative effects of various fibre types (Table 34.3). These general categories, based on the physiochemical properties of the materials, indicate that various fibres can provide desirable and complementary effects. This observation will have increasing relevance as we explore opportunities to improve specific public health concerns, such as diabetes, irritable bowel syndrome and colorectal cancer.

34.4 Use of resistant starch in food

Although RS is normally formed to some degree in starchy foods when they are cooked (Siljestrom & Asp 1985; Rabe & Sievert 1992; Liljeberg *et al*. 1996), the availability of RS, in the form of granular +80% amylose maize starches, provided the opportunity to evaluate these ingredients for use in the elevation of dietary fibre and RS in a wide range of food applications. The results of these investigations indicated that they were particularly useful in providing a natural white source of dietary fibre, and that RS could be added to foods such as bread, buns, breakfast cereals, extruded foods and snacks, pasta, biscuits and yoghurt without adversely affecting their taste or texture. The comparatively low level of water absorption of RS compared with other fibres means that they have less effect on the structure of food matrices, so providing high-fibre foods with greater organoleptic appeal to consumers. The potential contributions of resistant starch to food functionality are listed in Table 34.4 (Brown *et al*. 1995).

The identification of a RS suitable for use as an ingredient in foods, such as the +80% amylose maize starch, has permitted experiments to be conducted to gauge the extent of its suitability for use in general foods and those designed to meet the needs of individuals with special dietary concerns. The suggestion that people consuming a 'Western' diet should increase the amount of resistant starch that they eat by two- to three-fold in order to receive positive physiological benefits (Baghurst *et al*. 1996) would indicate that an appropriate ve-

Table 34.3 Comparison of some large bowel physiological effects of resistant starch with soluble and insoluble dietary fibre.

Nutritional property	Resistant starch	Soluble fibre	Insoluble fibre
Water insolubility	+	–	+
Fermentability	+++	+++	–
Short-chain fatty acid production	+++	+++	–
Increased butyrate production	+++	++	–
Reduced caecal pH	+++	+++	–
Increased faecal moisture	++	++	+
Increased faecal mass (dry)	+++	+	+++
Reduced faecal transit time	++	–	+++
Reduced plasma lipids	++	++	+

+, indicates the degree of agreement

Table 34.4 Functional properties of +80% amylose maize starch.

• Natural
• Source of dietary fibre and resistant starch
• White
• Fine particle size (<10 μm average particle size)
• High gelatinisation temperature
• Low water absorption compared with other dietary fibres
• Extrudes well with excellent film-forming properties
• Can give low bulk density high-fibre products
• Improves coating crispness
• Improves the bowl-life of breakfast cereals
• Provides an opportunity to make innovative foods
• Possible nutritional benefits

hicle for fortification of the diet with resistant starch is in staple foods, such as bread, breakfast cereals, pasta and noodles. The acceptance of RS being included in such foods is dependent on the RS surviving food processing and consumers not being able to detect the presence of the RS in the food. It is well known, that for many people their preference in the choice of foods is predominantly influenced by the organoleptic properties of the food, but with nutritional concerns having a major secondary influence.

34.4.1 *Resistant starch in bread and baked foods*

One of the major staple foods consumed globally is bread. Although fibre-rich wholemeal and multigrain breads are available, many people still prefer to consume white bread, due mainly to its superior palatability. The development of high-fibre white breads has been pursued with vigour during the past decade. The focus of this research has been the development of white bread where the increased fibre content does not adversely affect the organoleptic qualities of the bread. This condition is particularly important, since the consumers of white bread react negatively to changes in texture, colour and taste caused by the addition of fibre. The inclusion of +80% amylose maize starch, and more recently, enhanced dietary fibre granular starches as a source of dietary fibre, has addressed these issues and produced white bread that has achieved a high level of consumer acceptance. The preparation of breads using the rapid dough technique indicated that the level of dietary fibre could be increased in a controlled manner, even in the presence of high levels of sugar or α-amylase (Brown *et al*. 2000). The quality of the breads prepared using +80% amylose maize starch (Brown *et al*. 1995) and enhanced dietary fibre granular starch (Yue & Waring 1998) was excellent in terms of crumb structure and texture. The low level of water absorption by RS compared with other sources of fibre (Ranhotra *et al.* 1999) also lessened the degree of disruption to the bread matrix and provided a structure suitable for freezing and subsequent later use. The loaf volume, crumb colour, texture and other quality parameters of the white bread were not compromised, and in many cases were improved, when RS was used to increase the dietary fibre level (Kiriyama 1996; Yue & Waring 1998).

The inclusion of RS in foods can beneficially alter their nutritional parameters. For example, since +80% amylose maize starch is only partially digested in the small intestine, it can slow the rate at which glucose is released into the blood stream when compared with foods

that contain rapidly digested starch. Significant reductions in glycaemic index (GI) was reported for people consuming white breads containing 10% and 20% Hi-maize™ (based on replacement of flour), compared with normal white bread, when an equal quantity of bread was consumed (Muir *et al*. 1994). The GI was reduced from 100 for white bread to 74 and 53 respectively, for the RS-fortified breads.

The first significant use of RS in white bread occurred in Australia in 1994 when Quality Bakers Australia included Hi-maize™ in Wonder White® bread. Health authorities recommend that Australians should consume more dietary fibre, and that they should also increase their intake of bread. However, the problem is that some people – particularly children – refrain from consuming the high-fibre, multi-grain and wholemeal breads because of their preference for white bread. With this in mind, Quality Bakers Australia formulated a high-fibre white bread, incorporating Hi-maize™, that contained 5.6% dietary fibre on an as-consumed basis, compared with 2.9% for conventional white bread (Brown *et al*. 1995). Other types of dietary fibre have always coloured the bread, changed the texture by making it more fibrous or chewy, decreased freezing and toasting quality, or reduced the softness and volume of the loaf. For the first time, Hi-maize™ has allowed the preparation of a soft, high-fibre, white bread, with excellent keeping qualities, to be made available for the consumption and enjoyment of consumers. The fact that Hi-maize™ can be invisibly incorporated into the bread matrix, so that it not detectable by either children or an adult is a major benefit. Consumers have enthusiastically accepted Wonder White®, and this was demonstrated when it acquired a 12% share of the Australian packaged white bread market in the first 20 weeks of its release. During this time the size of the white bread market increased by some 8% (Brown *et al*. 1995). More importantly, Wonder White® encouraged more people to eat bread in accordance with dietary guidelines. Market share has continued to increase, and Wonder White® is now arguably the largest selling packaged white bread in Australia. The success of Wonder White® has led to the release of similar high-fibre white bread containing RS in New Zealand, called Natures Fresh Fibre White®.

The ability to increase the dietary fibre and RS content of foods without altering their taste or texture led to the decision in 1997, by McDonalds Australia to incorporate Hi-maize™ in all of their buns and muffins. Each McDonalds bun or breakfast muffin contains 3 g of dietary fibre, which is sufficient to allow for a 'good source of dietary fibre' claim to be made (Brown *et al*. 1998a).

RS can also have a positive textural effect on other types of baked foods, including biscuits, cookies, crackers, crispbreads, crumpets, muffins and low-fat cakes, while increasing their dietary fibre content (Yue & Waring 1998). Many of these products are now commercially available in Australia.

34.4.2 *Resistant starch and extruded foods*

A range of other foods has been made using RS for functional reasons as well as a source of dietary fibre. Breakfast cereals are prepared by a variety of techniques including extrusion. The versatility of RS in the preparation of breakfast cereals has been demonstrated through the inclusion of +80% amylose maize starch in a series of formulations that were processed by precooking, extrusion, flaking and toasting (Brown *et al*. 2000). The dietary fibre of the resultant breakfast cereals, depending on the formulation, was increased from 3.9% to 12.4% or from 4.0% to 20.7%. The conditions employed during extrusion can involve high tempera-

ture, pressure and shear forces. Research is underway to provide sources of RS that contribute higher levels of RS under these adverse conditions.

The inclusion of RS also provided a range of textural properties in extruded breakfast cereals that are not found through adding other fibres, which included increased expansion (Yue & Waring 1998), reduced bulk density, improved crispness and longer bowl life (Brown *et al*. 1995). This latter property permits the breakfast cereal to retain its crispness and shape for a longer period of time in the presence of milk. The multifunctional contribution of RS to extruded foods has led to the inclusion of RS, as Hi-maize™, in a number of breakfast cereals in Australia including the Uncle Toby's products called Healthwise™, Team Australia™ and Grinners™.

RS can be utilised to increase invisibly the dietary fibre content of noodles, including udon, soba and Chinese types (Kiriyama 1996) and pasta (Brown *et al*. 1995). Pasta containing up to 15% RS can be made with little or no effect on dough rheology during extrusion (Yue & Waring 1998). The impact of replacing a portion of the semolina by RS provided pasta with an even, light colour, which provided a firm 'al dente' texture when cooked for the same time taken as pasta without added fibre (Yue & Waring 1998). In Australia, Heinz has used RS, in the form of Hi-maize™, in its range of canned spaghetti and dried 'Kidz Pasta'. RS has also been included in extruded fruit snacks, such as Heinz 'Kidz Fruit Fingers'.

34.5 Resistant starch and probiotics

Prebiotics are non-digestible food ingredients that can stimulate the growth and activity of beneficial colonic bacteria and thus attempt to improve the health of the host (Gibson & Roberfroid 1995). It has been observed that RS – particularly high-amylose maize starch and its derivatives – can act as prebiotics (Brown *et al*. 1998b). Apart from being fermented and stimulating the growth of specific beneficial, or 'probiotic', indigenous colonic microorganisms (Wang *et al*. 1999a), including bifidobacteria (Brown *et al*. 1997), RS appears to be able to protect the viability of introduced probiotic bacteria in foods, such as yoghurt. This protection is afforded, both during the shelf-life of the product and during their passage through the upper regions of the gastrointestinal tract (Brown *et al*. 1998b; Wang *et al*. 1999b). The impact of fermentable prebiotic carbohydrates on human health have been proposed to include reduced risk of colon cancer, improved mineral absorption, reduced serum lipids and cholesterol, reduced infectious disease and an improved immune status and treatment of hepatic encephylothopy (Crittenden 1999). This broad range of potential effects is currently being studied. It has even been suggested that RS may have a positive impact on bacterially induced diseases found higher in the gastrointestinal tract, such as dental caries and stomach ulcers (Bird *et al*. 2000).

Opportunities now exist to incorporate RS ingredients as a prebiotic in foods, including yoghurt, ice cream and other dairy products, non-dairy and soy-based foods, confectionery, extruded foods, snacks and baked foods. As consumers become more educated about the beneficial effects of ingesting probiotic microorganisms, there will be an increasing need to maintain an acceptable number of viable bacteria at the time of ingestion of the food to ensure a physiological probiotic effect (Rohm *et al*. 1990). A number of countries have already set standards which establish the minimum requirements for the number of probiotic bacteria to be present in yoghurt at the time of consumer purchase (Ishibashi & Shimamura 1993; Lee & Salminen 1995). The use of industry codes of practice or government regulation will need to

be more extensively employed in order to provide some measure of guarantee to consumers. Insoluble prebiotics, such as resistant starch, appear to have an important role to play in the controlled provision of viable probiotic bacteria to the colon and the stimulation of both the beneficial, introduced and indigenous, probiotic bacteria in the colon.

34.6 Resistant starch and other applications

RS has been specifically developed to provide dietary fibre, resistant starch and functional properties in a wide range of commercial foods (Brown *et al*. 1995). Apart from those mentioned earlier, these include confectionery, snacks and beverages. Some of these products are already available in Australia, such as high-fibre fruit deposited gel confections, soy- and dairy-based yoghurts and soy-based breakfast drinks. In this last example the insoluble RS is suspended in the viscous opaque drink and thoroughly dispersed so that the small starch granule size does not cause a gritty mouthfeel.

In addition, it is anticipated that further applications for RS in the health food and pharmaceutical industry, in powder and tablet form (as a functional tableting base), will be become commercially available. The initial development has been the commercialisation by the Sigma Pharmaceutical Company in Australia of the registered bulk laxative product 'Nucolox' in 1998, which contains a proportion of RS. The versatility of resistant starch, in both a nutritional and a functional sense will see the range of food, pharmaceutical and animal feed applications continue to expand.

34.7 Commercialisation

The launch and development of RS ingredients, such as Hi-maize™, has been based on a detailed commercialisation programme that has a number of features, which can be illustrated through the example of Wonder White® bread. After it was identified that consumers required a high-fibre white bread, it was necessary to obtain a source of fibre that did not change the organoleptic qualities of white bread. Detailed evaluation in bread of the numerous types of dietary fibre revealed that only RS provided the desired nutritional and functional qualities. Next, the physiological properties of the resistant starch and the results of published clinical trials were presented to health professionals, along with the reasons for providing the high-fibre white bread. The presence of RS in the bread and its nutritional value was communicated to consumers through the use of a distinctive logo, prominently located on the product packaging and in the ingredient declaration. Continued clinical research, communication of research findings and consumer advertising of RS has further strengthened the brand name.

This commercialisation strategy has continued over the years in Australia, and there is now a general understanding of RS among health professionals and recognition of the Hi-maize® logo by a significant proportion of consumers. During this time, the number of foods and pharmaceuticals containing resistant starch ingredients has continued to expand. The successful use of RS ingredients in Australia has recently encouraged the introduction and promotion of these ingredients to health professionals and food manufacturers in other countries, where nutrition is an important consumer requirement.

34.8 Conclusions

The availability of RS starch ingredients, supported by clinical assessment and innovative food engineering, has combined to provide consumers with foods of greater nutritional quality, while meeting their demands for organoleptic acceptability. Nutritional research is continuing to identify new uses for RS ingredients, alone, or in combination with other dietary components, to address public health concerns. The key to gaining the widespread application of RS ingredients in foods, pharmaceuticals and animal feeds is through the identification of public health concerns, targeted clinical research, the dissemination of clinical results, the development of appropriate foods and other products, identification and communication of the relevance of these foods to consumers and finally, the continued monitoring of the public health issue that is being addressed. RS in its many forms has the potential to play a significant role in improving a number of public health issues in the years ahead. The added benefit has been that RS can be incorporated into a broad range of foods, while maintaining their high organoleptic quality.

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35 *In-vivo* and *In-vitro* Digestion of Resistant Starch

Stephen G. Haralampu

35.1 Introduction

The recognition of resistant starch (RS) has stirred considerable controversy during the past decade, in topics ranging from how it impacts on total dietary fibre (TDF) assays to how it behaves physiologically. It has been defined in physiological terms (Champ 1992) as, 'the fraction not digested in the small intestine.' RS differs from other components of dietary fibre in that it is a physical state of an otherwise highly digestible carbohydrate polymer. RS has been classified into three major groups (Englyst *et al*. 1992): (1) RS_1, being physically entrapped and unavailable for digestion, such as unmilled grains; (2) RS_2, being the raw, ungelatinised starch granules; and (3) RS_3, being retrograded starch (RS_4 represents chemically modified starches, which are rendered not digestible due to the modification). RS_3 is particularly interesting, because it can be manipulated for enhanced properties such as thermal stability, and function as a food ingredient. The structure of RS_3 has been identified as predominantly retrograded amylose (Berry 1986).

Since the resistance of starch to normal digestion is derived from particular physical states, an understanding of how these states form and their properties is crucial in understanding the fate of RS physiologically and in assays. There is no standardised analytical definition for RS. Consequently, direct comparisons between RS studies are not always possible. In particular, the temperature of an RS assay can have a tremendous effect on the measured value.

35.2 Resistant starch structures

The important structures comprising RS are the ordered structure of the raw granule (RS_2) and retrograded starch (RS_3). In both cases, the starch molecules order in helical structures with limited available water. Galant *et al.* (1992) reviewed specific details of the native starch granule structure, and the molecular arrangement of the enzyme-resistant regions. In contrast, fully hydrated starch molecules are completely amorphous, and are quickly digested.

The most thermally stable resistant starch structure is retrograded amylose. Retrograded amylose is comprised of a double-helical structure (Wu & Sarko 1978; Imberty & Pérez 1988). The helices are capable of packing together to form small crystallites. A simple model for the retrograded amylose structure is that of the fringed micelle, i.e. portions of amylose chains involved in the ordered, helical bundles, fringed by amorphous regions (Morris 1990). Resistance is presumed to be imparted by the ordered regions hindering amylase attack.

Typically, starch hydrates at temperatures ranging from 40° to 120°C, depending on the source of the starch and its amylose content. Hydration destroys the ordered starch structure, and renders it easily digested. RS_3 is formed upon cooling, when starch undergoes a relatively slow re-association of the linear molecular regions. The association process can be driven

further by dehydration. Retrograded starch is found in many foods, including cereal products and stale breads. RS_3-based commercial ingredients are also available as a source of RS.

The thermal stability of retrograded amylose regions is thought to be linked to the size of helices (number of glucose units involved) and the number of imperfections in the structure (Sievert & Würsch 1993). Differential scanning calorimetry (DSC) of retrograded amylose shows thermal activity anywhere from 100° to 165°C, depending upon molecular weight and how the amylose was retrograded (time–temperature history). Generally, thermal cycling between the glass transition temperature and the melting temperature of the microcrystallites tends to perfect the ordered regions, and increase the peak temperature and enthalpy.

Although amylose retrogradation drastically inhibits accessibility to amylases, it does not prevent their attack. Over long periods (days) α-amylases can almost completely digest some retrograded amylose (Ring *et al.* 1988). Since the helices are not static entities, molecular motion allows the amorphous ends to move, possibly assembling and disassembling the helices, allowing for enzymatic attack. Certainly, temperature plays a role in the motion and disassembly of some ordered regions, rendering them accessible to enzymes. Therefore, the term 'resistance' has temperature and time components associated with it.

Other structures which may be part of RS are amylose–lipid complexes and retrograded amylopectin. Amylose–lipid complexes are single-helix starch structures around a linear lipid (Biliaderis 1992). These complexes are thermally stable, exhibiting a DSC peak from 90° to 130°C. Amylose–lipid complexes are digested by amylase, but more slowly than amorphous amylose (Seneviratne & Biliaderis 1991; Kitahara *et al.* 1996). Retrograded amylopectin, like retrograded amylose, is comprised of double-helical structures, but the thermal stability is very low. Retrograded amylopectin exhibits a DSC peak between 40° and 80°C. The low temperature is presumably due to the relatively few glucose units involved in the helices, caused by the steric hindrance of the branch points within the molecule. There continues to be some controversy whether the helical structures of retrograded amylose are parallel or anti-parallel. Helices within amylopectin would necessarily be parallel, and this also could account for thermal stability differences.

35.3 RS and TDF assays

RS is comprised of a number of physical states, which may be thermally disassembled into the more common, rapidly digested, amorphous form of starch. It is also possible for amorphous starch to retrograde, induced by temperature fluctuations. Quantification of dietary fibre, which contains a portion of RS, is very technique-dependent, because of the way the assay affects the stability and/or formation of helical regions. Thermal history, assay temperature and amylase reaction time are all important in the quantification procedure. Dietary fibre analytical methods have been reviewed by Baghurst *et al*. (1996), and the influence of analytical procedure on RS estimates has been reviewed by Haralampu (2000).

The most universally accepted analytical procedure for dietary fibre is the Prosky method (AOAC methods 985.29 and 991.43 and AACC methods 32-05 and 32-07). This assay quantifies only a portion of the starch, which would pass the ileum, because the α-amylase step is conducted at 95–100°C. Many structures resistant to enzymatic hydrolysis at 37°C are 'melted' in the assay. In fact, even the 5°C variability in incubation temperature allowed by the procedure can give differences in RS quantification. The elevated temperature of the

enzymatic digestion means that the Prosky method underestimates the portion of starch passing the ileum.

Englyst *et al.* (1992) have proposed an assay for resistant starch, which is run at physiological temperature (37°C). In this assay, starch is categorised as rapidly digestible (RDS, digested within the first 20 min), slowly digestible (SDS, digested in the next 100 min), and resistant (RS, the remaining starch).

Table 35.1 compares RS values obtained by AOAC methods 985.29 and 991.43 and by the method of Englyst *et al.* (1992). Clearly the low, 37°C, assay temperature gives higher estimates of RS. For some products there is good agreement between the methods. Generally, the more thermal activity observed by DSC between 37° and 95°C, the greater the discrepancy between the AOAC and Englyst methods. There can be a difference between the two AOAC. For the values given in Table 35.1, the amylase digestion in AOAC 985.29 was run at 95°C, the lower end of the allowable temperature range, while AOAC 991.43 was run at 100°C, as specified by the method. Differences in the buffer systems may also affect the relation between enzyme activity and availability of resistant starch.

Other researchers have employed various other methods for RS, all of which are based on 'exhaustive' digestion by amylase (see for example, Champ 1992). Each method can provide different quantification, depending upon the time–temperature histories of the assays and enzyme types. In general, higher digestion temperatures and longer digestion times favour lower estimates of RS. Thermal fluctuations can promote or destroy RS structures, due to the nature of starch retrogradation. Jane and Robyt (1984) demonstrated that different α-amylases have different capabilities for digesting close to a helical zone. Therefore, the enzyme type utilised in a procedure is important in RS quantification.

35.4 Physiological impact of RS

Observations in animal systems are consistent between research groups, but the magnitudes of some findings differ, due to the lack of specificity in the RS definition. On many occasions, apparent RS levels in diets are confounded by the analytical method used for quantification, and it is sometimes unclear whether researchers use RS_2 or RS_3 in their studies.

Numerous studies in rats (Gee *et al.* 1991; Ranhotra *et al.* 1991; McIntyre *et al.* 1994; Rickard *et al.* 1994; de Deckere *et al.* 1995; Younes *et al.* 1995; Gordon *et al.* 1997) have shown that RS escapes digestion in the small intestine, and is slowly fermented in the large intestine. The general behaviour of RS physiologically is similar to soluble, fermentable fibre, like guar. The most common findings include increased faecal bulk and lower colonic pH.

Table 35.1 Comparison of resistant starch (RS) methods (values are percentages).

	AOAC 985.29	AOAC 991.43	Englyst *et al.* 1992
Hylon® 7*	18	11	20
CrystaLean®†	37	32	39
Test RS_3 Product[3]‡	32	26	41

* Raw amylomaize VII, National Starch and Chemical Company, Bridgewater, NJ, USA.
† Opta Food Ingredients Inc., Bedford, MA, USA.
‡ Prototype product, Opta Food Ingredients, Inc.

Additional observations suggest that RS, like soluble fibre, has a positive impact on colonic health by increasing the crypt cell production rate, or decreasing the colonic epithelial atrophy in comparison with no-fibre diets. There is indication that RS, like guar, influences tumorigenesis, and that serum cholesterol and triglyceride levels are reduced.

Further studies in humans support the rat model findings (see Muir *et al.* 1994). In the small intestine, RS may be slowly absorbed, but, more importantly, is associated with an increased malabsorption of starch. This results in decreased postprandial glucose and insulin responses, which has significant implications for the use of RS in food formulations for people with certain forms of diabetes. Interference with starch absorption also implies long-term benefits in controlling hyperlipidaemia. RS has been reported to increase faecal bulk, and to lower colonic pH. The portion fermented by intestinal microflora produces a wide range of short-chain fatty acids (SCFAs), primarily acetate, propionate and butyrate (Muir *et al.* 1994; Kritchevsky 1995; Phillips *et al.* 1995; Silvester *et al.* 1995).

Findings in human subjects liken the physiological effects of RS to some fermentable dietary fibres. Metabolism of RS occurs 5–7 h after consumption (Muir *et al*. 1995), in contrast to normally cooked starch, which is digested almost immediately. A decrease in colonic and serum ammonia levels is observed in humans (Silvester *et al.* 1995).

The energy contribution of RS is reported to range almost the entire gamut from 0 to 100% digestible. Most studies indicate that 30–70% of RS is metabolised (Ranhotra *et al.* 1991, 1996; Behall & Howe 1995, 1996; Cummings *et al.* 1996), while the balance is excreted in the faeces. The variability is largely due to inconsistent definitions and assay procedures, and to effects caused by the malabsorption of other ingested starch.

Slow digestion of RS has implications for its use in controlled glucose release applications, particularly for diabetics. Digestion over a 5- to 7-h period reduces postprandial glycaemia and insulinaemia, and has the potential for increasing the period of satiety (Raben *et al.* 1994; Reader *et al.* 1997). In a human study (Reader *et al.* 1997) using a commercial RS_3 ingredient (CrystaLean®, Opta Food Ingredients, Inc., Bedford, MA, USA), the maximum blood glucose level and the area under the curve responses for serum glucose and insulin were significantly lower after consuming RS_3 than after other carbohydrates (simple sugars, oligosaccharides and common starch). The RS_3 was incorporated into a bar-type product. The glycaemic response of subjects with type II diabetes mellitus was compared with the responses in subjects fed a common candy bar or a nutrition bar. All three bars had similar macronutrient contents. The study showed that the RS_3-containing bar decreased postprandial blood glucose, and may play a role in providing improved metabolic control in type II diabetes (non-insulin-dependent). There may also be a benefit for diabetics by lowering lipid levels and prolonging satiety. Another study with CrystaLean® in dogs (Murray *et al.* 1998) supported the result that the postprandial area under the curve for glucose and insulin was reduced, and that RS in enteral formulas may improve gastrointestinal tract health status due to faecal bulking, potential dilution of toxins and greater production of SCFAs.

35.5 Fermentable dietary fibres

Some of the main benefits attributed to fermentable dietary fibres are linked to the production of SCFAs in the colon by the intestinal microflora. There are indications that butyrate, in particular, is a primary energy substrate for the colonic mucosa in humans (Roediger 1980; Cummings 1981). SCFA production has a positive impact on bowel health, including

increased absorption of magnesium and calcium, epithelial proliferation, balance of bacterial species, and bacterial metabolism of bile salts. Whether through the action on bile salts or through dilution effects, RS is thought to provide a degree of protection against bowel cancer through its effect on SCFA concentrations.

When the diet is supplemented with the live beneficial intestinal microflora, the supplement is called a 'probiotic'. Generally, these are the lactic acid bacteria and *Bifidobacteria*. 'Prebiotics' are the compounds not digested in the small intestine, which also selectively stimulate growth of beneficial microflora in the lower gut. Health benefits of probiotic organisms have been reviewed elsewhere (see for example Lee & Salminen 1995; Walker & Duffy 1998). Common claims associated with probiotics include enhancement of immunity, prevention of diarrhoea and colitis, anti-tumorigenic effects and cholesterol reduction. These effects seem to be a direct result of SCFA production, particularly butyrate.

35.6 RS as dietary fibre

The ability of certain physical forms of starch to pass the ileum imparts many of the same physiological benefits as dietary fibre. Increased faecal bulk is a characteristic of insoluble dietary fibre. RS is apparently fermented in the colon, and may have prebiotic potential. It has been suggested for use in probiotic compositions to promote the growth of such beneficial microorganisms as *Bifidobacterium* (Brown *et al.* 1996). Reduced colonic pH, increased faecal SCFA concentration and decreased ammonia concentration are all observed with diets rich in RS – typical benefits of fermentable soluble dietary fibres. Additionally, positive effects on serum triglycerides have been reported.

35.7 Characterisation of an RS_3 food ingredient

RS-based food ingredients have interesting potential within the food industry. As a source of dietary fibre, RS products do not interfere with structure and texture like traditional fibres. CrystaLean® has been shown not to interfere with the structure of an extruded, puffed cereal product, while substantially surviving the heat and shear rigors of an extrusion process (Haralampu 2000). Other applications with an RS_2 ingredient (Novelose™ 240, National Starch and Chemical Company, Bridgewater, NJ, USA) are given as a traditional fibre replacement (Yue & Waring 1998).

RS ingredients also have unique, niche applications. Haralampu *et al.* (1999) have reviewed CrystaLean® applications intended to modulate glucose release.

35.8 Prebiotic potential of CrystaLean®

Conceptually, it is easy to define RS as, 'the portion of ingested starch, remaining undigested by the small intestine'. However, it is much more problematic to identify exactly what physical forms of starch meet this definition. The lack of a practical definition for RS poses a problem attributing specific literature to performance of a specific RS product. In this study, the behaviour of a particular commercial RS_3 product (CrystaLean®) was examined. It has already been shown that this form of RS_3 survives most normal food processing operations, and

is a useful functional food ingredient. DSC shows its increased thermal stability, in comparison with its RS_2 parent starch (Haralampu 2000).

The prebiotic potential of RS_3 was examined for CrystaLean®, which is a highly retrograded maltodextrin derived from debranched, high-amylose cornstarch. The RS level of the ingredient used was 34.4% as measured by the Prosky method (AOAC 991.43). The prebiotic effect of RS_3 was compared with inulin.

The probiotic organisms chosen for this study were *Lactobacillus acidophilus*, *Lactobacillus casei* ssp. *paracasei* and *Bifidobacterium bifidum* (all obtained as freeze-dried cultures from Chr. Hansen Inc., Milwaukee, WI, USA). Growth was monitored in an MRS-type broth in which the utilisable carbon source was replaced by the test source, i.e. RS, inulin or glucose. Each carbohydrate was added at the same total solids level (40% of the media's dry weight). Cultures were grown anaerobically at 37°C for up to 3 days, and microbial activity was monitored by qualitative assessment of turbidity and by pH drop. Lactic acid and SCFA production was measured after 1 and 3 days by HPLC.

The HPLC technique utilised a BioRad (Hercules, CA) Aminex HPX-87H cation exchange column (300 × 7.8 mm). Elution was at 60°C with 5 mM H_2SO_4 at 0.7 ml/min. The culture media were prepared only by filtration prior to being directly injected.

CrystaLean® was tested after partial amylase digestion, simulating *in vitro* a relatively long passage through the small intestine. Partial digestion was accomplished by incubating CrystaLean® with a mixture of porcine pancreatin, invertase and amyloglucosidase at 37°C for 16 h, following the concepts of the method used by Englyst *et al.* (1992). The extent of digestion was compared with maltodextrin (Maltrin® M40, Grain Processing Corporation, Muscatine, IA, USA) and gelatinised common corn starch (Fig. 35.1). The RS fraction of CrystaLean® was recovered by centrifuging the undigested material, resuspending the pellet in RODI (reverse osmosis, deionised) water and re-centrifuging. This simulation of digestion in the small intestine indicated that 46.3% of the CrystaLean® would pass the ileum, while RS assayed by the Prosky method indicated 34.4% TDF. This is a reflection of the different amylase digestion temperatures.

The probiotic organisms tested were found to grow poorly on starchy substrates, and all three organisms required exogenous amylase to enable starch utilisation. Therefore, it was necessary to add some pancreatin mixture to the cultures, simulating carryover of amylases from the small intestine, or the production of amylases by other species found in the colonic

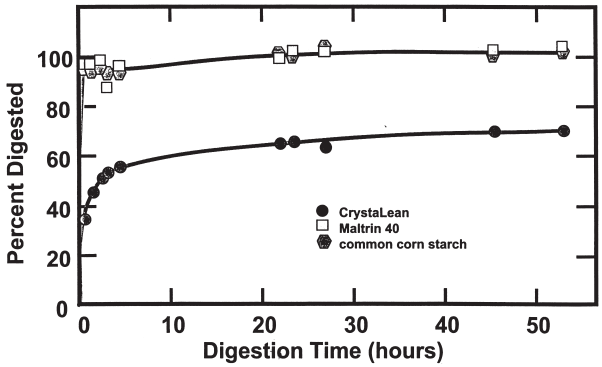

Fig. 35.1 Extent of digestion by the pancreatin mixture.

microflora. Growth was monitored after 1 day by the pH drop (Table 35.2). RS_3 can support growth of the probiotics, although not quite to the extent of glucose or inulin. This reflects the fact that RS_3 is not 100% available over the 3-day culturing period. The reduced acid production is attributed to the overall lower amount of available carbon.

Organic acid production in the cultures is presented in Table 35.3, where it can be seen that lactic acid is the predominant acid produced. Lactic acid formation probably chiefly accounts for the observed pH reductions. Comparing the lactic acid production with that of the glucose controls, it is estimated that approximately 50% of the RS_3 was available. RS may also induce SCFA production from these cultures, although SCFAs are not a primary metabolic product of these microorganisms. A high background acetic acid level prohibited any quantification of its production. Propionic and butyric acid production was detected from each of the carbon sources tested. In general, RS_3 favoured an even distribution between propionic and butyric acids, while inulin favoured butyrate production. For both *L. acidophilus* and *L. casei* the propionate and butyrate produced from RS was considerably greater than that produced from inulin. When propionic and butyric acid production are normalised by the lactic acid concen-

Table 35.2 pH after 1 day culturing.

Organism	No carbohydrate source	No carbohydrate plus pancreatin mixture	Glucose	Washed RS_3 plus pancreatin mixture	Inulin
B. bifidum	6.5	6.4	4.0	4.8	4.5
L. acidophilus	6.2	5.9	3.7	4.4	4.1
L. casei	6.4	5.8	3.9	4.6	3.7

Table 35.3 Short-chain fatty acid concentration (values are percentages).

	Acetic acid	Propionic acid	Butyric acid	Lactic acid
B. bifidum				
No carbohydrate	0.18	0.01	ND	0.06
Glucose	0.16	0.01	0.30	0.75
Washed RS_3	0.17	0.10	0.09	0.44
Inulin	0.17	0.02	0.14	0.33
L. acidophilus				
No carbohydrate	0.18	0.02	ND	0.05
Glucose	0.17	ND	ND	0.48
Washed RS_3	0.18	0.11	0.11	0.52
Inulin	0.17	0.02	ND	0.19
L. casei				
No carbohydrate	0.29	ND	ND	0.05
Glucose	0.15	0.02	0.02	1.17
Washed RS_3	0.18	0.06	0.03	0.56
Inulin	0.17	0.02	0.01	1.10

ND, none detected.

tration, i.e. assuming that lactic acid is an indicator of carbon source availability, available RS is as good or better than inulin in promoting SCFAs (Table 35.4).

Since it is likely that SCFA production is linked both to availability of the carbon source and the rate of growth, future investigations are necessary to elucidate the impact of exogenous amylase concentrations on SCFA. Enzyme concentrations are important to relate *in-vitro* studies to the carryover of amylase from the small intestine *in vivo*. The results so far are positive, and indicate that further *in-vitro* and *in-vivo* testing is warranted. *In-vivo* tests are necessary to complete the complex picture of enzyme concentrations, transit times and the competitive microbial environment of the colon.

35.9 Final remarks

Resistant starch has been shown to have many of the positive attributes of dietary fibre, and should continue to be quantified, at least in part, by the Prosky method. Many forms of RS exist, some which are not very thermally stable. Only the most retrograded, thermally stable RS_3 products are fairly quantified by the Prosky method as insoluble dietary fibre, but are still about 50% available for fermentation in the colon. Variability in RS structures and the ability of various assays to quantify RS requires some level of vigilance before comparisons can be made between studies on an equal basis.

RS can be a useful food ingredient, because it can boost TDF levels in a food without imparting some of the negative textural attributes associated with traditional fibres. It also provides a modulated release glucose source, enabling food formulations with reduced postprandial glucose spiking. RS_3 ingredients are important because they possess the thermal stability capable of withstanding most food processes, and can be manipulated by processing to have superior functional performance.

It seems that RS can be an important prebiotic. Exogenous amylase is necessary to enable fermentation by the probiotics tested. Additionally, RS may induce these organisms to produce SCFAs. The next step needs to identify whether RS stimulates growth of beneficial

Table 35.4 Normalised propionate and butyrate production normalised for lactic acid.

	Propionate:lactate	Butyrate:lactate
B. bifidum		
Glucose	0.01	0.40
Washed RS_3	0.23	0.20
Inulin	0.06	0.42
L. acidophilus		
Glucose	0.00	0.00
Washed RS_3	0.21	0.21
Inulin	0.11	0.00
L. casei		
Glucose	0.02	0.02
Washed RS_3	0.11	0.05
Inulin	0.01	0.00

microorganisms in the competitive environment of the colon. Within the framework of all future research, information on the physiological benefits of RS must be developed with a uniform basis for the definition of resistant starch, which implies a unified analytical definition.

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36 Resistant Starch: Health Aspects and Food Uses

Gur Ranhotra

36.1 Introduction

Whether naturally occurring or formed during or after food processing, resistant starch (RS) is starch that is not broken down by human enzymes and, thus, enters the large intestine undigested. RS is measured as fibre by the method widely used in North America to determine fibre in food products (AOAC 1998; Ranhotra *et al.* 1999a). Although a definition of fibre which may find wide acceptance has not yet been agreed upon, RS is generally considered as one of the components that make up total dietary fibre (TDF).

RS is classified into three different types based on a number of natural and processing phenomena (Englyst *et al.* 1992), and occurs naturally in many grain-based and other foods, but usually in small amounts. Only commercially prepared concentrated sources may contain high enough levels of RS to allow use in processed foods to take advantage of the material's health benefits and functional characteristics. Several concentrated sources, mainly of type 3 RS (RS_3), are now available for food use.

36.2 Health aspects of resistant starch

Although not digested in the small intestine, RS, like other fibre components, undergoes some degree of bacterial fermentation (degradation) in the large intestine. Short-chain fatty acids (SCFAs) that are produced as a result of this fermentation may provide some energy to humans, although this contribution may be negligible. Thus, current interest in RS centres on the health benefits that it may provide, and these are briefly discussed below.

36.2.1 *Energy value*

Under the nutrition labelling regulations currently in effect in the United States, RS and other insoluble fibre components are considered as non-caloric food components. An in-vitro model study (Mathers 1992) assigns RS an energy value of 2 cal/g; however, the model requires, as suggested by the author, further testing to predict the energy value of RS from simple *in-vitro* measurements. In a study conducted using an animal model, RS was found to have no caloric value (Ranhotra *et al.* 1996). In this study, the net increases in carcass energy observed in animals fed RS did not differ from the group of rats fed fine silica, a non-caloric material (Table 36.1).

36.2.2 *Hypolipidaemic effect*

A number of studies, most of which have been performed in animals, show RS as effectively

Table 36.1 Increase in carcass energy in rats.*

Material/diet	Total carcass energy† (cal)	Net increase in carcass energy‡ (cal)	Relative increase or (decrease) in carcass energy (cal)§
Resistant starch A	128 ± 10b	85 ± 10b	(3)
Resistant starch B	128 ± 5b	85 ± 5b	(3)
Fine silica (sand)	131 ± 3b	88 ± 3b	
Pre-gelatinised starch	201 ± 11a	158 ± 11a	70

* Values are averages ± SD for eight rats per diet. Within a column, values not sharing a common letter are significantly different ($P < 0.05$).
† Based on body composition of rats after 3 weeks on diet.
‡ Total carcass energy minus baseline (0 day) carcass energy (43 cal).
§ Relative to diet based on silica.
Source: Ranhotra *et al*. 1996.

lowering blood cholesterol and triglyceride levels. In a study with rats fed low- or high-RS diets, the high-RS diet reduced serum total cholesterol and triglyceride concentrations significantly as compared with the low-RS diet (De Deckere *et al.* 1993). RS may be an even more effective cholesterol-lowering agent than cholestyramine, a drug used specifically for this purpose (Younes *et al*. 1995). In studies using hamsters as the test model, RS was shown to lower serum total cholesterol significantly (Ranhotra *et al*. 1997), though the effect was not RS dose-dependent (Table 36.2).

36.2.3 *Glycaemic responses*

In a study with healthy, normal-weight males, a significant reduction in postprandial glycaemia and insulinaemia was observed in subjects fed 50 g pre-gelatinised potato starch, as compared with those fed 50 g of raw potato starch (type 2 RS) (Raben *et al*. 1994). Adding RS to a food staple such as bread may also modify the product's glycaemic index, as results in Table 36.3 show (Brown *et al*. 1995). This use should widen food choices for diabetics to control their blood sugar levels.

Table 36.2 Serum lipid levels in hamsters fed resistant starch for 4 weeks.*

	Resistant starch (% in diet)				
Measurement	0	5	10	15	20
Total dietary intake (g)	202 ± 1a	202 ± 1a	201 ± 1a	202 ± 1a	202 ± 0a
Body weight gain (g)†	41 ± 6a	39 ± 6a,b	36 ± 6a,b	34 ± 6b,c	28 ± 8c
Serum total cholesterol (mg/dl)‡	339 ± 36a	295 ± 36b,c	315 ± 41a,b	297 ± 41b,c	267 ± 38c
Serum triglycerides (mg/dl)‡	217 ± 81a	179 ± 47a,b	215 ± 25a	179 ± 26a,b	161 ± 46b

* Values are averages ± SD for 10 hamsters per diet. Within a row, values not sharing a common letter are significantly different ($P < 0.05$).
† Initial body weight 49 ± 5 g.
‡ Initial (0-day) values: total cholesterol 152± 3 mg/dl; triglycerides 67 ± 23 mg/dl.
Source: Ranhotra *et al*. 1997.

Table 36.3 Glycaemic index of bread containing resistant starch.

Bread	Glycaemic index
White bread (no resistant starch)	100
White bread (5% resistant starch)	96
White bread (10% resistant starch)	74
White bread (20% resistant starch)	53

Source: Brown *et al*. 1995.

36.2.4 *Colonic health*

Among the SCFAs produced in the gut, butyric acid is reported to inhibit the growth of tumour cells in the colon. As results listed in Table 36.4 show, RS fermented in the gut not only produces more butyric acid but also lowers faecal pH and increases faecal bulk (Phillips *et al*. 1995), both of which are accepted markers of colonic health. In a 4-week study where rats were fed normal wheat starch (containing 0.5% RS) or heat-treated wheat starch (containing 11.5% RS), faecal bulk increased seven-fold due to the feeding of heat-treated starch. Faecal bulk increased still further when rats fed treated starch were also fed antibiotics to reduce the bacterial population in their gut and thus reduce degradation of the RS (Table 36.5) (Ranhotra *et al*. 1991).

Table 36.4 Effect of resistant starch on fermentation-dependent events in humans.

Measurement	Low RS (5 g/day) diet	High RS (39 g/day) diet
Faecal output (g wet weight/day)	138 ± 22	197 ± 37
Faecal pH	6.9 ± 0.1	6.1 ± 0.1
Butyric acid (mmol/day)	3.1 ± 1.0	6.2 ± 1.7

Source: Phillips *et al*. 1995.

Table 36.5 Faecal bulk in rats fed resistant starch for 4 weeks, with or without antibiotics.*

Diet/measurement	Diet A	Diet B	Diet C
Starch type†	Native	Treated	Treated
Antibiotics	No	No	Yes
Total faecal wet weight (g)	5.1 ± 0.4a	37.2 ± 7.5b	95.8 ± 4.4c
Digestibility of RS (%)	49.5 ± 5.8a	37.1 ± 12.9b	14.3 ± 3.7c

*Values are averages ± SD of 8–10 rats per diet. Within a row, values not sharing a common letter are significantly different ($P < 0.05$).
†Isolated wheat starch containing 0.5% resistant starch (native starch) or 11.5% resistant starch (starch treated through several heating and cooling cycles).
Source: Ranhotra *et al*. 1991.

36.3 Food uses of resistant starch

36.3.1 *Natural levels of RS*

In processed foods containing naturally occurring RS, the natural levels rarely exceed approximately 4%. This is evident from analytical values listed in Table 36.6 (Baghurst *et al*. 1996), and from the results of a recent study where 70 grain-based foods were analysed for TDF and RS (Ranhotra *et al*. 1999a, b). Although natural levels may be increased substantially by modifying certain parameters during food processing, only concentrated sources of RS may add substantial amounts to these foods.

36.3.2 *Functional characteristics of concentrated RS sources*

Concentrated RS sources containing 40–60% RS are now commercially available. As an ingredient, RS is highly functional. Since it holds little water (Yue & Waring 1998), concentrated sources of RS are well suited for use in intermediate- and low-moisture products such as cookies, crackers and extruded products. In pasta products and noodles, RS permits tolerance to overcooking, and the cooked products tend not to stick together. RS also imparts desired expansion, crispness and mouthfeel to RS-fortified foods such as breakfast cereals and snack items (Table 36.7) (Yue & Waring 1998). RS also causes an increase in the bowl-life of breakfast cereals by reducing moisture uptake and thus giving the products extra 'crunch'.

The non-caloric nature of RS may allow foods containing this ingredient to be labelled as 'reduced-calorie' (one-fourth reduction) or 'light' (one-third reduction) products under the

Table 36.6 Naturally occurring resistant starch in major categories of foods.

Food category	Resistant starch (g/100 g food)
Beans/lentils/sweet corn, boiled	2–5
Crispbreads/crackers	2–4
Banana	2
Cooked potatoes, cooled	2–4
Breakfast cereals/breads/biscuits	0.5–3
Rice/pasta/peas/baked beans in sauce	1

Source: Baghurst *et al*. 1996.

Table 36.7 Expansion of extruded oat cereal containing resistant starch.

Cereal	Expansion ($inch^3$; cm^3)	Change from control (%)
Control (no RS)	0.295 (4.83)	–
RS (40%)	0.347 (5.69)	+17.6
Oat fibre + RS (1:3)	0.284 (4.65)	–3.7
Oat fibre + RS (1:1)	0.265 (4.34)	–10.3

Source: Yue & Waring 1998.

Table 36.8 High-fibre white bread made with different fibre sources.

Fibre source	Total quality scores (maximum 100)
Wheat fibre	54.8
Cellulose	60.7
Oat fibre	61.1
Resistant starch (40% RS material)	65.3

Source: Yue and Waring 1998.

United States' labelling regulations. Such use would also increase the fibre content of foods, and may even allow fibre 'claims' (content and health claims).

36.3.3 *Food uses of concentrated RS sources*

Concentrated sources of RS are now used in a wide variety of foods: bakery products, breakfast cereals, extruded snacks, pasta and noodle products, nutrition bars, beverages, baby foods, and even confectionery items such as 'jelly beans' and 'gummy bears'. In most cases, this use improves product quality as well as nutritional characteristics. Products can also be formulated with RS to address the needs of certain population groups, such as diabetics (products with a lower glycaemic index) and athletes (energy bars and beverages).

In a study where four different fibre sources were tested in white bread, bread quality scored the highest when RS was used as the fibre source (Table 36.8) (Yue & Waring 1998). This bread can also be labelled as 'high in fibre' as it provides a minimum of 20% of the daily value (25 g) for fibre per 50-g serving. Formulas have also been developed for several other products such as muffins, crackers, low-fat and regular cakes, pasta products and various snack items which contain substantial amounts of RS (Yue & Waring 1998).

36.4 Conclusions

Resistant starch occurs naturally in many processed foods, but generally in only small amounts. As a concentrated source, resistant starch holds great promise as an ingredient for use in processed products. This use may provide many health benefits and also greatly improve the quality of the finished products. Unlike other fibre sources, resistant starch is relatively functional in processed foods, even when used at a substantial level.

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37 Structural Features of Resistant Starch

Yong-Cheng Shi and Roger Jeffcoat

37.1 Introduction

Starch is a storage carbohydrate in plants and serves as the major source of energy in most humans. Regular starch is comprised of amylose and amylopectin. Amylose is essentially a linear polymer of 1,4-α-linked D-glucosyl residues, while amylopectin is a branched polymer composed of linear chains of 1,4-α-linked D-glucosyl residues that are linked by 1,6-α-D-glucosidic bonds. The size of amylose varies between plant species, ranging from 800 to 1600 glucose units in amylose from wheat starch, up to 4900 glucose units in amylose from potato starch (Hizukuri 1996). Amylopectin is much larger than amylose, the number of component glucose units being approximately $2.5–3.0 \times 10^6$, based on the reported molecular weight of $4–5 \times 10^8$.

Starch occurs naturally as granules, the size of which is dependent on the plant species and the cultivar. Amylopectin is arranged radially within the granules and the branch chains are clustered (French 1984). How amylose is distributed within granules is not exactly known, but it is thought to be polydispersed throughout the granule (Jane *et al.* 1992). Some evidence suggests that the amylose is more concentrated at the periphery than at the core of the granule (Jane & Shen 1993).

Native starch granules are partially crystalline. They contain 15–45% crystallinity and 38–53% double helix as determined by X-ray diffraction and solid-state NMR, respectively (Gidley & Bociek 1985; Zobel 1988). The melting of crystallites in granules is dependent on the amount of water and the source of the starch (Van den Berg 1981). Heating or cooking can destroy granules, cause total loss of crystallinity, and result in complete digestion of starch. However, incomplete gelatinisation, or retrogradation after gelatinisation, produces a starch that is not totally digestible.

Nutritionally, starches can be grouped into glycaemic starches and resistant starches. Glycaemic starches (or starches for metabolism) are starches that are digested in the small intestine into maltose and glucose, by α-amylase secreted by the pancreas. These sugars are absorbed through the wall of small intestine into the blood stream, and function as the main source of energy (~4.0 kcal/g) for metabolism. Resistant starches (RS) are the sum of starches and products of starch degradation that resist digestion and absorption in the small intestine of healthy individuals. These starches pass into the colon where they are used as nutrients and fermented by colonic microflora, releasing short-chain fatty acids (acetate, propionate, butyrate) and gases (CO_2, CH_4 and H_2).

RS has been classified into four types (Englyst & Cummings 1987; Eerlingen & Delcour 1995). Type RS_1, is starch which is physically inaccessible due to entrapment of granules within a protein matrix or within a plant cell wall. Type RS_2 is composed of native starch granules and is found in uncooked food or food containing uncooked starch. Type RS_3 is retrograded, non-granular starch as formed by heat-moisture treatment of starch, and may be

present in products such as cooked and cooled potatoes. Type RS_4 comprises chemically or thermally modified starch that contains glycosidic bonds other than α-1,4 or α-1,6 linkages.

All RS types are assumed to have the same metabolic fate in the large intestine. They are fermented, but the site, magnitude and diversity of biochemical and physiological actions may differ along the large intestine. Therefore, the concept of RS is foremost from a physiological standpoint, but the physical characterisation of RS is also very important. Any knowledge obtained about the physicochemical properties of RS should be useful in understanding the molecular origin of resistance, and should help the food technologist in designing better starch-based foods for improved shelf stability and consumer acceptance.

In this work, very pure resistant starches (HPRS) were made from high-amylose-based starch products by pancreatic α-amylase digestion. In order to understand fundamentally the structure of RS, light microscopy, scanning electron microscopy (SEM), X-ray diffraction, differential scanning calorimetry (DSC) and gel permeation chromatography (GPC) were used to characterise these very pure RS materials.

37.2 Materials and methods

High-amylose starch, HYLON VII® starch, and the resistant starch products, NOVELOSE® 240 and NOVELOSE® 330, were commercially available from National Starch and Chemical Company (Bridgewater, NJ, USA). Pancreatin from porcine pancreas was purchased from Sigma Chemical Company (Product Number P 7545).

Highly pure RS was prepared as follows. A high-amylose starch product (200 g) was slurried in 4400 ml of 50 mM Na_2PO_4 containing 4% NaCl. The pH was adjusted to 6.9 and the temperature to 37°C. Pancreatin (0.8% w/w based on starch sample weight) was added. After 8 h of digestion, the enzyme was deactivated. The sample was filtered, washed and dried, and ground to pass through a 40-mesh sieve.

Total dietary fibre (TDF) content was determined using the TDF Test Kit commercially available from Megazyme International, Ireland, Ltd. which is based upon the AOAC method 985.29.

The amount of RS was determined as follows. Starch sample (2 g dry weight) was placed in a jar. Phosphate buffer (50 mM) containing 4% NaCl was added to a total weight of 42 g. The solution was capped, mixed, and held in a 37°C shaker bath for 5 min. Pancreatin solution (8 ml, 5%) was added, mixed, capped tightly, and incubated in shaker bath at 37°C. After 6 h, 1.5 ml of sample was pipetted into a microcentrifuge tube, and centrifuged at ~5000 *g* for 2 min. The concentration of sugars in the supernatant were measured using a refractometer which had been zeroed with deionised water. A blank which contains all reagents except the starch sample was run and analysed concurrently. From the percent solubles, the amount of starch digested was calculated. Resistant starch was that amount of starch that remained undigested.

DSC measurements were performed using a Perkin-Elmer DSC-7 (Norwalk, CT, USA). The instrument was calibrated with indium. Samples (~10 mg of starch) at a starch:water ratio of 1:3 were prepared and heated at 10°C/min from 5°C to 160°C. An empty stainless-steel pan was used as a reference. GPC was performed as described in Shi *et al*. (1998), using a flow rate for the mobile phase of 0.7 ml/min.

37.3 Results and discussion

37.3.1 *TDF and RS content*

The TDF and RS contents of high-amylose-based starch products, and of the HPRS made from them, are listed in Table 37.1. For the base starches, NOVELOSE 240 had the highest TDF value. After treatment with pancreatin, HPRS products made from NOVELOSE 240 and NOVELOSE 330 starches had a high RS content as well as high TDF contents. The TDF measurements were performed at high temperature (97°C), whereas RS incubations were performed at 37°C. This suggests that even after cooking, HPRS products are still highly resistant to digestion by α-amylase.

In addition to supplying HYLON V and HYLON VII – starches that contain 50–55% and 70–75% amylose content, respectively – National Starch and Chemical Company (Bridgewater, NJ, USA) has also developed a maize hybrid in which the starch has an amylose content of approximately 95% (Fergason *et al.* 1994). Using butanol fractionation and GPC, this newly developed, low-amylopectin starch (LAPS) was shown to contain less than 10% amylopectin. Literature evidence supports the positive correlation between amylose content and the level of RS in cereal and grains (Pomeranz 1992). LAPS has a higher gelatinisation temperature, lower swelling power in hot water, and is more resistant to acid digestion than is HYLON VII starch (Shi *et al*. 1998). The TDF content of LAPS is approximately 32%, higher than that of HYLON VII starch (24.1%). Furthermore, NOVELOSE 240 and NOVELOSE 330 type products made from LAPS are higher in TDF than those made from HYLON VII starch.

37.3.2 *SEM and light microscopy*

SEM pictures of HYLON VII, NOVELOSE 240 and NOVELOSE 330 starches are shown in Fig. 37.1a–c, and the corresponding pictures for enzyme-digested, HPRS are shown in Fig. 37.2. Samples of base HYLON VII and NOVELOSE 240 starches show granular structures. The shape of most granules varies from polygonal to almost spherical. One unique granular feature of these two samples is that both samples contain materials with elongated, tubular, or rod-like structures. By counting the granules, these rod-like materials represent about 5% of the total number of granules. Interestingly, in the HPRS samples made from HYLON VII and NOVELOSE 240 starches, these rod-like materials were not observed. This implies that these rod-like materials are preferentially attacked by pancreatic α-amylase and disappeared

Table 37.1 Total dietary fibre (TDF) and resistant starch (RS) content of HYLON VII, NOVELOSE 240 and NOVELOSE 330 starches and highly pure resistant starch (HPRS) products made from them.

Sample	TDF (%)	RS (%)
HYLON VII starch	24.1	65.0
NOVELOSE 240 starch	62.0	54.0
NOVELOSE 330 starch	33.3	54.0
HYLON VII starch (HPRS)	14.2	80.0
NOVELOSE 240 starch (HPRS)	50.3	90.0
NOVELOSE 330 starch (HPRS)	48.2	92.5

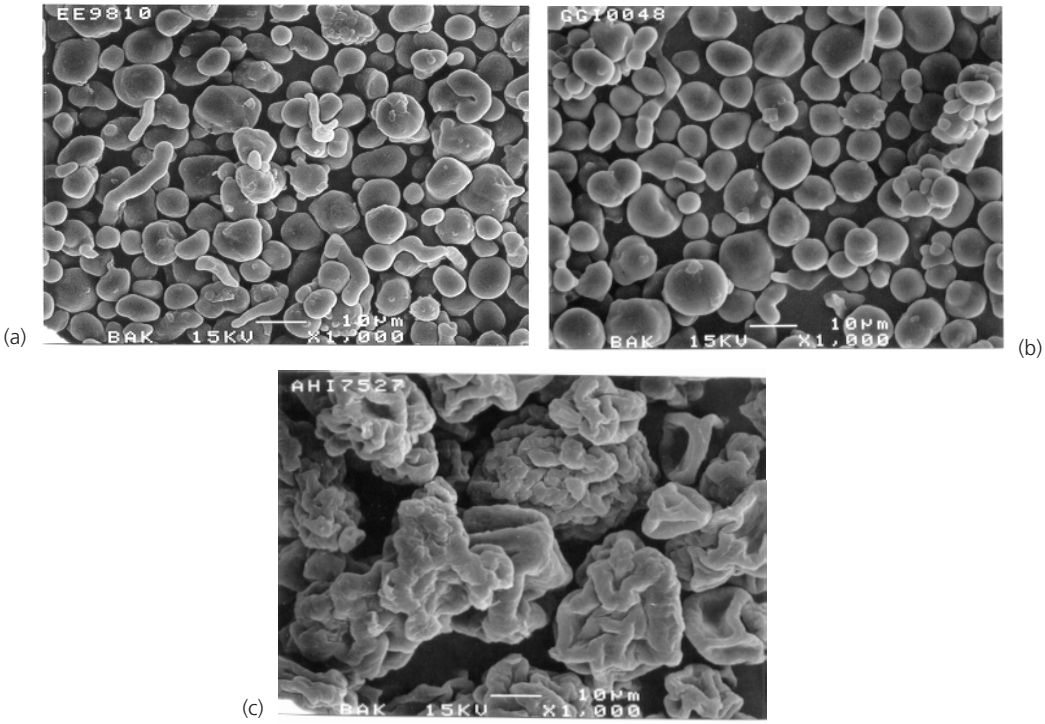

Fig. 37.1 Scanning electron micrographs of: (a) HYLON VII starch; (b) NOVELOSE 240 starch; and (c) NOVELOSE 330 starch.

in the two HPRS samples. In addition, overall surface roughness appears to be increased for HPRS samples.

When base HYLON VII and NOVELOSE 240 starches were viewed by light microscopy, similar granular features were observed. When viewed under polarised light, most granules show birefringence in the form of a typical Maltese cross. The birefringence is evident because these granules have a high degree of molecular order. It is interesting to note that the rod-like materials do not show any Maltese cross feature when viewed under polarised light, indicating that these materials have a lesser degree of molecular order. As a result, it would be expected that they would be more susceptible to α-amylase digestion and be removed after such treatment.

With NOVELOSE 330 starch, granules were destroyed during jet-cooking and a highly convoluted morphology was observed in SEM. HPRS is made from NOVELOSE 330 starch by treatment with α-amylase, filtration to recover the undigested material, air-drying and milling. In SEM, particles of varying size were observed. Because the granular structure of NOVELOSE 330 and HPRS NOVELOSE 330 is destroyed during processing, particles of either do not show the Maltese cross when viewed under polarised light.

37.3.3 *X-Ray diffraction and DSC*

HYLON VII, NOVELOSE 240 and NOVELOSE 330 starches give B-type X-ray diffraction patterns, as shown in Figs 37.3–37.5. Following enzymatic treatment, all HPRS products

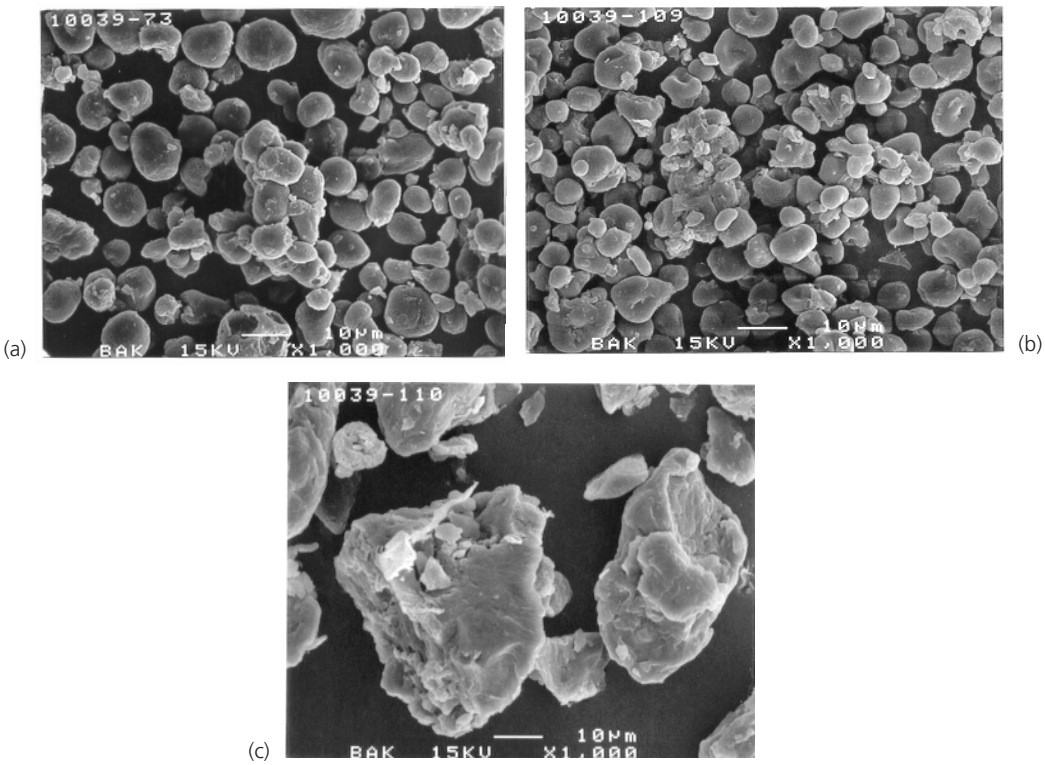

Fig. 37.2 Scanning electron micrographs of HPRS made from: (a) HYLON VII starch; (b) NOVELOSE 240 starch; and (c) NOVELOSE 330 starch.

show enhanced X-ray crystallinity. For example, the peak at 2θ of 17 for HPRS HYLON VII starch is higher and narrower (sharper) than that for the base HYLON VII starch (Fig. 37.3). This suggests that the amorphous regions are preferentially digested by pancreatic α-amylase.

The results of the DSC studies show that HPRS products have higher ΔH (enthalpy) values than their corresponding base materials (Table 37.2). For instance, the ΔH value for HPRS NOVELOSE 240 starch is 33.0 J/g, which is much higher than the ΔH value (19.8 J/g) for NOVELOSE 240 starch. This confirms that the amorphous regions are preferentially removed, and that the HPRS is more crystalline than the base starch. Immediate rescanning of these starches in DSC revealed peaks at approximately 95°C. These peaks are due to the melting of amylose–lipid complexes, indicating that these complexes are at least partially resistant to α-amylase digestion.

The high melting temperature and ΔH values indicate that these HPRS products are highly process-tolerant. They can at least maintain – and possibly even increase – their enzyme resistance and TDF content during processing under typical processing temperatures.

37.3.4 *Gel permeation chromatography (GPC)*

To characterise the changes in molecular structure in the preparation of HPRS, HYLON VII,

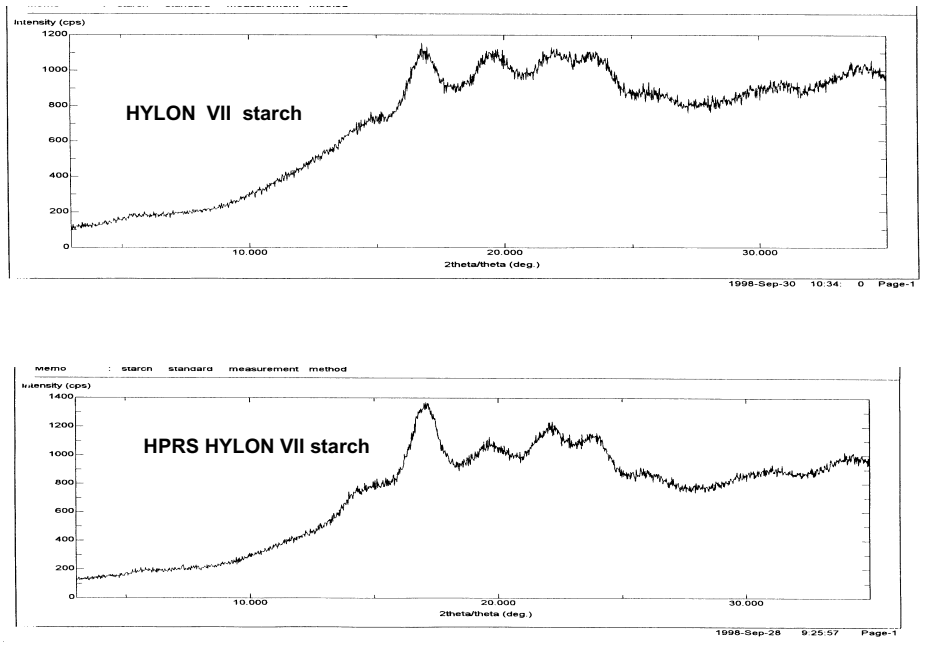

Fig. 37.3 X-Ray diffraction patterns of HYLON VII starch and HPRS made from HYLON VII starch.

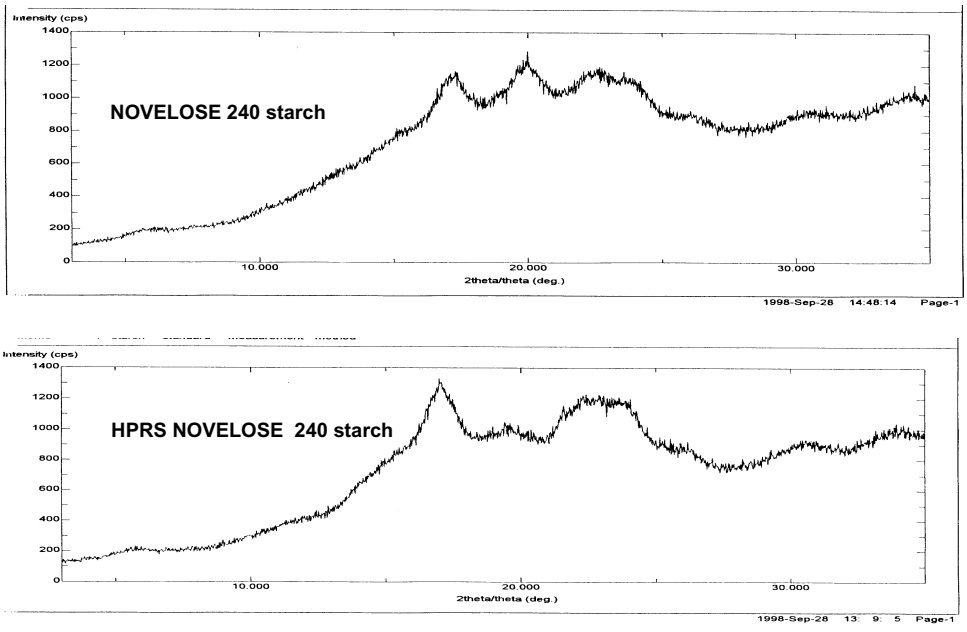

Fig. 37.4 X-Ray diffraction patterns of NOVELOSE 240 starch and HPRS made from NOVELOSE 240 starch.

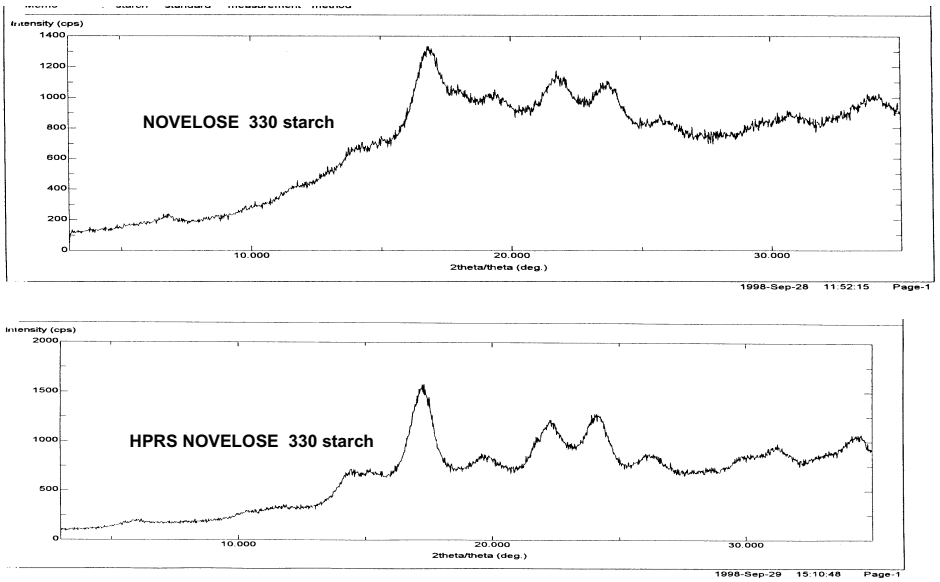

Fig. 37.5 X-Ray diffraction patterns of NOVELOSE 330 starch and HPRS made from NOVELOSE 330 starch.

Table 37.2 Differential scanning calorimetry (DSC) results of HYLON VII, NOVELOSE 240, NOVELOSE 330 starches and their corresponding highly pure resistant starch (HPRS) products.

Sample	Onset (°C)	Peak (°C)	End (°C)	ΔH (J/g)
HYLON VII starch	68.6	88.3	107.3	13.0
HPRS HYLON VII starch	74.4	96.4	126.2	17.2
NOVELOSE 240 starch	94.7	107.3	132.5	19.8
HPRS NOVELOSE 240 starch	99.6	121.5	158.5	33.1
NOVELOSE 330 starch	104.6	120.7	130.4	19.9
HPRS NOVELOSE 330 starch	110.2	125.5	137.3	32.8

NOVELOSE 240, NOVELOSE 330 starches and their corresponding HPRS products were examined by GPC (Figs. 37.6–37.8). For HPRS from HYLON VII starch, the amylopectin fraction was reduced compared with that in the base HYLON VII starch; however, a small amount of amylopectin was still present. Furthermore, a new, low-molecular weight peak was observed in HPRS from HYLON VII starch. The slope of the intrinsic viscosity curve for this fraction was slightly lower than that for the original amylose peak in HYLON VII starch, indicating that this fraction may contain some branched molecules.

For HPRS NOVELOSE 330 starch (RS_3), the large molecular weight fraction in the original NOVELOSE 330 starch was absent. A relatively low-molecular weight peak was generated, as was observed in GPC. Compared with HPRS HYLON VII and HPRS NOVELOSE 240 starches (RS_2 products), HPRS made from NOVELOSE 330 starch (RS_3 product) has one peak and a relatively narrower molecular weight distribution (Fig. 37.9). This suggests

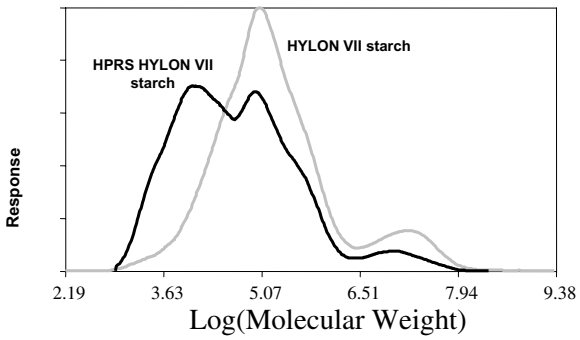

Fig. 37.6 Molecular weight distribution of HYLON VII starch and HPRS HYLON VII starch.

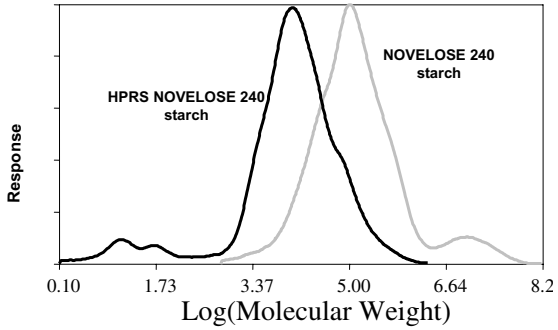

Fig. 37.7 Molecular weight distribution of NOVELOSE 240 starch and HPRS NOVELOSE 240 starch.

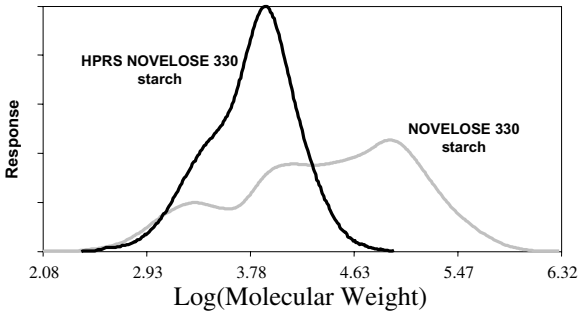

Fig. 37.8 Molecular weight distribution of NOVELOSE 330 and HPRS NOVELOSE 330.

that HPRS products made from RS_2 and RS_3 do not necessarily have the same molecular structure, even though they all resist α-amylase digestion.

As a RS_3 starch, NOVELOSE 330 is crystallised (retrograded) from essentially linear molecules derived from HYLON VII starch. The isolated HPRS made from NOVELOSE 330 starch has a peak molecular weight of 10 000 Da, i.e. the degree of polymerisation (DP) is about 60. This is in agreement with previous studies on the molecular size of retrograded material obtained on enzyme treatment of starch or amylose. In these studies, the products

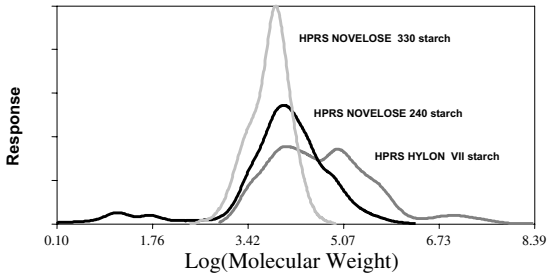

Fig. 37.9 Molecular weight distribution of highly pure resistant starches.

had DPs ranging from 30 to 65, depending on the source of the starch or amylose, and on the preparation conditions used (Jane & Robyt 1984; Berry *et al*. 1988; Russell *et al*. 1989; Leloup *et al*. 1992, Eerlingen *et al*. 1993). As suggested by Gidley *et al*. (1995), chain lengths with a minimum DP of 10 are necessary for double-helix formation, and the maximum chain length is about 100 glucose units. In addition, it has been reported (Eerlingen *et al*. 1993) that the yield of RS_3 increases with an increase in the chain length up to DP 100.

All of the above results indicate that HPRS products have a reduced molecular weight compared with the base starch, but that they are more highly crystalline. This could be explained by the fact that pancreatic α-amylase is an *endo*-enzyme and thus reduces the molecular weight. However, since α-amylase does not cleave α-1,6 linkages (branch points), HPRS products may still contain some branched molecules. During cleavage, the enzyme preferentially attacks the amorphous regions, resulting in a highly crystalline material.

At present, the exact molecular origins of resistant starch are unclear. However, intrinsic factors of molecular ordering such as chain length, double helices, type of crystals, degree of crystallinity, and the conformation of starch chains at the surface, are clearly involved. Other factors such as the porosity of the starch, the accessible of its surface to enzyme attack, and adsorption of the enzyme to the starch may also affect resistance to enzyme digestion.

For granular starches such as HYLON VII and NOVELOSE 240, part of the amorphous region in amylopectin appears to be accessible to α-amylase digestion, as evidenced in the reduction of the amylopectin fraction in the GPC curves. Amylose may occur in two forms in granules. One form is lipid-complexed amylose, and the other is lipid-free amylose. Both forms of amylose are amorphous in native starches which contain less than 30% amylose. However, in high-amylose maize starches, at least part of the amylose complexes with lipid in a crystalline form, as indicated by a V-type X-ray diffraction pattern. The lipid-complexed amylose is at least partially resistant to α-amylase digestion, and is still present in HPRS products made from HYLON VII starch, NOVELOSE 240 as well as NOVELOSE 330 starches. With regard to the lipid-free amylose in the amorphous regions of the granule, these molecules may be susceptible to α-amylase hydrolysis initially, but the fragments released during the hydrolysis could associate rapidly into the very insoluble and enzyme-resistant double-helical form. Moreover, because of the low level of amylopectin in high-amylose starches, a fraction of the amylose molecules could be in a double-helical structure in native starch granules (Shi *et al*. 1998), and inherently resistant to enzyme hydrolysis.

For NOVELOSE 330 starch, which is a type III RS, granules are destroyed during processing. The HPRS product is crystallised (retrograded) from essentially linear molecules derived from the HYLON VII. The main structures of HPRS made from NOVELOSE 330 starch ap-

pear to be substantially double-helical linear chains, arranged into aggregates with B-type crystal packing geometry, together with some lipid inclusion complexes.

Compared with HPRS made from NOVELOSE 330 starch, the broad molecular weight distribution of HPRS products made from HYLON VII and NOVELOSE 240 starches suggests that, in addition to molecular microstructure, granular structure is another factor contributing to enzyme resistance, possibly related to the limited diffusion of the enzyme into granules.

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Part 11

Other Polysaccharides

38 Nutritional Benefits of Larch Arabinogalactan

Ramona Robinson, Jennifer Causey and Joanne L. Slavin

38.1 Introduction

Arabinogalactan (AG) is a non-starch polysaccharide (NSP) that has been approved as a dietary fibre by the Food and Drug Administration. It is classified as a dietary fibre since it resists digestion by enzymes of the saliva and small intestine and enters the large bowel intact, where it is fermented by resident microflora. Because arabinogalactan has a highly branched structure it is soluble in water and has low viscosity (D'Adamo 1990). Larch arabinogalactan is also known as larch wood sugar or larch gum. It is most similar to gum arabic because it is highly branched, extremely water-soluble, and high concentrations can be produced with very low viscosities (Furia 1972). AG contains approximately 90% total dietary fibre as analysed by AOAC Method 985.29.

Many plants are rich sources of arabinogalactan and arabinogalactan proteins. The carbohydrate component is the primary constituent of arabinogalactan proteins, and is generally composed of the sugars arabinose and galactose. Arabinogalactan is commonly consumed by humans in such foods as carrots, tomatoes, radishes, pears, maize, wheat and red wine (D'Adamo 1990). In addition, several herbs have been found to contain significant amounts of AG, including *Echinacea purpurea*, *Angelica acutiloba* and *Curcuma longa* (Kiyohara *et al.* 1989; Egert & Beuscher 1992; Gonda *et al.* 1993).

The Western larch (*Larix occidentalis*) and Mongolian larch (*Larix dahurica*) provide major commercial sources of AG (Odonmazig *et al.* 1994). Arabinogalactan can be extracted from a variety of purified concentrated sources, although the commercial form is extracted from the butt wood of the Western larch. Arabinogalactan derived from trees of the genus *Larix* (larch) is a hemicellulosic product that is easily extractable by water in a pure form, from the lower portions of de-barked larch logs. Wood chips from the logs are steam-heated and the aqueous extract is evaporated to obtain a purified form of arabinogalactan. This procedure is unique because harsh chemicals are not needed to release the polysaccharide from the plant matrix, and therefore the product remains pure and structurally unaltered.

38.2 Arabinogalactan structure

The first studies on the structure of larch arabinogalactans were conducted early in the twentieth century (Timell 1965; Wilkie 1985; Whistler and Chen 1991). Since that time, many structural studies have been conducted involving many species of the genus *Larix*, including the species *occidentalis*, *desidua*, *laricina*, *lyallii*, *leptolplepis* and *korean*. Constituent groups of sugar residues attached to the (1→3)-linked D-galactan chains are similar for all species of larch. The empirical formula for the chemical structure of arabinogalactan is $[(C_5H_8O_4)\ (C_6H_{10}O)_6]_x$ (Fig. 38.1). Arabinogalactan is a complex, highly branched polymer

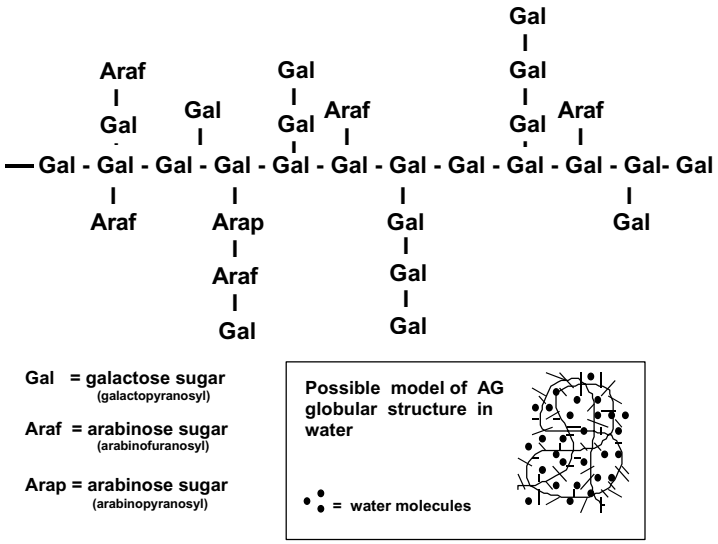

Fig. 38.1 The chemical structure of arabinogalactan (AG) and a possible model of AG globular structure in water.

of arabinose and galactose in a ratio of 1:6. It is composed of a major, highly branched, β-galactan fraction with a molecular weight of approximately 100 000 Da, and a minor arabinogalactan fraction with a molecular weight of approximately 16 000 Da in a 4:1 ratio (Furia 1972).

The arabinogalactan content of wood is affected by seasonal influences, and varies according to tree type and the part of the tree from which AG is extracted. Concentrations from *Larix occidentalis* can be as high as 5–25% at the lowest end of the tree in the butt wood (Furia 1972). Arabinogalactans harvested by Larex Incorporated from the butt wood of Western larch have indicated an aggregating behaviour and average molecular weight between 15 000 and 25 000 Da (Dexter 1997).

Commercially available AG (Larex Inc.) consists of a fine white powder, which can be incorporated into beverages due to limited sensory impact. AG has little impact on viscosity, colour, odour and taste. In addition, it is a low-calorie additive (1.4 kcal/g) which can boost the fibre content of food and potentially enhance the gastrointestinal environment.

38.3 Safety of arabinogalactan

A variety of toxicity studies were conducted during the early 1960s to determine safe doses of arabinogalactan. Short-term studies conducted with rats suggested that AG was safe for consumption when fed for up to 30 days at up to 25% of their ration (Nazareth *et al.* 1961). The Wisconsin Alumni Research Association (1964) conducted a longer-term, 6-month trial feeding arabinogalactan to beagle dogs. They found no significant clinical differences between control dogs and dogs fed diets consisting of 1, 5 or 10% arabinogalactan.

38.4 Physiological effects of AG

AG is a soluble fibre and would be expected to share many of the physiological effects of soluble dietary fibres. Additionally, AG may have immunomodulating activity. Experimental research indicates that larch arabinogalactan stimulates natural killer cell cytotoxicity. It may be cytotoxic to tumour cells and virus-infected cells, and therefore potentially therapeutic for a variety of diseases. It has also been shown to have properties capable of stimulating the immune system, such as the ability to stimulate phagocytosis and potentiate reticuloendothelial system action (Kelly 1999).

38.5 Gastrointestinal effects of AG

38.5.1 *Microflora*

AG may influence colonic bacterial populations. Jeraci *et al.* (1993) suggested that arabinogalactans were fermentable and degraded by several predominant anaerobic bacterial species found in the human intestine. Salyers *et al.* (1976) evaluated the ability of 10 *Bacteroides* species, found in the human colon, to ferment mucins and a variety of plant polysaccharides including arabinogalactan. Plates were inoculated with bacterial cultures and incubated at 37°C in an anaerobic chamber for 7 days. A bacterial strain was considered to have fermented if it decreased the pH of the medium to below 6.0. Many of the strains were found to ferment the highly branched polysaccharide larch arabinogalactan. The authors suggested that these strains may have de-branching enzymes which allowed them to degrade arabinogalactan effectively.

Salyers *et al.* (1981) further analysed the digestion of larch arabinogalactan by *Bacteroides thetaiotamicron* growing in a continuous culture. Continuous cultures were used to provide an environment similar to the normal habitat of intestinal microflora. Bacterial growth yields were 80, 82, 58 and 50 g of cells per mole of utilised arabinogalactan at growth rates of 3.5, 6.3, 11.6 and 27.7 h per generation. These yields were quite high, and similar to those attainable when monosaccharides such as glucose or galactose were the substrate.

Crociani *et al.* (1994) found that arabinogalactan was fermented by several strains of *Bifidobacterium.* They evaluated the ability of 29 strains of *Bifidobacterium* to ferment a variety of complex carbohydrates. Seventeen strains of *Bifidobacterium longum* were found to degrade arabinogalactan. Macfarlane and Macfarlane (1997) evaluated whether *Bifidobacterium adolescentis* could compete with *Bacteroides thetaiotaomicron* for arabinogalactan in a carbon-limited continuous culture. Measurements of residual arabinogalactan in pure and mixed cultures showed that *Bacteroides* species extensively degraded the galactose backbone, and to a lesser extent, degraded the polymer's arabinosyl side chains. There was evidence of arabinogalactan degradation under a variety of growth conditions, indicated by the accumulation of arabinose monomers and oligosaccharides. Mixed culture studies found that *Bacteroides* was better able to degrade arabinogalactan than Bifidobacteria. This suggested that *Bacteroides* was better able to compete for substrate degradation than Bifidobacteria under environmental and physiological conditions similar to that of the human colon. Bifidobacteria were better able to degrade substrate at higher pH and at high specific growth rates. The authors suggested that this might have been due in part to the ability of *Bifidobacterium* to produce substances that were toxic to *Bacteroides*.

Bacteria growing in human faecal samples have also been evaluated for their ability to degrade arabinogalactan (Englyst *et al.* 1987). The arabinose side chains and the galactose backbone of arabinogalactan were simultaneously degraded by faecal bacteria. Arabinogalactan-degrading activity was apparent at 3 h, and suggested that faecal bacteria appeared to be able to adapt to the presence of new substrates by rapidly synthesising catabolic enzyme systems. Because these enzymes appeared so rapidly, they may have been produced by bacteria already present in the culture. Mazur *et al.* (1993) also evaluated the ability of human faecal bacteria to ferment a variety of substrates. Homogenised faecal samples from five healthy non-smoking humans were used as the source of inoculum. Arabinogalactan was found to be slowly fermented by human colonic flora. The highly branched nature of arabinogalactan may have hindered access of glycosidases to the glycosidic linkages, thus slowing their degradation.

Vince *et al.* (1990) evaluated the effect of arabinogalactan on bacterial populations *in vivo*. Five healthy human subjects were studied, first while eating their regular diets, and later while consuming their regular diets with the addition of arabinogalactan for 2 days. Prior to, and following the consumption of arabinogalactan, human faecal homogenates were incubated anaerobically for 48 h with and without added arabinogalactan. Consumption of arabinogalactan alone did not change bacterial populations, while a 48-h faecal incubation with the addition of arabinogalactan did change bacterial populations. The consumption of arabinogalactan alone did not significantly affect faecal counts of total anaerobes, total aerobes, Gram-negative anaerobes, Gram-positive anaerobes, enterobacteria, enterococci or lactobacilli. Arabinogalactan consumption followed by arabinogalactan supplementation to faecal incubates led to significant changes in faecal bacterial counts. After 48 h of incubation, Gram-negative anaerobes, Gram-positive anaerobes and enterobacteria populations fell significantly, while no significant changes in enterococci or lactobacilli counts were seen.

Robinson (1999) found that consumption of either 15 or 30 g/day AG significantly increased total faecal anaerobes. A significant increase in *Lactobacillus* spp. was seen when subjects consumed AG for a total of 6 weeks, regardless of dose.

38.5.2 *Short-chain fatty acids and pH*

The predominant short-chain fatty acids (SCFA) in the human colon are acetate, propionate and butyrate. SCFAs produced upon substrate fermentation are readily absorbed from the gut lumen into the intestinal mucosa, and then into the blood. At least 95% of SCFAs produced in the colon are absorbed and therefore cannot be seen in faecal samples; this may be why the consumption of dietary fibre does not consistently produce significant changes in faecal SCFAs (Fleming 1993).

Concentrations of major SCFAs upon fermentation of various substrates are listed in Table 38.1. Acetate was found to be the major anion produced as a result of the fermentation of most substrates, and pectin produced the largest amount of acetate. Arabinogalactan and guar produced the least acetate, but were good sources of propionate. Starch and oat produced large amounts of butyrate, while pectin was shown to be a poor source.

Arabinogalactan is utilised by colonic microflora as a source of energy and produces SCFAs upon fermentation. Salyers *et al.* (1981) analysed the *in-vitro* fermentation pattern of larch arabinogalactan by a polysaccharide-degrading human colonic bacterium (*Bacteroides thetaiotamicron*) growing in a continuous culture. The authors reported that as the rate of

Table 38.1 Molar ratios of major fermentation products from various carbohydrates.

Substrate	Acetate	Propionate	Butyrate
Starch	62	15	23
Arabinogalactan	50	42	8
Guar	58	29	13
Pectin	84	14	2
Gum arabic	66	25	8
Oat bran	57	21	22

Source: adapted from Cummings 1995.

bacterial growth decreased over time, the pattern of fermentation end products changed: acetate and propionate concentrations increased and the concentration of succinate decreased.

Wang and Gibson (1993) evaluated the effects of *in-vitro* fermentation of a variety of substrates. Faecal slurries were incubated for 24 h with the addition of each carbohydrate substrate. Arabinogalactan fermentation was found to produce various gases, including hydrogen, methane and carbon dioxide. It was also found to produce SCFAs at concentrations similar to several other carbohydrate substrates. Molar ratios of SCFAs produced following the fermentation of arabinogalactan were: acetate, 68; propionate, 24; and butyrate, 8.

Englyst *et al.* (1987) examined the fermentation of starch and three NSPs; pectin, xylan and arabinogalactan. These substrates were found to be fermentable by mixed populations of human faecal bacteria in batch culture. Starch and pectin were rapidly degraded, while arabinogalactan and xylan were degraded more slowly. The molar ratios of SCFAs produced were dependent on the polysaccharide tested. Arabinogalactan was found to produce acetate, propionate and butyrate at ratios of 50:42:8, respectively (Table 38.2).

Vince *et al.* (1990) evaluated the effect of lactulose, pectin, arabinogalactan and cellulose on the production of organic acids. Five healthy human subjects were studied, first while eating their regular diets, and later while consuming their regular diets with the carbohydrate additions for 2 days. Prior to and following the consumption of carbohydrates, human faecal homogenates were incubated anaerobically for 48 h with each of the added carbohydrates. Lactulose, pectin and arabinogalactan were shown to be vigorously fermented in the faecal incubates, and produced similar amounts of SCFAs within 48 h. Due to the generation of organic acids, the pH of all samples fell during incubation.

Following the consumption of excess carbohydrate, butyrate exceeded propionate in all incubates, regardless of whether or not additional carbohydrates were added to faecal incu-

Table 38.2 Short-chain fatty acids (SCFA) produced by mixed populations of human faecal bacteria after 48 h growth on different polysaccharide substrates.*

Polysaccharide	Acetate	Propionate	Butyrate	Total
Starch	0.25 (50)	0.13 (22)	0.21 (29)	0.59
Arabinogalactan	0.19 (50)	0.20 (42)	0.04 (8)	0.43
Xylan	0.42 (82)	0.10 (15)	0.02 (3)	0.54
Pectin	0.27 (84)	0.06 (14)	0.01 (2)	0.35

*Bacteria were grown anaerobically in batch culture at 37°C. Values in parentheses are molar ratios of SCFA.
Data are from Englyst *et al.* 1987.

bates, and acetate was the major SCFA in all samples at all times. Because butyrate concentrations were found to exceed propionate in all faecal samples following consumption of these three carbohydrates, the authors concluded that the consumption of these carbohydrates might be of clinical benefit if similar changes in SCFAs occurred within the large intestine.

Bradburn *et al.* (1993) evaluated the colonic fermentation of complex carbohydrates within patients with familial adenomatous polyposis (FAP). This study was conduced with subjects who had a genetic predisposition to developing colonic tumours. Patients with FAP who had tumours were compared with patients with FAP who had not developed tumours, and to controls (patients without FAP). Normal controls ($n = 11$) and FAP patients ($n = 20$) provided stool samples which were incubated anaerobically with various carbohydrates, including arabinogalactan. Faecal SCFA profiles were evaluated by gas chromatography following *in-vitro* fermentation with starch, arabinogalactan, glucose and raffinose.

Arabinogalactan was found to produce less total SCFAs than other carbohydrate substrates. Fermentation of arabinogalactan produced larger molar proportions of propionate and smaller proportions of butyrate.

38.5.3 *Ammonia*

Of all the ammonia found in bodily fluids, the amount found in the human faeces is second only to that found in the urine (Wrong *et al.* 1985). Most of the ammonia in the large intestine is thought to be produced by the deamination of amino acids (Macfarlane & Macfarlane 1997). Wrong *et al.* (1985) conducted a study designed to determine whether endogenous urea was the main precursor of faecal ammonia concentrations. They infused the plasma of healthy human volunteers for several days with ^{15}N-labelled urea and found that only about 8% of the labelled urea reached the large bowel. Thus, they determined that the large bowel was relatively impermeable to ammonia, and that the majority of faecal ammonia was not likely to be produced from endogenous urea. They suggested that faecal ammonia is derived from intestinal secretions, shed epithelial cells, and the deamination of protein from dietary substrates.

High levels of ammonia in the large intestine appear to contribute to detrimental health conditions. Ammonia can alter cell replication, increase viral infections in poultry, and depress immune response. Ammonia has also been shown to affect the intermediary metabolism and DNA synthesis of mucosal cells (Visek 1972, 1978). Ammonia levels as low as 5 mmol/l can have cytopathic effects on colonic epithelial cells. Because ammonia has been shown to be toxic toward epithelial cells, it leads to increased cell turnover. Rapidly dividing cells are reported to be the most vulnerable targets for carcinogenic action (Warwick 1971).

Visek (1978) reported that fibre consumption could reduce the levels of potentially toxic ammonia in the large bowel. Lactulose is fermented in the colon by bacteria that utilise ammonia as a nitrogen source, thus decreasing colonic ammonia concentrations. These bacteria produce SCFAs in the form of anions, and therefore the colonic pH is lowered and the anions may subsequently 'trap' the ammonia.

Normally, ammonia is absorbed from the colon and detoxified by the liver, where it is converted to urea. In patients with liver disease, ammonia can build up and spread systemically, and this in turn may cause hepatic coma. These patients have been successfully treated with antibiotics and lactulose. Weber (1979) evaluated the effect of lactulose on urea metabolism and nitrogen excretion in cirrhotic patients, and found lactulose to increase ammonia utilisa-

tion by colonic microflora. For this reason, lactulose has been used in the treatment of patients with liver insufficiency.

There is research to support the theory that the consumption of arabinogalactan leads to decreased ammonia levels. In the study by Vince *et al*. (1990), lactulose significantly decreased ammonia levels when added to faecal incubates. Pectin and arabinogalactan significantly decreased net ammonia production, but only if subjects had been pre-fed these carbohydrates. Cellulose did not affect ammonia generation. Robinson (1999) found that either 15 or 30 g/day of AG significantly reduced faecal ammonia content.

38.5.4 *Stool weight*

Stephen and Cummings (1980) reported that a fermentable fibre could increase faecal output. They evaluated the mechanism of action of dietary fibre in the human colon by comparing faecal weights following the consumption of two types of dietary fibre. Six healthy male subjects were fed a controlled diet with the addition of 18 g dietary fibre for 3 weeks. It was found that wheat fibre remained largely undegraded, while faecal output was increased due to retention of water in the gut lumen. In contrast, cabbage fibre was extensively fermented and increased faecal output by increasing bacterial mass.

In addition to the fermentability of a fibre, other possible factors may cause increases in faecal weight, including the particle size of the dietary fibre or 'personality' factors associated with the subject. Larger fibre particles may lead to increased stool weights (Wrick *et al.* 1983), as the coarser fibre particles may be degraded more slowly and survive colonic transit, in turn allowing them to absorb more water and increase faecal weight.

38.5.5 *Transit time*

Increases in faecal weight generally lead to reductions in transit time. However, fibre type, fermentability and viscosity may also affect transit time. Insoluble fibres and fibre sources that are poorly fermented are expected to contribute to increased faecal weight, which in turn may lead to accelerated transit time. Because they are not utilised as an energy source, they (and the water they retain) exit the system quickly by stimulating intestinal propulsion and thereby inhibiting their absorption.

Some research indicates that dietary fibres that are largely fermented may reduce intestinal transit time. Possible reasons for such a reduction include the production of acids and gases by substrate fermentation. These gases may contribute to colonic distension and lead to increased colonic propulsion. Edwards *et al.* (1988) found that a fermentable substrate that was completely degraded was associated with accelerated transit time, while non-fermentable fibres did not affect transit time. These authors suggested that the best bulk laxatives might be partially fermentable fibres which accelerate transit and promote bacterial fermentation, yet remain partially structurally intact so that they retain fluid.

It is clear that many factors determine whether faecal weight and transit time will be altered by the consumption of dietary fibre. Since arabinogalactan is a soluble fibre, it is believed to be largely fermented, and therefore not likely to lead to increased faecal weight. Furthermore, arabinogalactan is a fibre with relatively low viscosity and is therefore unlikely to slow intestinal transit time. Robinson (1999) found no significant changes in faecal composition or transit time when 15 or 30 g/day of AG was consumed by normal subjects.

38.5.6 *Cholesterol lowering*

Lower blood cholesterol levels, which are associated with protection against coronary heart disease, may be linked with increased fibre consumption. Marlett (1997) reviewed various studies with dietary fibre, and reported that the viscosity of fibre was inversely correlated with blood cholesterol levels. A review of a comprehensive range of studies by Jenkins *et al*. (1986) reported that viscous fibres (e.g. guar, pectin and locust bean gum) have been shown to produce a hypolipidaemic effect most consistently. These authors suggested that the increased viscosity of the fibres reduced the rate of ileal absorption, which led to increased bile acid losses. Such a mechanism of action is similar to that of cholestyramine, a drug used to reduce serum cholesterol levels.

Another mechanism believed to be involved in the cholesterol-lowering effects of dietary fibre is associated with elevated levels of SCFAs. When dietary fibres are fermented, SCFAs are produced, and high caecal levels of these acids are associated with decreased blood lipid levels, with propionate thought to be the hypocholesterolaemic agent. Diets supplemented with propionate have been shown to lower blood cholesterol in rats (Illman *et al*. 1988). In evaluating a range of studies, Marlett (1997) concluded that the viscosity of dietary fibre – and not its fermentation – was the most likely mechanism responsible for lowering blood cholesterol levels. Since arabinogalactan is a relatively non-viscous soluble fibre, it may not contribute to decreased cholesterol levels. Robinson (1999) found no changes in serum lipids with AG feeding, although individuals with initially elevated blood lipids did see individual lipid-lowering results.

In conclusion, AG is an exciting soluble dietary fibre that is easily incorporated into the diet, changes the gut microflora, and decreases faecal ammonia levels. Current research is directed at defining the immunoenhancing properties of AG.

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Part 12

Oligosaccharides

39 Non-digestible Oligosaccharides and Polysaccharides: Their Physiological Effects and Health Implications

Diederick Meyer and Bryan Tungland

39.1 Introduction

Non-digestible oligosaccharides (NDO) and polysaccharides (NDP) occur naturally in many plants, mainly fruits, vegetables and whole grains. They resist hydrolysis and absorption in the human alimentary system and are partially or completely fermented by the colonic microflora in the large intestine. As such, these carbohydrates are part of a broad class of carbohydrate-based compounds called dietary fibre. NDO and NDP exhibit a variety of chemical and physiological properties.

It is the purpose of this chapter to provide an overview of the most important oligosaccharides and polysaccharides that are classified as dietary fibre, to describe their occurrence and structures, various physiological effects (both at a local and a systemic level) and the scientific evidence for these effects. The evidence for the health effects associated with these physiological effects will also be discussed. Finally, some food delivery systems for specific and general use will be described.

As a general guide for this review, the primary elements of a recent dietary fibre definition developed by an *ad hoc* AACC task force are used. These primary elements include being made up of edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine. Further, the compounds being included in the definition of dietary fibre should provide beneficial physiological health effects, such as laxation, and/or blood glucose attenuation, and/or blood cholesterol attenuation.

Fibres are an integral part of the foodstuffs that humans consume on a daily basis, and the main food sources in which these fibres occur are listed in Table 39.1. Nowadays, it is also possible to enhance the fibre content of food products by the addition of suitable fibre sources (e.g. inulin or hydrolysed guar gum are suitable for this application). Adding fibres with the desired properties offers the opportunity of increasing the physiological value of these foodstuffs.

39.2 Classes of dietary fibres

The physicochemical characteristics can be used to differentiate various types of fibres (Table 39.1). Based on their simulated intestinal solubility (an aqueous enzyme solution representative of the human alimentary enzymes), dietary fibres are classified as either insoluble or soluble. Soluble fibres are further classified based on their precipitation with an ethanol:water (4:1, v/v) mixture. However, this method does not successfully measure a number of oligosaccharides, including polydextrose and inulin. Included among the insoluble fibres are lignin, cellulose and hemicellulose, while examples of those fibres in the soluble

Table 39.1 Source of various fibre components.

Characteristic	Fibre component	Main food source
Insoluble	Cellulose	Plants (vegetables, sugar beet, various brans)
	Hemicellulose	Cereal grains
	Lignin	Woody plants
Soluble	β-Glucans	Grains (oat, barley, rye)
	Pectins	Fruits, vegetables, legumes, sugarbeet, potato
	Gums	Leguminous plants (guar, locust bean)
	Inulin	Chicory, Jerusalem artichoke, onions, wheat

classification include native pectins, β-glucans (mainly oat and barley), galactomannan gums and inulin. The two types of fibres – insoluble and soluble – exhibit somewhat different physiological effects (Table 39.2).

39.3 Physicochemical characteristics and physiological effects of various fibres

Dietary fibres can have rheological effects: some are viscous (such as guar gum), some form gels (pectins), while others have a high water-retention capacity (e.g. cellulose). A changed rheology of the intestinal contents can also have physiological effects: a high viscosity is generally connected with a delayed gastric emptying and increased small intestinal transit time. Fibres with a high water-binding capacity can increase stool mass as they have a stool-bulking effect.

Connected with the solubility of a fibre is its fermentability. Almost all of the soluble fibres are fermented, albeit to varying degrees, by the microorganisms present in the colon. The most notable exceptions are cellulose derivatives such as carboxymethyl cellulose, which is soluble but almost non-fermentable by the human colon flora. Insoluble fibres are mostly poorly fermented in the human colon.

In the stomach and small intestine, large quantities of food are digested and absorbed. Dietary fibres have a significant influence on the rate and effectiveness of nutrient digestion and absorption. Insoluble fibre (fibre as part of a plant cell wall) may impose a barrier for hydrolytic enzymes such as amylases, and this results in a slower digestion of starch. Insoluble fibre generally has a high water-holding capacity, and can further bind enzymes and cations, in turn affecting its availability for digestion and absorption. For example, some of these

Table 39.2 Physiological effects of various fibre types.

Effects	Soluble	Insoluble	Mixed type	Non-digestible oligosaccharides
Gastric emptying	Lower rate	None	?	No effect
Glucose absorption curve	Flattening	Unknown	?	Flattening
Fermentation in colon	Large extent	Hardly	Variable	Completely
Stool bulking	No	Yes	Yes	Yes
Blood cholesterol	Lowering	No effect	Variable	Lowering

fibres may have a negative impact on calcium absorption. Insoluble dietary fibre, through its increased water holding capacity, promotes laxation by increasing stool mass and softness. This fibre is only slowly fermentable in the colon.

Water-soluble fibre generally promotes slow transit through the stomach and increases the small intestinal transit time (Gurr & Asp 1994). This fibre is rapidly fermented in the large intestine. However, just as not all fibres fit one analytical method, not all fibres fit a neat physiological-effect definition. Some soluble fibres increase viscosity (xanthan gum, pectin, guar gum), which in turn slows the transit of chyme in the upper gastrointestinal tract and results in slower absorption rates, lower blood concentrations of nutrients and altered hormonal responses to the absorbed nutrients (e.g. glucose). Some dietary fibre can sequester bile salts and thus may indirectly affect lipid absorption. Other non-viscous soluble fibres such as inulin have little effect on gastric emptying, but are effective through fermentation in producing specific short-chain fatty acids (SCFAs), which themselves change the intestinal microflora, modulate intestinal pH and appear to affect blood glucose and cholesterol levels by regulating hepatic enzyme activities (see Table 39.2).

The major physiological effects of dietary fibres originate from the interactions with the colonic content: the fermentation of dietary fibres by the anaerobic colonic microflora is the basis for the physiological effects of fibre consumption. The physical and chemical nature of the fibre determine the extent of the fermentation, which may range from completely fermented (as for inulin) to no fermentation (as for cellulose particles). An example is shown in Fig. 39.1; in this figure it is clear that the chemical structure affects the fermentability of a fibre. Cellulose composed of β-1,4-linked glucose is hardly fermented, whereas starch – which has α-1,4 and α-1,6 linked D-glucosyl residues – is much more susceptible. It is also clear that amylose film shows limited fermentation due to its limited accessibility to digestive enzymes, whereas an amylose gel, being much more accessible to enzymes, is fermented to a large extent.

Soluble fibres are fermented to a varying degree by the anaerobic colonic microflora, and the physiological effects originating from this colonic fermentation are diverse. The fermentation leads to an increased biomass and hence to an increase in faecal mass (stool-bulking effect). The fermentation products – SCFAs (mainly acetate, propionate and butyrate; see Fig. 39.1) and lactic acid – lead to a decrease in intracolonic pH, whereas the gas from the fermentation may lead to stimulation of peristalsis.

Insoluble fibres are only fermented poorly once they reach the colon. Their water retention exerts a stool-bulking effect that causes shorter transit times and a larger faecal mass. This means that both soluble and insoluble fibres have a stool-bulking effect, but that the mechanisms are quite different. The data provided in Fig. 39.1 show the differences in fermentation products from a variety of fibres in *in-vitro* experiments with human faecal slurries. Not only does the amount of fermentation products differ, but qualitative differences are also found. Thus, with poorly fermentable cellulose very little acid is formed, and this is composed of just acetic acid; in contrast, in the case of fermentable fibres larger quantities of SCFA are formed, including propionic, butyric and acetic acids.

At the colonic level, the fermentation may exert local health effects such as inhibition of growth of pathogens, increased mineral absorption or production of vitamins. The SCFAs are absorbed into the blood stream of the host and reach other organs where they can affect metabolism. This can lead to systemic effects such as changes in glucose and fat metabolism, and can effect glycaemia and lipidaemia. SCFAs are also metabolised by the host, delivering a certain amount of energy. The energy content of a fibre is, from a scientific point of view,

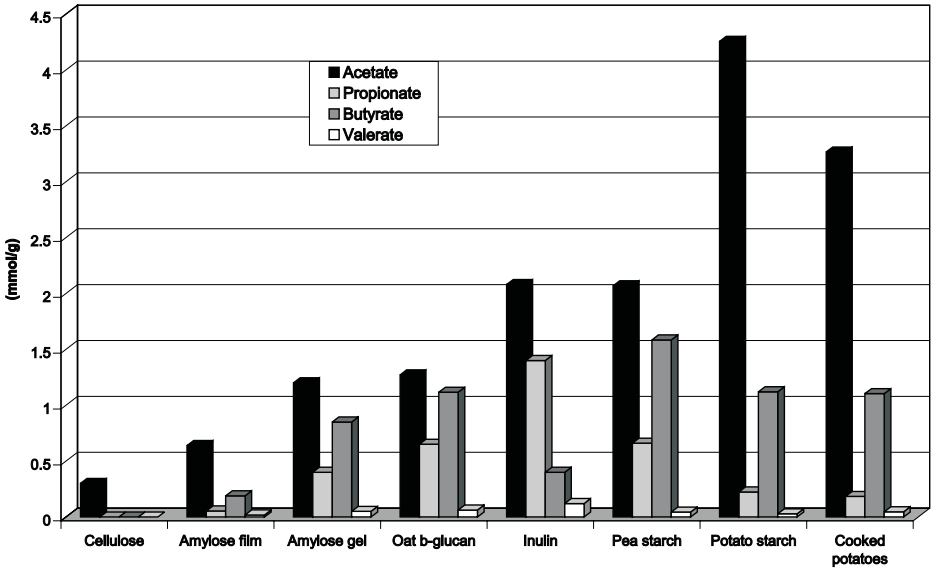

Fig. 39.1 *In-vitro* fermentation of various fibres by human faecal slurries. (Data from Botham *et al*. 1998.)

dependent of the degree of fermentation. Fibres that are not fermented at all have a caloric content of 0 kcal/g, while complete fermentation leads to a maximum of about 2 kcal/g. It should be noted that legal values for nutritional labelling can be different.

The SCFAs play a pivotal role in the physiological effects. This means that fermentable fibres, in particular, potentially lead to a range of effects. An improved colonic physiology may give rise to a variety of health effects, some of them at a local level (e.g. restoration of a normal intestinal flora and improved mineral absorption from the colon) and others at a systemic level (e.g. lowering of serum lipids). The latter is an example of a potential health effect; a high serum lipid level is connected with an increased risk for cardiovascular disease. This risk may be lowered by the consumption of fermentable fibres (Fig. 39.2).

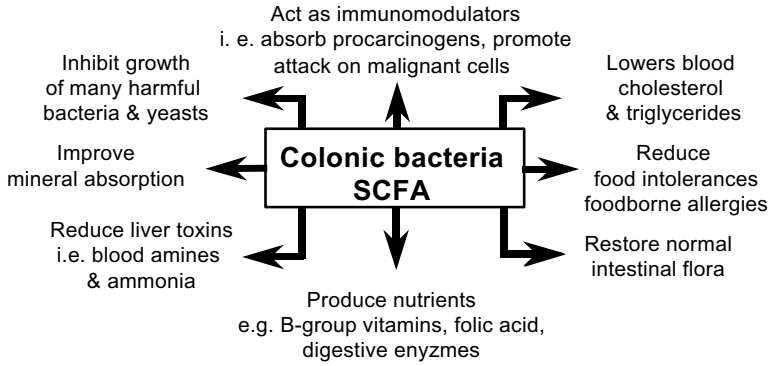

Fig. 39.2 Schematic representation showing the relationship between colonic bacteria and short-chain fatty acid (SCFA): related physiological effects.

The relationship between dietary fibre and colon carcinogenesis should also be mentioned here. Both soluble and insoluble fibres may exert their own effects; the latter by assuring a proper disposal of potentially carcinogenic waste products, thereby limiting exposure of colonocytes to these products. The fermentation of soluble fibre leads to butyric acid, which is a preferred substrate for colonocytes, preventing them from conversion to tumour development. Fibres may also affect other parameters related to colon cancer. Enzymatic activity converting procarcinogens into carcinogens can be decreased, as well as the secondary bile acid concentration and the cytotoxicity of faecal water. All of these factors may contribute to a diminished risk of colon cancer, but other factors such as age and genetic background also play an important role (Chaplin 1998).

Further details on the relation between gastrointestinal physiology and function, and food science is discussed by Salminen *et al*. (1998).

39.4 Physicochemical properties and effects on human physiology of selected NDP and NDO

An overview of many different NDOs, their chemical make-up and occurrence, and a summary of the effects on the human intestine is given in Table 39.3. The physical and chemical structures of selected NDOs and NDPs will now be described, with particular focus on possible physiological effects of these materials based on human studies.

39.4.1 *Resistant starch*

According to Asp (1992), resistant starch is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals. It therefore reaches the colon intact, where it may be (partly) fermented. Several types of resistant starch are discerned: starch that is physically inaccessible to α-amylase (cf. the amylose film in Fig. 39.1), uncooked starch granules, and retrograded starch, all of which can exist in the same food. Resistant starch is part of our daily diet (3–6 g/day; see Dysseler & van Hoffem 1995), or can be added as an ingredient to various food products.

The colonic fermentation of resistant starch in humans is well documented (Heijnen *et al*. 1998). *In-vitro* experiments with human faecal slurries corroborate this (e.g. Botham *et al.* 1998). Stool-bulking effects were also found, probably as a consequence of the increased bacterial mass in the faeces. Whether consumption of resistant starch leads to a stimulation of the growth of specific bacteria in the colon remains unclear.

To date, the health effects of resistant starch consumption are less clear. In a human study, no effect on serum lipid level was observed (Heijnen *et al*. 1996). Neither were any effects found on the apparent absorption of magnesium, calcium and phosphorus (Heijnen & Beynen 1998). Much attention nowadays is focused on biomarkers related to colon cancer. Fermentation of resistant starch gives rise to relatively high amounts of butyric acid (Phillips *et al*. 1995) which, as a preferred substrate for colonocytes may prevent tumorigenesis. However, Heijnen and Beynen (1998) could not find either any increase in faecal butyric acid or change in secondary bile acid concentrations. Animal model studies, on the other hand, show that resistant starch inhibits chemically induced carcinogenesis in the colon of rats.

Table 39.3 Non-digestible oligosaccharides.

Name	Type of bond and composition	DP	Occurrence and production	Human intestinal effects
Cellobiose and cellodextrins			Not in nature, degradation of cellulose	No studies on prebiotic effects
Cellobiose (CEL)	4-O-β-D-glucopyranosyl-D-glucose	–		
Cellodextrins	$((4\text{-O-}\beta\text{-D-glucopyranosyl})_n\text{-D-glucose})$	3–6		
β-Cyclodextrins	cyclic molecules of α-1-4 linked D-glucose, (α-cyclodextrin-hexamer, β-cyclodextrin-hetamer and γ-cyclodextrin-octamer)	–	Not in nature	May have effect based on fermentation, no published studies on effects
Fibersol-2	Mixed & random glycosidic llinkages	3–10	Produced by pyrolysis of corn starch	Partially digestible
Gentiooligosaccharides (GeOS)	mixture of β-1-6 linked D-glucose oligomers	2–5	Not in nature, in Japan by transglucosylation of glucose, marketed by Gentose	Scarce data, no conclusion on effects on intestinal microflora
Glucooligosaccharides (α-GOS)	mixture of α-D-glucose	2–6	by transglucosidation using an α-glucosidase from *Leuconostoc mesenteroides*, marketed in France by BioEurope (Solabia Group)	Animal studies show influence of intestinal microflora. No human studies to date
Isomaltooligosaccharides	linear α-1-6 linked glucose residues, some 1-4 linkages	2–8	Occur in miso, soy sauce and sake, honey. Prepared commercially by transglucosylation of glucose residue. By transglucosidase (α-glucosidases). Commercially available as Isomalto-900 in Japan. Produced by Showa Sangyo, Niihon Shokuhin Kako, Nikken Kagaku, Hayashibara	Both animal and human studies indicate influence of intestinal microflora
Isomaltose – IMA	6-O-α-D-glucopyranosyl-D-glucose			
Isomaltotriose – IMT	6-O-α-D-glucopyranosyl-(1-6)-α-D-glucopyranosyl-D-glucose			
Panose – PAN	6-O-α-D-glucopyranosyl-(1-6)-α-D-glucopyranosyl-(1-4)-D-glucose			
Leucrose	(5-O-α-D-glucopyranosyl-D-fructopyranoside	–	By-product of microbial dextran production, found in foods fermented by *L. mesenteroides*, Transglucosylation of sucrose	No data available on fermentation by intestinal bacteria or effect on activity of intestinal flora
Leucritol	Mixture of 60% glucosyl-sorbitol and 40% glucosyl-mannitol	–	Not in natural products. Prepared from leucrose by catalytic hydrogenation	No data available on fermentation by intestinal bacteria or effect on activity of intestinal bacteria

Maltitol – MAT	6-O-α-D-glucopyranosyl-D-sorbitol	–	Prepared from maltose by catalytic hydrogenation	Fermentation by several intestinal strains of bacteria, may have an effect on composition or activity, but not studied in humans
Palatinose or isomaltulose – PAL	6-O-α-D-glucopyranosyl-D-fructose	–	In beet sugar processing, honey, cane juice. Produced from sucrose by α-glucosidase reaction of *Protaminobacter rubrum*	Data show fermentation by intestinal flora, potential influence
Palatinose condensate – (PCO)	mixture of oligosaccharides (tri- to octamers) of glucose and fructose	2–7	By heating palatinose under suitable conditions	Studies with human volunteers have shown effects on bacterial composition
Palatinit	equimolar mixture of 6-O-α-D-glucopyranoside-D-mannitol and 6-O-α-D-glucopyranoside-D-sorbitol (maltitol)	–	Prepared from palatinose by reduction	Data on fermentation by intestinal strains and effects are not available
Theanderose	O-α-D-glucopyranosyl-(1-6)-O-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside	–	Naturally occurring sugar, present in honey, commercially produced using fungus *Mucor javanicus*	Human trial suggests partial indigestiblity with intestinal bacteria modification
D-Tagatose	D-*lyxo*-hexulose	–	Produced from whey, lactose or galactose by alkaline isomerization. Commerically marketed by Biospherics Inc. MD	Studies indicate fermentation
Lactitol (LTOS)	4-O-β-D-galactopyranosyl-D-sorbitol	–	Not in nature. Hydrogenation of lactose	Fermented rapidly and likely effect on intestinal metabolism. No studies yet conclude effect on composition of intestinal microflora
Lactosucrose (LAS)	O-β-D-galactopyranosyl-(1-4)-O-α-D-glucropyranosyl-(1-2)-β-D-fructofuranoside	–	Not yet detected in any natural food product. A raffnose isomer, by action of a fructofuranoside from *Arthrobacter* sp. on a mixture of lactose and sucrose. (Ensuiko Sugar Refining Co. LTD, Japan)	Several Japanese studies show effect on either the composition or the metabolism of the intestinal microflora

Name	Type of bond and composition	DP	Occurrence and production	Human intestinal effects
α-galactooligosaccharides (raffinose, stachyose/other soy oligosaccharides)	RAF, O-α-D-galactopyranosyl-(1-6)-α-D-glucopyranosyl-β-D-fructofuranoside	3–4	Extraction from soybeans, legumes. Marketed by Calpis Food Industry Co. Ltd	May have significant effect on composition and activity of intestinal flora
β-galactooligosaccharides or transgalacto-oligosaccharides (TOS)	β-D-galactopryanosyl-(1-6)-[β-D-galactopyranosyl]$_n$-(1-4) α-D-glucose	2–8, [3]	Likely some in nature, enzymatic transgalactosylation of lactose. Produced in Japan (Oligomate, Yakult Ltd, San-ei Sucrochemical Co Ltd) and Borculo Whey Products, NL	Studies indicate intestinal flora effects on composition and activity
Lactulose (LAT)	4-O-β-D-galactopyranosyl-D-fructose	–	Not in nature. Alkaline isomerisation of glucose moiety of lactose to fructose, marketed as laxative and gut health aid. Morinaga Nyugyo, Nikken Kagaku-Japan	Gas production is relatively large, due to fermentation by clostridia, Kl. Pneumoniase. Studies suggest effects on bacterial composition and activity
Lactitol-oligosaccharides (LTOS)	4-O-β-D-[galactopyranosyl]$_n$-D-sorbitol	3–8?	Not in nature. Transgalactosylation of lactitol using *Asperigillus oryzae* β-galactosidase	A study in rats indicates effects on composition and metabolic activity of intestinal flora, no human data are available
4'-galatosyllactose (GLL)	O-D-galactopyranosyl-(1-4)-O-β-D-galactopyranosyl-(1-4)-D-glucopyranose	–	Not in nature. Produced in Japan by action of *Crytococcus laurentii* on lactose. Marketed as Cup-oligo powder or syrup by Nisshin Sugar Mtg. Co., Japan	Unaffected by human enzymes. Likely to have effect on composition and metabolic activity in human intestinal microflora
Synthetic galactooligosaccharides		–	Prepared chemically by the Koenigs-Knorr reaction	No animal or human data exists. Unknown if these products are digested in the upper GI tract
Neogalactobiose (NGB)	β-D-galactopyranosyl-β-D-glucopyranoside			
Isogalactobiose (IGB)	β-D-galactopyranosyl-α-D-glucopyranoside			
Galsucrose (GAS)	α-D-galactopyranosyl-β-D-glucopyranoside			
Isolactose I (IL1)	α-D-galactopyranosyl-β-D-fructofuranosyl-(2-6)-β-D-fructofuranoside			
Isolactose II (IL2)				
Isolactose III (IL3)				
A lactose trimer				

Fructans				Data indicate that addition of inulin-type fructans affect the bacterial composition and the metabolic pattern of the intestinal microflora. Studies have shown production of short chain fatty acids and a relatively high production of propionate and butyrate, necessary for colonic health and systemic influences on blood glucose and lipids
Levan-type	β-D-(2→6)-fructofuranosyl)$_n$ α-D-glucopyranoside	2–3 M	Produced by *Bacillus polymyxa* on sucrose Naturally occurring	
Inulin-type	β-D-((2→1)-fructofuranosyl)$_n$ α-D-glucopyranoside	2–65	Naturally occurring in J. artichokes, chicory, onion, etc. Produced by extraction from plant material, Produced by Sensus, ORAFTI, Cosucra	
Fructooligosaccharides	mixtures of 1- kestose, nystose and 1f-β-fructofuranosylnystose	3–5	Produced by transfructosylation of a β-fructosidase of *Aspergillus niger* on sucrose, Meiji Seika Kaisha LTD-Japan	
	β-D-((2→1)-fructofuranosyl)$_n$ β-D-fructofuranoside	1–7	Partial enzymatic degradation of native plant inulin, ORAFTI	
Xylooligosaccharides	β-D-((1→4)-xylose)$_n$	2–4	Partial enzymatic hydrolysis of polyxylan by xylanase from *Trichoderma* sp.	Not hydrolysed by human enzymes, utilised in colon by several species, including bifidiobacteria, *E. coli*, clostridia, lactobacilli and *Bacteroides* sp. Changes in metabolic pattern of intestinal flora have been observed in rats, but not humans.
Alginate oligosaccharides	High in mannuronic and guluronic residues	–	Produced by enzymatic degradation of alginate, INRA Nates and CEVA (Centre d'Etudes et de Valorisation des Algues, France (non-commercial)	Limited data, algal oligosaccharides have an effect on either the metabolism or composition of the intestinal microflora.
Guar gum oligosaccharides	Galactomannan residues	–	Produced by partial hydrolysis of guar gum, Benefiber®-Novartis Corporation	Fermented by colon microflora.
Mannoligosaccharides	Mannoligosaccharide mixture containing less than 50% oligosaccharides	–	By *Saccharomyces cerevisiae* on sucrose. Produced by Bio Mos, Alltech, Inc.	Used as growth promoter in animal feed industry. No data available on fermentation either by bacterial strains or from human trials

39.4.2 *Pectins*

Pectins are present in plant cell walls, and are polysaccharides composed of an α-1,4-linked D-galacturonic acid backbone with a variable amount of neutral sugars (arabinose, galactose, xylose) present as side chains. Rhamnose may also be present in the backbone. The galacturonic acid residues are substituted with acetyl and methyl ester groups (Schols & Voragen 1996). The main applications for pectins as food additives are as gelling and thickening agent in many food products. Most pectins for food use are extracted from citrus or apple.

Due to their gelling behaviour, these soluble polysaccharides may decrease the rate of gastric emptying and influence small intestinal transit time, properties which explain their hypoglycaemic effects (Jenkins *et al*. 1978).

Various human studies show that pectins are fermented to a large extent in the colon (e.g. Cummings *et al*. 1979; Hillmann *et al*. 1983). No effects on stool weight are observed (Eastwood *et al*. 1986), but pectins effect a decrease in serum cholesterol (Kay & Truswell 1977).

39.4.3 *Guar gum*

Guar gum is a galactomannan isolated from the seed of *Cyamopsis tetragonolobus* (guar). In its unmodified form this food additive is used as a thickener in a large variety of food products. Partial enzymatic hydrolysis results in a product that can be used as a soluble dietary fibre. The physiological effects of this fibre source comply with what might be expected from a soluble fibre:

(1) It is readily fermented by the human faecal microflora (Salyers *et al*. 1977) and it has bifidogenic effects (at least with enteral feeding; Okubo *et al*. 1994).
(2) It improves bowel functioning (reduces diarrhoea in enterally fed patients; Homann *et al*. 1994) and relieves constipation in patients (e.g. Takahashi *et al*. 1994).
(3) It shows a hypolipidaemic effect in humans, lowering both serum cholesterol and triglycerides (Takahashi *et al*. 1993).
(4) It reduces postprandial glycaemia (Wolever *et al*. 1979).

39.4.4 *Gum arabic*

This exudate from the acacia tree is a complex arabinogalactan polysaccharide in admixture with a glycoprotein. It has a high molecular weight, and it is used as an additive in many food applications as a stabiliser and emulsifier. The physiological effects from human studies include:

(1) It is fermented completely in the human colon, with indications for a bifidogenic effect (McLean-Ross *et al*. 1983; Wyatt *et al.* 1986; Michel *et al*. 1998).
(2) It lowers serum triglyceride and cholesterol levels (McLean-Ross *et al*. 1983).

39.4.5 *Fructans*

Fructans can be divided in two classes, levan and inulin. Levans are β-2,6-linked fructans with variable degrees of β-2,1-linked side chains that are produced by a large variety of

bacteria. Inulins, on the other hand, are composed of a backbone of β-2,1-linked fructosyl units, and are produced by many dicotyledonous plants as a reserve carbohydrate. Side chains linked via β-2,6-links, may be present in some plant inulins (Vijn & Smeekens 1999). Inulin is present in a wide variety of plants and vegetables and form a part of the daily western diet (van Loo *et al*. 1995). The background consumption from wheat, onions, leek and other vegetables ranges from 3 to 10 g/day.

Levans are not commercially available, and only limited data on their physiological or health effects are available. However, it should be noted that in a rat study, a lowering of serum cholesterol level was found when levan was included in the diet (Yamamoto *et al*. 1999), whereas no breakdown by colonic bacteria was observed.

Inulins are commercially produced by extraction from chicory roots. Fructo-oligosaccharides (FOS) are produced, either by enzymatic hydrolysis of inulin or by trans-fructosylation from sucrose. Inulin is used as a food ingredient for a variety of reasons, including fat and sugar replacement, as a low-caloric bulking agent and as a texturising agent. It is also used for its physiological features of being a soluble dietary fibre and having prebiotic characteristics.

The physiological effects of inulin and FOS are identical. Since the β-2,1-bonds are not susceptible to hydrolysis in the human gastrointestinal tract, these compounds reach the colon intact, where they are completely fermented, especially by Bifidobacteria and Lactobacilli (the growth of which are stimulated by these fructans) (Causey *et al*. 1999; Kruse *et al*. 1999). The physiological effects originating from this fermentation are both local and systemic.

Inulin and FOS are perhaps the most intensively investigated soluble dietary fibres. During the 1980s, studies were performed in Japan that indicated the physiological effects. The bifidogenic potential (Hidaka *et al.* 1986), hypolipidaemic effects (Mitsuoka *et al*. 1986), and improved bowel function (Hidaka *et al*. 1986). Later studies not only with FOS but also with inulin have shown the following physiological effects:

- Stimulation of colonic Bifidobacteria and Lactobacilli (e.g. Gibson *et al*. 1995; Kleessen *et al.* 1997; Kruse *et al*. 1999).
- Improvement of bowel function (Kleessen *et al*. 1997).
- In some studies, a lowering effect on serum lipids was found (e.g. Causey *et al*. 2000); however, these results are debatable as other studies showed no effect (e.g. Pedersen *et al*. 1997; van Dokkum *et al*. 1999; Meyer 1999).
- FOS (van den Heuvel *et al*. 1999) and inulin (Coudray *et al*. 1997) stimulate calcium absorption in humans.
- Activity of faecal enzymes is changed (Buddington *et al*. 1996), and the decrease in β-glucuronidase activity indicates a decreased potential for forming carcinogens (Causey *et al*. 1999; van Dokkum *et al*. 1999).
- In animal model studies inulin prevented early neoplastic lesions after chemically induced carcinogenesis (e.g. Reddy *et al*. 1997).

39.4.6 *Galacto-oligosaccharides*

These oligosaccharides are produced from lactose by the transglycosylating activity of β-galactosidase. They consist of a number of β-1,6-linked galactosyl residues linked to a terminal glucose unit via an α-1,4-bond.

Galacto-oligosaccharides are not digested in the human alimentary tract. However, reports have been made showing a change in colon flora composition and activity following consumption of these compounds (Ito *et al*. 1990; Alles *et al*. 1999), showing the prebiotic nature of these compounds. Various human studies show that these oligosaccharides may relieve constipation, improve calcium absorption, and retard the development of colon cancer in rat model systems (for a review, see Schoterman & Timmermans 2000).

Another type of galacto-oligosaccharides is isolated from soybeans. These α-galacto-oligosaccharides (galactosyl-sucrose oligosaccharides) include raffinose, stachyose and verbascose and consist of galactose residues linked α-1,6 to the glucose moiety of sucrose. The physiological effects of these oligosaccharides appear to be similar to the β-linked galactose oligomers; they are bifidogenic (Oku 1996), and hence other effects can be expected from this change in colon flora. However, no further data are available at present.

39.4.7 *Lactulose*

Lactulose is a disaccharide of D-galactose linked 1,4-β to fructose. It is manufactured from lactose, where alkali isomerisation is used to convert the glucose moiety in the lactose into a fructose residue. The disaccharide is not digested by humans, and promotes growth of bifidobacteria in the colon (Modler 1994; Strohmaier 1996). Lactulose is mainly used as a pharmaceutical to prevent constipation, and also in portosystemic encephalopathy. It is not allowed for food use in Europe but seems to find its way into the food supplement market (at least in The Netherlands). Although it is clearly used for physiological reasons and it has well-established health effects (prevents constipation), these effects cannot be exploited in the food industry in the EU because it is not allowed for food use. The physiological effects of lactulose are exploited in Japan (Mizota 1994).

39.4.8 *Other oligosaccharides*

As indicated in Table 39.3, a wide variety of oligosaccharides, all of which have effects on the activity and/or composition of the human colon flora, have been described in the literature (Oku 1996; Playne & Crittenden 1996).

There is a variety of fibre ingredients available that can be used to influence the physiology of the human digestive process in a beneficial way. Placing emphasis on the physiological properties of a fibre has several advantages, particularly if these properties define the basis of the dietary fibre complex, rather than being driven by a set of analytical criteria. By establishing a clear distinction of the physiological basis of particular fibre components within the dietary fibre complex, one provides a means by which to increase the number of sources of dietary fibre to be used by various food manufacturers. These new sources of fibre provide the tools required to create healthier foods with enhanced organoleptic properties, this being particularly true for inulin. Increasing the number of new fibres for food texture and functional food use also creates new research opportunities in both nutrition and food science. In addition, focussing on the physiological health aspect of dietary fibre, and the types of fibres meeting a physiological definition, will help to communicate further to the consumer that fibre is important in their diets. This in turn will educate the public – which currently is not consuming adequate levels of fibre – on where to obtain additional sources. However, it should be realised that not all of the potential ingredients listed are currently permitted for food use. For example, inulin, FOS and β-galacto-oligosaccharides are permitted for food use

in most European countries, the US and Canada, but a much wider range of oligosaccharides may be used in Japan.

39.5 Applications

The main technological functions of fibre polysaccharides (such as pectin and guar) in food has already been mentioned. These polysaccharides are used mainly to modify the texture of the food product, which explains why they are mostly listed in handbooks as thickeners, gelling agents, emulsifier and stabilisers rather than as fibres.

In addition to guar and pectin, a number of other polysaccharides are available to modify or stabilise the texture of a food product [e.g. alginates, carrageenans, cellulose and derivatives (modified) starch]. These polysaccharides are not treated in this overview, because the physiological effects of most of them are limited; their main or only function is in rheology modification and stabilisation. Furthermore, they are often used in low concentrations in food products, such that physiological effects cannot be expected to occur. However, most of these are to be considered as dietary fibres according to the definition given in Section 39.1.

In contrast to the fibre polysaccharides, NDO – and more specifically inulins – are used in food products because of their physiological functionality as prebiotic fibre, as well as their technological characteristics as both sugar and fat replacers, texturiser and gelling agent.

The texturising and gel-forming features of inulin, in addition to its neutral taste, allow its use as a fat and sugar replacer or texturiser in a wide variety of food products. Inulin is used in an ever-increasing range of products such as dairy foods (yoghurt drinks, desserts, ice cream, cheese products), bakery products (bread, cookies), spreads, beverages, cereals and confectionery. In these products the physiological functions as well as the technological functions can be exploited. For instance, in yoghurt drinks aimed at 'balancing your gut flora', inulin will also function to improve texture and mouthfeel, resulting in a healthy product with a perfect taste sensation.

In spreadable products, like a very low-fat spread, inulin offers the opportunity to reduce the fat content even below 5%, while maintaining the spreadability and mouthfeel of the product. In addition, health claims can be made referring to the presence of prebiotic fibres.

In addition to the above applications, which can benefit the general population, it is also possible to formulate products for specific target groups. The anti-constipation effects of inulin and other NDO can be exploited in food products for the elderly (most of the studies showing this effect were carried out with elderly people). The improved calcium absorption brought about by inulin or lactulose can be used to make products with these ingredients for people requiring a high calcium uptake, such as adolescents or post-menopausal women.

At present, labelling of such applications requires much care, as the labelling must avoid statements related to curing or preventing diseases, as this is not allowed for food products. This is more or less the current situation in Europe. It is to be anticipated that health claims on food products will become possible in the future, as legal frameworks will be set up. In the USA, nutrient content or structure–function claims are already allowed. With regard to fibre, health claims on 'fibre-containing products', fruits and vegetables, and cancer are allowed, as well as on the use of fruits, vegetables and grain products that contain fibre (particularly soluble fibre) and the risk of coronary heart disease. For these claims to be applicable to NDO these should legally be considered as fibres. This is not always the case, as NDO escapes detection by internationally recognised official analytical methods for fibre determination. It

is to be expected however, that the fibre features of NDO, such as those described above, will convince legal authorities that NDO should be seriously considered for dietary fibre labelling status. This will allow the food industry to fully exploit all of the properties of NDO, both technological and physiological.

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40 Development and Beneficial Effects of Fructo-oligosaccharides (Neosugar®)

Hidemasa Hidaka, Takashi Adachi and Masao Hirayama

40.1 Introduction

One of the most promising findings for the development of resistant oligosaccharides is thought to lie in their indigestibility in the small intestine. This characteristic promotes growth of *Bifidobacterium* and the production of short-chain fatty acids (SCFA) in the large intestine, both of which are understood to be key parameters associated with the beneficial effects of resistant oligosaccharides. Pioneering work in this area was initiated by our group in 1980, with the development of the mixture of oligosaccharides [fructo-oligosaccharides (FOS); Neosugar®) (Hosoya 1982; Hirayama & Hidaka 1993). Neosugar is a mixture of 1-kestose (GF_2), nystose (GF_3) and 1-fructofructanosylnystose (GF_4). The first target in this research was to develop materials which would lower cariogenicity, and FOS were shown to have the desired effect (Ikeda *et al*. 1990). However, during the course of such evaluations, the indigestibility of these oligosaccharides in the ileum – but fermentation in the large intestine – was discovered. Subsequently, the potential physiological properties in the colon have been used to develop a novel area of functional foods (Hidaka 1983). In this chapter, we describe the beneficial properties of the resistant oligosaccharides which have already been developed, and those expected from further research.

40.2 Research and development of resistant fructo-oligosaccharides

40.2.1 *Natural occurrence and commercial production through enzymatic transglycosylation*

Fructo-oligosaccharides are widely found in many types of edible plant, including onion, garlic, edible burdock, wheat and bananas. In the United States, the daily intake of FOS from common food items has been estimated to be approximately 800 mg (Spiegel *et al*. 1994).

FOS can also be prepared from sucrose, through the transfructosylating action of either β-fructofuranosidases (EC 3.2.1.26) or β-fructosyltransferases (EC 2.4.1.100). The ability of the former enzyme to hydrolyse sucrose has been known for many decades; however, it was not realised that some of these enzymes have sufficient transfructosylating activity to produce oligosaccharides as the major reaction products. A fungal enzyme from *Aspergillus niger* ATCC 20611 has been applied successfully to the industrial production of Neosugar® from a sucrose syrup of 50–60% (w/v), because of its higher transfructosylating (Hidaka *et al*. 1988; Hirayama *et al*. 1989) (Fig. 40.1). Two types of FOS are commercially available, Neosugar® G and Neosugar® P. The former (Neosugar® G) consists of 35% (w/w) of glucose (Glc) and fructose (Fru), 10% (w/w) of sucrose (GF), and 55% (w/w) of FOS. The latter is produced from Neosugar® G by removing monosaccharides, and contains greater than 95%

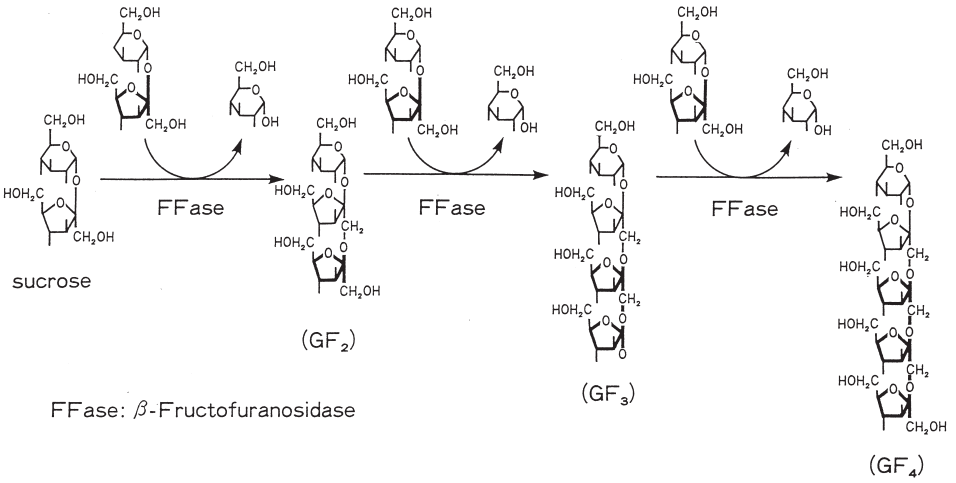

Fig. 40.1 Enzymatic preparation of fructooligosaccharides, 1-kestose (GF_2), nystose (GF_3), and 1^F-β-fructofuranosylnystose (GF_4).

(w/w) of FOS. The sweetness of Neosugar® G and P is about 60% and 30%, respectively, of that of sucrose, and both are stable at neutral pH at temperature up to 140°C.

40.2.2 *Discovery and confirmation of resistant oligosaccharides*

The lack of hydrolysis of FOS by the digestive enzymes of rat and humans (such as the disaccharidases of intestinal mucosa and α-amylase of pancreatic homogenates) was shown by Oku *et al.* (1984). Sugar tolerance tests on healthy subjects indicated that FOS ingestion did not cause any increase in either plasma glucose or fructose, or in the degree of insulin response, despite there being a rapid increase of both parameters following sucrose ingestion (Yamada *et al.* 1990) (Fig. 40.2). Thus, these results showed that FOS were neither digestible nor absorbable.

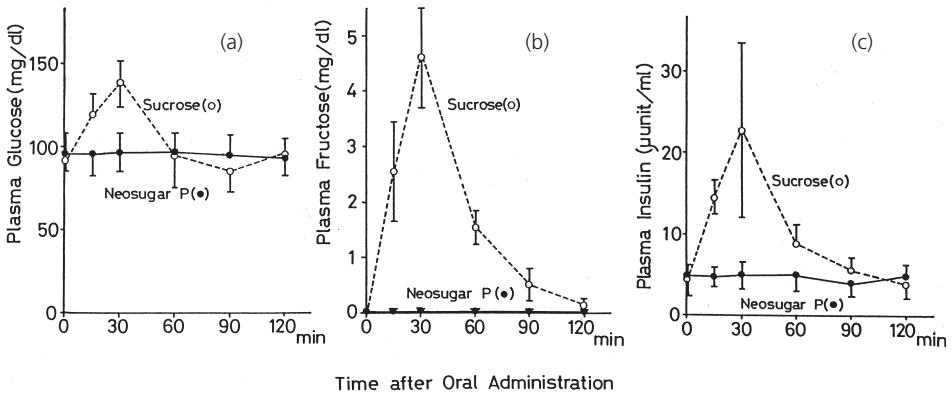

Fig. 40.2 Plasma glucose (a), fructose (b) and insulin (c) after oral administration of fructo-oligosaccharides (25 g) (●) or sucrose (25 g) (○) to healthy men. Values are given as the mean ± SD of six subjects.

The bioavailability of FOS in rats (Tokunaga *et al*. 1989) and humans (Hosoya *et al.* 1988) was investigated in *in-vivo* radiorespirometry studies using [U-^{14}C]-FOS, together with *in-vitro* faecal incubation. When labelled FOS were ingested by healthy men, 49% and 55% of the administered radioactivity was detected in the expired $^{14}CO_2$ after 24 and 48 h, respectively (Fig. 40.3a). Maximal $^{14}CO_2$ excretion was reached at 7 h after administration, which differed somewhat from the situation found with digestible saccharides, when maximal $^{14}CO_2$ excretion occurred after 3–4 h. During anaerobic incubation of the labelled FOS with the faeces of the subject, FOS were catabolised primarily to SCFA (57.8%), including acetic, propionic and butyric acids, and to $^{14}CO_2$ (9.6%) (Fig. 40.3b). These results show that FOS pass through the small intestine and are fermented by intestinal bacteria in the colon to yield CO_2 and SCFA, both of which are absorbed and metabolised, appearing as CO_2 in the expired air (Fig. 40.4).

The key characteristic of resistant oligosaccharides is considered to be their indigestibility in the small intestine, which results in their passage into the large intestine where they are subsequently fermented by intestinal bacteria to produce a healthy microflora. Intestinal fermentation promotes useful characteristics through bifidogenic effects and SCFA production.

40.3 Useful characteristics obtained through intestinal fermentation

40.3.1 *Selective utilisation by healthy intestinal bacteria*

Intestinal bifidobacteria and lactobacilli are well known as 'favourable' bacteria, while some other bacteria such as *Escherichia coli* and *Clostridium perfringens* are 'unfavourable' for humans and animals, both in terms of health and nutrition (Mitsuoka 1982). The *in-vitro* utilisation of FOS compared with several other carbohydrates by intestinal bacterial species is shown in Table 40.1 (Hidaka *et al*. 1986). FOS were utilised by all bifidobacteria except for *Bifidobacterium bifidum*, but were not utilised by *Lactobacillus fermentum*, *E. coli* or *C.*

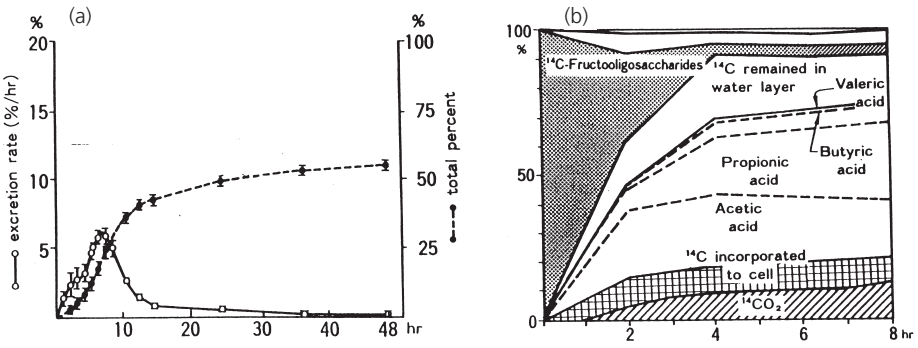

Fig. 40.3 (a) $^{14}CO_2$ expiration after ingestion of [U-^{14}C]fructo-oligosaccharides in six subjects. The open circles indicates the $^{14}CO_2$ expiration rate (% of ingested radioactivity per hour), and the closed circles show the cumulative amount of $^{14}CO_2$. The circle and vertical bars represent the mean of six subjects and the standard error of the mean, respectively. (b) Degradation profile of [U-^{14}C]fructo-oligosaccharides during anaerobic incubation with faeces of the subjects.

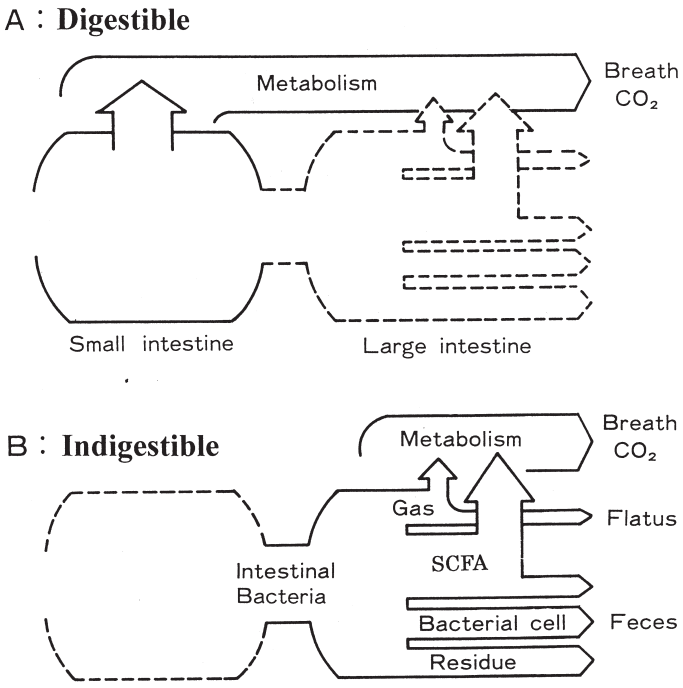

Fig. 40.4 Metabolic pathways of digestible and indigestible (resistant) oligosaccharides.

perfringens. Hence, FOS are used more selectively by bifidobacteria than are other carbohydrates. As a typical example, administration of FOS to senile humans for 2 weeks (8 g/day) resulted in a significant increase in the population of faecal bifidobacteria (Mitsuoka *et al*. 1987), the average number of bacteria per gram of stool increasing from $10^{8.8} \pm 10^{1.1}$ to $10^{9.7} \pm 10^{0.5}$.

40.3.2 *Production of SCFA and suppression of putrefactive substances*

After FOS administration (8 g/day for 2 weeks) to healthy subject (Hidaka *et al*. 1991), the increase in faecal bifidobacterial counts was paralleled by an increase in the amount of SCFA and the suppression of putrefactive substances in the faeces. In the group ($n = 3$) in which the faecal bacterial counts increased from $10^{8.8} \pm 10^{1.6}$ to $10^{9.6} \pm 10^{0.6}$, the average amounts (mg/g dry stool) of total SCFA and their components (acetic, propionic, butyric acids) were found to increase from 25.3 ± 19.1 (14.6 ± 10.2, 4.9 ± 3.5 and 5.3 ± 5.7 for acetic, propionic and butyric acids, respectively) to 43.9 ± 30.7 (29.4 ± 20.1, 6.1 ± 4.1 and 7.6 ± 6.0), respectively). In the same group, the amounts of total putrefactive substances and their individual components (indole, skatole, *p*-cresol and phenol) were seen to decrease.

40.3.3 *Effect on gastrointestinal condition*

FOS have been shown to relieve constipation in patients (Kameoka *et al*. 1986). Similar beneficial effects were also found in healthy subjects, especially among those people having

Table 40.1 Utilisation of several saccharides by intestinal bacteria.*

	Saccharide		
Bacterial species†	Glucose	Lactulose	Fructo-oligosaccharides
Bifidobacterium adolescentis (4)	++	++	++
B. longum (3)	++	++	++
B. breve (3)	++	++	+
B. infantis (2)	++	++	++
B. bifidum (2)	++	++	–
Lactobacillus acidophilus (3)	++	++	–
L. fermentaum (4)	++	++	–
L. salivarius (2)	++	++	+
L. casei (1)	++	++	–
L. plantarum (1)	++	++	+
Eubacterium aerofaciens (1)	++	++	+
Eu. limonus (1)	++	–	–
Eu. lentum (1)	–	–	–
Propionibacterium acnes (1)	++	–	–
Bacteroides fragilis (4)	++	++	++
Bact. thetaiotaomicron (3)	++	++	++
Bact. vulgatus (2)	++	++	++
Bact. dislasonis (1)	++	++	++
Bact. ovatus (1)	++	++	++
Bact. melaninogenicus (1)	++	–	++
Fusobacteriun varium (2)	++	–	–
Megamonas hypermegas (2)	++	++	++
Mitsuokella multiacidus (2)	++	++	v
Escherichia coli (2)	++	++	–
Klebsiella pneumoniae (1)	++	–	++
Enterococcus faecalis (1)	++	+	+
Enterococcus faecium (1)	++	++	+
Streptococcus intermedius (2)	++	++	++
Peptostreptococcus prevotii (1)	++	–	–
Peptostreptococcus pervulus (1)	++	–	++
Clostridium perfringens (4)	++	++	–
Cl. difficile (2)	++	–	–
Cl. paraputrificum (2)	++	+	–
Cl. clostridiiforme (2)	++	++	+
Cl. ramosum (2)	++	++	+
Cl. butyricum (1)	++	++	++
Veilonella dispar (2)	–	–	–
Megasphaera elsdenii (1)	–	–	–

*Judgement of bacterial growth: ++, same level of growth compared with glucose; +, weaker growth compared with glucose; –, no growth; v, variable (strains may be either + or –); superscripts indicate the result of occasional strains in the species.
†Values in parentheses indicate number of strains.

a stool frequency of less than once each day. Daily intake (1, 3 and 5 g) of FOS was shown to have a promotional effect on both stool frequency and on defaecation conditions (frequency, quantity, colour, odour and feeling) (Tokunaga *et al*. 1993). FOS syrup and powders were registered in Japan in 1993 as 'Foods for Specified Health Use' (FoSHU) on the basis of

their beneficial effect on gastrointestinal conditions. In a recent placebo-controlled study (Tominaga *et al.* 1999), a FOS-intake (3 g/day) was reconfirmed as having a promotional effect on stool frequency (Fig. 40.5).

40.3.4 *Promotion of mineral absorption*

In a recent study (Ohta *et al*. 1998), FOS intake and subsequent fermentation were found to improve the bioavailability of minerals (Ca, Mg, Fe), with the promotional effect being shown to take place mainly in the large intestine. Gastrectomised rats were found to develop both anaemia and osteopenia. FOS ingestion led to a rapid recovery from anaemia (compared with the control) and an increase in bone mineral density (Fig. 40.6). Mineral deficiency is a major nutritional problem in Japan, and in 1999 FOS was approved under FoSHU for its promotional effect on Ca absorption, this being the first such registration of resistant (indigestible) oligosaccharides (RO) with regard to its benefits on mineral absorption.

40.4 Uses as food ingredients and feedstuffs

The beneficial effects of FOS, both physically and physiologically, have been so pronounced that they have been developed as an attractive material for functional foods having low-energy sweetness, a bifidogenic effect, and promotional effects on both defecation and mineral absorption. In Japan, FOS were first used commercially in candies in 1983, but are now used in many types of foods and confectioneries. In the FoSHU system in Japan, 171 types of Japanese functional foods were listed in December 1999. Among these foods, FOS has been one of the leading functional ingredients, and 14 types of FOS foods have been registered in two functional items of health claim labelling, namely, their benefits on gastrointestinal condition (1993) and their promotion of mineral absorption (1999).

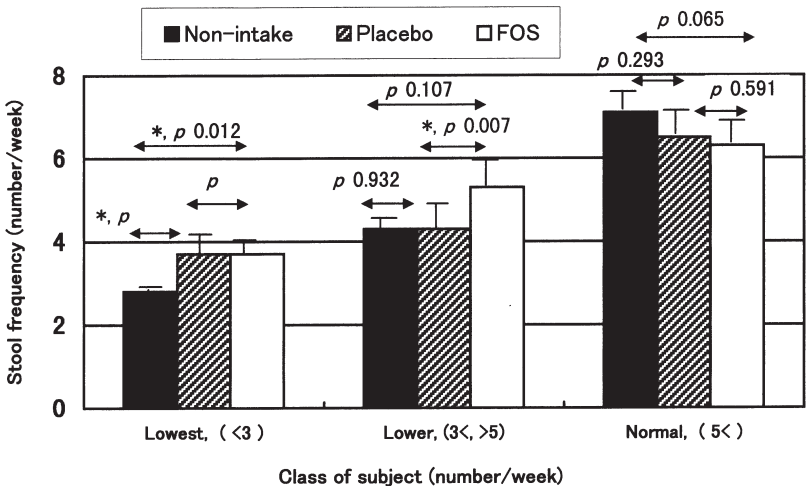

Fig. 40.5 Effect of ingested fructo-oligosaccharides on stool frequency for three classes of healthy female volunteers. Each bar represents the mean ± SE, and numbers shown above arrows show statistical *P*-values (Wilcoxon, non-parametric) between groups.

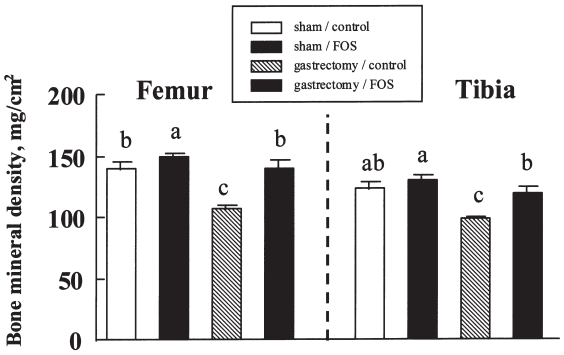

Fig. 40.6 Bone mineral density of the femur and tibia on completion of the experiment in sham-operated or gastrectomised rats fed diets either with or without (control) fructo-oligosaccharides. Each bar represents the mean± SE, and bars not sharing a common superscript letter are significantly different, $P < 0.05$ (ANOVA).

FOS have also been found to be useful for increasing the body weight gain and the feed conversion of piglets (Table 40.2) (Fukuyasa *et al.* 1987), and are added to the feed of domestic animals.

40.4.1 *Postulated and expected effects*

In addition to the established functions described above, FOS have been shown to have the following effects (representatives of animal or clinical studies):

- the prevention of colon carcinogens (*Min* mice; Pierre *et al*. 1997).
- a reduction in serum levels of triacylglycerols and cholesterol (positive report on hyperlipidaemia (Hata *et al*. 1983), and no effect on healthy subjects (Luo *et al*. 1996).
- the prevention of intestinal disorders such as ulcerative colitis (Umemoto *et al*. 1998) and *E. coli* O-157 infection (Oike *et al*. 1999).

Table 40.2 Growth-promoting effects of fructo-oligosaccharides (FOS) on piglets.*

Experimental group	No. of piglets	Body weight (mean ± SD)		Weight gain (mean ± SD)	Feed conversion
		Initial†	Final‡		
Control-1	10	6.688 ± 1.02	15.728 ± 2.48	9.048 ± 1.78	1.62
Control-2	10	7.198 ± 0.67	17.428 ± 2.13	10.238 ± 2.12	1.49
Mean§	9.648 ± 2.00	1.56			
FOS-1	10	7.108 ± 1.03	21.138 ± 1.22	14.038 ± 0.61**	1.66
FOS-2	10	6.298 ± 0.53	17.348 ± 1.25	11.058 ± 1.03**	1.36
Mean§	12.548 ± 1.82	1.48			

*FOS were administered at 0.25% in the feed.
†Initial age 25 days.
‡Final age 53 days.
§Mean of two experimental groups.
**, significant difference, $P < 0.01$.

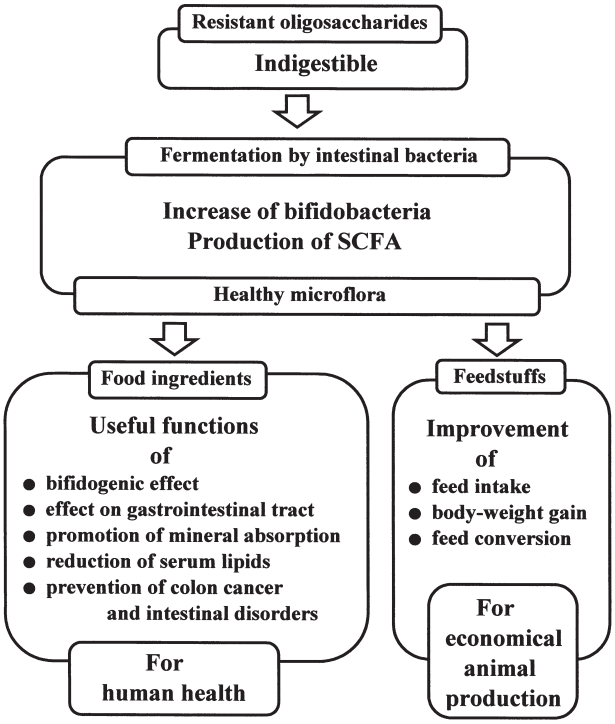

Fig. 40.7 Functional effects of resistant oligosaccharides.

Following the success of FOS, a range of other indigestible oligosaccharides have been developed, including galacto-oligosaccharides, soybean-oligosaccharides (galactosyl-sucrose oligosaccharides), xylo-oligosaccharides, and others. These resistant oligosaccharides also have useful potential in improving gastrointestinal functions and physiology (Fig. 40.7). These functions – including those already established and these presently postulated – are expected to be widely utilised in the promotion of human health and economical animal production.

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41 Fructo-oligosaccharides and Other Fructans: Chemistry, Structure and Nutritional effects

Francis R.J. Bornet

41.1 Chemistry, structure and origin

Fructans, such as inulin and fructo-oligosaccharides (FOS) are carbohydrates. They are a group of linear glucosyl $\alpha(1\rightarrow2)(\text{fructosyl})_n\beta(2\rightarrow1)$fructose polymers with a degree of polymerisation (DP) ranging from 3 up to 60 (Fig. 41.1). By definition, if oligosaccharides have a DP lower than 9, they are named fructo-oligosaccharides. The main fructo-oligosaccharides are 1-kestose (GF_1), nystose (GF_2) and fructosylnystose (GF_3) (GF = glucosylfructo-oligosaccharide). The fructans components with a higher DP are named inulin.

41.1.1 *Natural occurrence of fructans*

Fructans occur in a number of plants such as onions, Jerusalem artichokes, asparagus, wheat, rye and garlic (Clevenger *et al.* 1988). Onion has the highest content of FOS, ranging from 25 to 40% (dry matter basis) of which 97% are short-chain FOS (GFn, n <5). Garlic and chicory root have a low FOS content (Table 41.1). In Western countries, the average daily intake of FOS from these natural sources is about 1 g.

Fructans of artichoke globe are mainly composed of long-chain polymers with a DP >40. Chicory, inulin and Jerusalem artichoke have shorter polymers, with a main DP ranging from 20 to 40 (Table 41.2).

Table 41.1 Natural occurrence and distribution of fructo-oligosaccharides (GFn, $n < 9$) in edible plants.

		Proportion of GF (%)				
Substance	% Fructan (DS)	GF_2	GF_3	GF_4	GF_{5-8}	Total
Onion	25–40	61	25	10	3	100
Wheat	1–4	30	13	6	50	100
Chicory	15–20	4	5	5	16	30
Jerusalem artichoke	16–20	DP <9: 50%				
Garlic	25–35	DP <9: 10–20%				

DS, dry substance; DP, degree of polymerisation.

Table 41.2 Natural occurrence and distribution of fructan polymers (inulin; GFn, $n \geq 9$) in edible plants.

		Degree of polymerisation (%)		
Substance	% Fructan (DS)	10–20	20–40	>40
Chicory	15–20	24	45	2
Jerusalem artichoke	16–20	22	20	6
Globe artichoke	2–9	0	13	87

DS, dry substance.

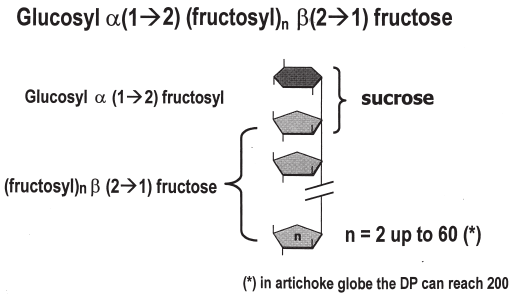

Fig. 41.1 General chemical structure of fructans.

41.1.2 *Structure and composition of commercial FOS ingredients*

FOS are produced on a commercial scale by two different processes, either from sucrose using a food-grade fungal fructosyltransferase (ACTILIGHT®; Béghin Meiji Industries, France), or from inulin by partial hydrolysis using *endo*-inulinase (Orafti, Belgium).

In FOS synthesis from sucrose, the sucrose plays the dual role of fructose donor and fructose acceptor (Fishbein *et al*. 1988). The first reaction on two sucrose molecules leads to kestose and glucose. The action of the fructosyltransferase on kestose produces nystose, and on nystose produces fructosylnystose. The reaction is stopped to optimise the ratio between $GF_2/GF_3/GF_4$ at 37%/53%/10%. A chromatography step ensures the purification of short-chain FOS (sc-FOS). The composition of commercial product is shown in Fig. 41.2. FOS from sucrose are composed only of glucosyl $\alpha(1\rightarrow2)(\text{fructosyl})_n\beta(2\rightarrow1)$fructose, with n = 1 to 3.

The short-chain FOS mixture has a taste profile similar to that of sucrose, without any cooling effect. The sweetness of the purified short-chain FOS mixture is 30% of that of sucrose, while the water-retention capacity is higher than that of sucrose. Being non-reducing sugars, sc-FOS do not lead to Maillard reactions, and they are stable at pH values >3 and temperatures up to 130°C. According to their technological characteristics, sc-FOS may be used as ingredients in biscuits and cakes, breakfast cereals and cereal-filled bars, ices and desserts, dairy products, yoghurt and milk.

Chicory inulin is hydrolysed by *endo*-inulinase, producing a mixture of glucosyl $\alpha(1\rightarrow2)(\text{fructosyl})_n\beta(2\rightarrow1)$fructose with n = 1 to 6, and fructosyl $\beta(2\rightarrow1)(\text{fructosyl})_n$-

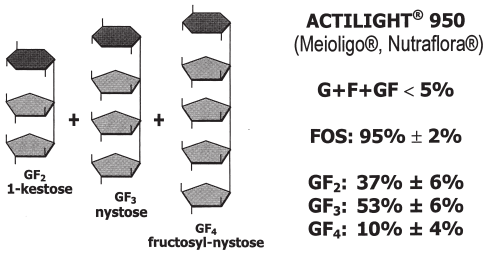

Fig. 41.2 Composition of commercial fructo-oligosaccharides ingredients from a sucrose source.

β(2→1)fructose with n = 2 to 7 (Fig. 41.3). A chromatography step ensures the purification of FOS. The composition of the commercial product is given in Fig. 41.4.

41.1.3 *Structure and composition of commercial inulins*

The commercial inulins are obtained by hot-water extraction from chicory roots. The composition of inulin extracts are variable: it is a function of many factors such as the source from which it was extracted, the climate and the growing conditions, the harvesting time and storage conditions. Figure 41.5 illustrates the average composition of a commercial inulin extract (Raftiline® ST, Orafti, Belgium). Different commercial products are available, one of which has a low FOS content (< 2%, Raftiline® HP; Orafti).

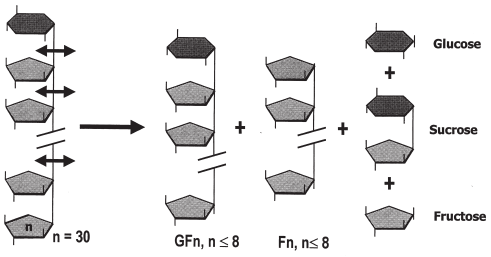

Fig. 41.3 Action of the *endo*-inulinase on chicory inulin.

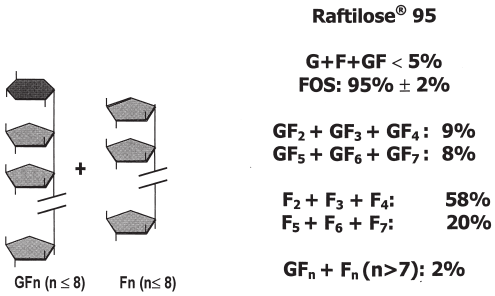

Fig. 41.4 Composition of commercial fructo-oligosaccharides ingredients from a chicory inulin source.

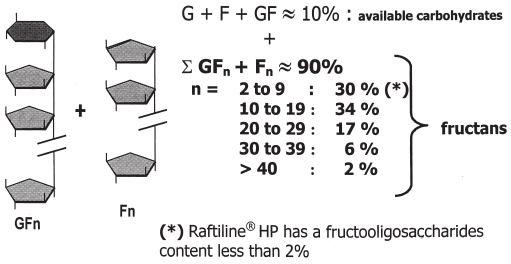

Fig. 41.5 Average composition of commercial inulin from chicory.

41.2 Methods to measure sc-FOS in food products

A recent survey conducted by Lee and Prosky (1995) concerning the definition of dietary fibre supports the view that the definition should be revised to include oligosaccharides that are resistant to hydrolysis by the alimentary tract, as are the sc-FOS.

The AOAC dietary fibre analytical method involves three enzymatic digestion steps with α-amylase, protease and amyloglucosidase. These enzymatic treatments do not modify the sc-FOS structures. However, the AOAC method does not measure sc-FOS because of their ethanol solubility. Ouarné *et al.* (1997) have developed a specific and reliable analytical method to measure sc-FOS in food products. The method involves an invertase hydrolysis step, followed by detection of sugars using a Dionex ion chromatograph. The minimal amount of quantifiable sc-FOS is 0.75 g per 100 g of food product.

Other methods for the measurement of sc-FOS are AOAC Method 997.08 (Hoebregs 1997) and AOAC Method 999.03 (McCleary *et al.* 2000). Both of these methods use inulinase enzymes to hydrolyse FOS to fructose and glucose. In the latter procedure, sucrose is selectively removed with a pure sucrase enzyme.

41.3 Nutritional aspects

The FOS have aroused interest during the past decade, mostly because of their nutritional properties. Fructans, to a large extent, escape digestion in the human upper intestine and reach the colon where they are totally fermented, mostly to lactate, short-chain fatty acids (SCFAs; acetate, propionate and butyrate), H_2 and CO_2. The most important property of FOS is their ability to stimulate bifidobacterial growth specifically while suppressing the growth of some other species in the colon, such as *Clostridium perfringens*. The demonstration of the potential health benefits of FOS constitutes an active field of research in human nutrition.

41.3.1 *Digestive fate of sc-FOS*

Numerous *in-vitro* studies have been conducted in animal models and in humans, showing the indigestibility of sc-FOS in the small intestine. Kestose (GF_2) and nystose (GF_3) are not significantly hydrolysed by pancreatic homogenate (Oku *et al.* 1984), purified sucrase-isomaltase complex (Oku *et al.* 1984), nor by small intestinal mucosa homogenate from either animals (Oku *et al.* 1984; Tsuji *et al.* 1986) or human (Molis *et al.* 1996). Long-term

ingestion of sc-FOS did not cause induction or suppression of the hydrolysing enzymes in the rat small intestine (Oku *et al.* 1984). In addition, sc-FOS did not influence the transmural potential difference of everted sacs prepared from the jejunum (Tokunaga *et al.* 1986). When injected intravenously into rats, sc-FOS are rapidly excreted in the urine, without degradation, suggesting that they are not used as an energy source in the body (Oku *et al.* 1984).

The percentage of ingested sc-FOS reaching the colon has been measured in six healthy volunteers using the intubation and the slow marker method (Molis *et al.* 1996). Some 90% of the ingested sc-FOS have been recovered at the end of the ileum. Moreover, the percentages of the constitutive sc-FOS (GF_2, GF_3 and GF_4) remain identical to those of ingested sc-FOS, showing that most unabsorbed sc-FOS were in an intact unhydrolysed form.

Utilisation of sc-FOS was studied *in vivo* in man using a radiorespirometry method, and *in vitro* by incubation with human faeces (Hosoya *et al.* 1988; Tokunaga *et al.* 1989). The studies showed that sc-FOS were fermented by intestinal microorganisms, mainly to SCFAs and CO_2, and that the SCFAs are absorbed by the colon and further converted to CO_2 in the body. Compared with other fermentable products, such as cellulose, pectin or lactulose, the fermentation of FOS produces higher percentages of propionic and butyric acid (Bornet *et al.* 1994; Luo *et al.*, 1996). SCFAs are absorbed in the colon, and are in part metabolised within the colon; the remainder is metabolised in the liver and peripheral tissues (Bornet *et al.* 1994).

41.3.2 *Caloric values of fructans*

Sc-FOS not hydrolysed in the upper gastrointestinal tract are completely fermented in the colon, such that none is found in the stools (Molis *et al.* 1996). The colonic fermentation of carbohydrates is responsible for SCFAs, lactic acid and gas production, bacterial maintenance and growth, and heat dissipation (Macfarlane & Cummings 1991), which results in a loss of energy for the host estimated to be equal to 50% of the energy content of carbohydrate (Van Es 1987; Hobbs 1988; Beaugerie *et al.* 1990).

Molis *et al.* 1996, using the ileal intubation method in healthy subjects, estimated that the caloric value of sc-FOS was 9.5 kJ/g, a value somewhat higher than the 6.3 kJ/g reported by Hosoya *et al.* (1988). Those authors used a radiorespirometry method in healthy subjects ingesting [U-^{14}C] sc-FOS, and also measured gas and SCFA production from labelled sc-FOS in anaerobic incubation with faeces. They postulated that SCFAs were completely absorbed in the large intestine, but they used a very low mean energy content for SCFAs (10.0 kJ/g) to calculate the energy value of sc-FOS. The heat produced by combustion of acetic, propionic and butyric acids is 14.6, 20.7 and 25.0 kJ/g, respectively (Blaxter 1989). Accordingly, the metabolisable energy from acetic, propionic and butyric acids is 10.9–12.6, 15.5–17.6 and 18.8–21.3 kJ/g, respectively. According to these figures, the energy value of FOS should range between 8.4 and 9.2 kJ/g, a value close to that found by Molis *et al.* (1996). Sc-FOS in healthy humans are only slightly digested in the small intestine and are fermented in the colon, resulting in reduced energy production (about one-half that of sucrose). The caloric value of inulin is claimed to be 1 kcal/g.

41.3.3 *Effects of sc-FOS on glucose and lipid metabolism*

The glycaemic, insulinaemic and fructosaemic responses to sc-FOS have been studied in

healthy and diabetic patients (Drevon & Bornet 1992). The postprandial plasma responses were not significantly modified after an oral load of 25 g sc-FOS.

The effect of sc-FOS on glucose tolerance was assessed by Yamashita *et al.* (1984). Eighteen non-insulin-dependent diabetes patients received 8 g per day of sc-FOS for 14 days. Fasting glucose levels were significantly reduced by 15 mg/dl under the sc-FOS diet. A double-blind, cross-over study in 12 healthy subjects (Luo *et al.* 1996) showed that over a period of one month, daily intake of 20 g sc-FOS significantly decreased basal hepatic glucose production.

The mechanism by which sc-FOS can improve glucose metabolism is unclear, although a role of SCFAs is suspected (Venter *et al.* 1990; Bornet *et al.* 1994). Among the SCFAs produced, propionate in particular was shown to improve hepatic glucose metabolism in both rats and healthy subjects. A recent study carried out in normal rats fed for 4 weeks with a diet enriched with sodium propionate (7.8 g/100 g food) showed a reduction in the fasting blood glucose (Boillot *et al.* 1995). Long-term oral propionate in healthy subjects (7.5 g sodium propionate/day, equivalent to 78 mmol propionate/day) decreased both fasting serum glucose and maximum insulin increment during a glucose-tolerance test, as shown by Venter *et al.* (1990). Todesco *et al.* (1991) have also noted a decline in fasting glycaemia in six healthy subjects whose diet was supplemented with propionate (9.9 g sodium propionate/day) for 7 days. Propionate is a gluconeogenerator, and has been shown to inhibit gluconeogenesis from lactate and to stimulate glycolysis in isolated hepatocytes (Anderson & Bridges 1984). Propionate enters the Krebs cycle at succinyl-CoA. The inhibiting effect of propionate on gluconeogenesis may be related to its metabolic intermediaries, methylmalonyl-CoA and succinyl-CoA, both of which are specific inhibitors of pyruvate carboxylase (Baird *et al.* 1980). In addition, propionate enhances glycolysis, probably by depleting hepatic citrate (Blaird *et al.* 1973), an important metabolic inhibitor of phosphofructokinase. Finally, propionate may also influence hepatic glucose metabolism indirectly by lowering plasma fatty acid concentrations, known to be closely related to gluconeogenesis (Foley 1992).

These results suggested that the effects of dietary fibre on glucose metabolism may be exerted, not only through their action on the upper digestive tract (reduction of gastric emptying, lengthening of digestion and absorption of digestible carbohydrates) but also through their fermentation products.

The hypolipidaemic effects of sc-FOS, described as a reduction of total cholesterol and triglycerides, have been studied in different subpopulations: non-insulin-dependent diabetics (Yamashita *et al.* 1984; Mitsuoka *et al.* 1986), and hyperlipidaemic out-patients (Hata *et al.* 1983; Mitsuoka *et al.* 1986). Again, this may involve propionate, as propionate inhibits cholesterol synthesis by inhibiting both 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) synthase (Bush & Milligan 1971) and HMG-CoA reductase (Rodwell *et al.* 1976). The hypolipidaemic effects of sc-FOS (20 g/day) have not been observed in healthy subjects (Luo *et al.* 1996).

41.3.4 *The bifidogenic effect of fructans*

Unlike other undigestible sugars, such as lactose or lactulose which are hydrolysed by a wide variety of gut bacteria, sc-FOS are only fermented *in vitro* by a limited range of microorganisms that include most species of bifidobacteria (except *B. bifidum*) (Hidaka *et al.* 1986; Mitsuoka *et al.* 1987; McKellar *et al.* 1993). Indeed, bifidobacteria have relatively high amounts of β-fructosidase, which is selective for the β-(1,2) glycosidic bonds present in sc-

FOS. After sc-FOS hydrolysis, fructose serves as an efficient growth substrate for the bifidus pathway of hexose fermentation, which is carried out almost exclusively by bifidobacteria (Scardovi 1965).

Numerous studies in humans showed that sc-FOS ingestion led to an increase of faecal bifidobacteria (Hidaka *et al.* 1986, 1991; Mitsuoka *et al.* 1986, 1987; Sano 1986; Takahashi 1986; McKellar *et al.* 1993; Tokunaga *et al.* 1993; Rochat *et al.* 1994; Williams *et al.* 1994; Bouhnik *et al.* 1996; Buddington *et al.* 1996). Recent studies (Rochat *et al.* 1994; Williams *et al.* 1994; Bouhnik *et al.* 1996, 1999) have been conducted in healthy subjects using a 'control' group and a double- or single-blind design. The main characteristics and results of the studies conducted in humans are summarised in Table 41.3.

The β-fructosidase activity in stools (determined by Bouhnik *et al.* 1996) increased significantly during the 12-day period of sc-FOS ingestion, and returned to the baseline level in the post-ingestion period. A significant correlation between faecal β-fructosidase activity and bifidobacteria counts was observed in subjects receiving sc-FOS, but not in those receiving placebo.

More recently, Bouhnik *et al.* (1999) have assessed the threshold dose of sc-FOS which could lead to a significant increase in faecal bifidobacteria levels, and suggested a dose-related effect. Forty healthy volunteers were allocated at random into five groups. All subjects ingested their usual diet and a supplement of sc-FOS for a period of 7 days (of which the amount differed for each group: 0 g, 2.5 g, 5 g, 10 g or 20 g/day, respectively). Faecal bifidobacteria counts were collected twice, before and at the end of the 7-day period. Faecal bifidobacteria counts did not vary in the group G_0 and $G_{2.5}$, but they increased significantly during sc-FOS ingestion in the G_5, G_{10} and G_{20} groups. A significant correlation between the dose of sc-FOS ingested and the faecal bifidobacteria counts was observed at the end of the 7-day period.

Table 41.3 Main characteristics and results of clinical studies conducted in healthy subjects on the prebiotic effects of the short-chain fructo-oligosaccharide (FOS), ACTILIGHT.

No. of subjects	Mean age (years)	Sc-FOS daily ingestion (g)	Study duration (days)	Bifidobacteria count in stools (log CFU/g; mean ±SEM)		*P*-value	Reference
				Before	After		
6	–	6	30	9.6	9.8	NS	Mitsuoka *et al*. 1986
23	73±9	8	14	8.8±1.1	9.7±0.5	<0.005	Mitsuoka *et al*. 1987
27 (9×3)	36.8±9 25.2±3.3	1	14	9.8±0.6	10.2±0.4	<0.05	Tokunaga *et al*. 1993
		3	14	9.9±0.6	10.4±0.4	<0.05	
		5	14	9.7±0.6	10.3±0.4	<0.01	
38	–	8	14	5.2±0.9	6.2±0.6	<0.01	Rochat *et al*. 1994
10	20–40	4	14	8.3±1.8	9.4±2.3	<0.05	Williams *et al*. 1994
10	22–39	12.5	12	7.9±0.5	9.1±0.3	<0.01	Bouhnik *et al*. 1996
32 (8x4)	29.6	2.5	8	8.0±1.1	8.2±1.1	NS	Bouhnik *et al*. 1999
		5	8	8.1±0.8	9.1±0.4	<0.05	
		10	8	8.0±1.3	9.5±0.3	<0.02	
		20	8	8.2±0.9	9.5±0.6	<0.002	

NS, not significant.

The relationship between chain length of fructans and the capacity to stimulate the growth of bifidobacteria has been recently studied in healthy subjects. ACTILIGHT® P950 (sc-FOS, 100% GFn; n <5) and long-chain fructans (Raftiline® HP, content of GFn; n <9 lower than 2%) were ingested during one week at a daily dose of 10 g and compared with a placebo (10 g/day maltodextrins) by three groups, each comprising eight healthy volunteers. The growth of bifidobacteria in the stool was significantly stimulated by ACTILIGHT® P950 (increase observed for seven subjects out of eight), but not by Raftiline HP (increase observed for three out of eight), nor by the placebo (increase in one subject only) (Fig. 41.6). In a second set of clinical assays, the effect of 10 g/day ACTILIGHT P950® and 15 g/day Raftiline® HP have been compared in two groups, each of 25 healthy volunteers, during a 2-week period. The β-galactosidase activities in stools that reflected the functional activity of the flora was measured at 1 and 2 weeks after ingestion of the two different fructans. The β-galactosidase activity was significantly increased within the 2-week period of ingestion of ACTILIGHT®, but not of Raftiline® HP (Fig. 41.7). The results suggest that the sc-FOS are the more effective components of the bifidogenic and the functional effects of fructans.

The stimulation of bifidobacteria growth is usually associated with a decrease of faecal pH (Hidaka *et al.* 1986; Mitsuoka *et al.* 1987; Rochat *et al.* 1994) and caecal pH (Koo & Rao 1991), an increase of faecal or colonic SCFA production (Hidaka *et al.* 1986, 1991), a decrease of *C. perfringens* (Hidaka *et al.* 1986; Rochat *et al.* 1994), and a decrease in the

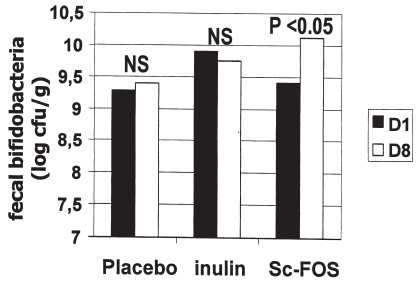

Fig. 41.6 Comparison of the bifidogenic effect of 10 g/day short-chain fructo-oligosaccharides (ACTILIGHT® P950) and 10 g/day long-chain fructan (inulin, Raftiline® HP) in three groups, each of eight healthy subjects after 8 days of daily consumption.

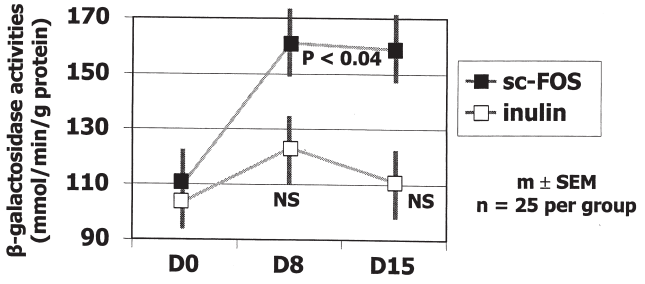

Fig. 41.7 Comparison of 10 g/day short-chain fructo-oligosaccharides (ACTILIGHT® P950) and 15 g/day long-chain fructan (inulin, Raftiline® HP) on functional activity of the faecal flora after 8 and 15 days of daily consumption in healthy subjects.

production of putrefactive substances in urine and stools (Hidaka *et al.* 1986). The stimulation of bifidobacteria growth in healthy subjects is inconsistently associated with significant modifications of faecal bacterial enzymatic activities such as β-glucuronidase, glycocholic acid hydroxylase, nitroreductase and azoreductase, or modification of faecal neutral sterols, and in particular of cholesterol (Bouhnik *et al.* 1996; Buddington *et al.* 1996).

41.3.5 *Bifidobacteria and immunopotentiating activity*

It has been reported that bifidobacteria have various immune functions, for example mitogenic activity (Kadooka *et al.* 1991), adjuvant activity (Kohwi *et al.* 1982; Sekine *et al.* 1994a), promotion of macrophages (Kadooka *et al.* 1991; Sekine *et al.* 1994b), stimulation of antibody production (Yasui & Ohwaki 1991; Yasui *et al.* 1992) and anti-tumour effects (Kohwi *et al.* 1982; Sekine *et al.* 1995). Lee *et al.* (1993) tested the immunopotentiating activity of 27 microorganisms *in vitro*, and showed that bifidobacteria strains have a higher immunopotentiating activity than do *L. casei* or *L. acidophilus* (i.e. to stimulate the proliferative response of murine immune cells). *Bifidobacterium adolescentis* M100-4, originally derived from human intestinal microflora, had the strongest mitogenic activity on the splenocytes and the Peyer's patches cells. This mitogenic activity developed by *B. adolescentis* M100-4 was increased after disruption of the cells by sonication, indicating the existence of an intracellular soluble immunopotentiator. Recently, Hosono *et al.* (1997) have isolated this soluble fraction, which exhibited mitogenic activity in an assay of murine splenocytes and Peyer's patch cells *in vitro*, in a dose-dependent manner. This fraction contained galactofuranosyl residues as characteristic constituents, which have not been previously detected in other soluble fractions from Gram-positive bacteria. *B. adolescentis* is well known as one of the major components of the microflora in the intestines of healthy humans (Mitsuoka 1984).

41.3.6 *Butyrate production for colonic maintenance*

During recent years, the increased interest in, and research on, fermentable fibre has focused on the colon and the products of colonic fermentation, in particular the SCFA. Colonocytes are purported to be sustained by SCFA derived from bacterial fermentation, with butyrate oxidation providing more than 70% of the oxygen consumed by human colonic tissue (Roediger 1980a). Limited evidence (Roediger 1980a) indicates that butyrate is the preferred energy substrate of rat colonocyte. Impaired utilisation of SCFA has been implicated in ulcerative colitis, suggesting the existence of an energy-deficient state (Roediger 1980b). Mucosal cells demonstrated an absence of butyrate oxidation, reflecting a metabolic defect in the mucosa of ulcerative colitis patients. Moreover, Harig *et al.* (1989) inferred that diversion colitis represented an inflammatory state resulting from a nutritional deficiency, and which might be effectively treated with enema containing SCFA – the missing nutrient.

Butyrate is not simply an energy source for the colonocyte. Butyrate salts are antiproliferative and differentiating agents that are effective *in vitro* on many cancer cells (Kruh 1982), the situation being particularly relevant to colon carcinoma cells (Whitehead *et al.* 1986; Garnet *et al.* 1992). From epidemiological and experimental studies, a correlation was recently shown between protection against colon cancer and the release of butyrate from dietary fibre (Weaver *et al.* 1988; McIntyre *et al.* 1993).

The direct antiproliferative effect of butyrate against tumour cells *in vitro* (Kruh 1982) could slow down the development of tumours *in vivo*. However, this effect is transient *in*

vitro, and rapidly disappears on removal of butyrate. It has been shown recently that butyrate stimulates the immunogenicity of cancer cells (Perrin *et al.* 1993, 1994). These authors have shown that the phenotype of the weakly immunogenic rat colon cancer PROb cells was modified with sodium butyrate. After 4 days sodium butyrate treatment *in vitro*, the lymphokine-activated killer cell sensitivity, the expression of Major Histocompatibility Complex class I, and the intercellular adhesion molecule 1 of PROb cells, were increased in a dose-dependent manner. Perrin *et al.* (1994) subsequently tested the efficiency of interleukin 2 (IL-2) and sodium butyrate, alone or in combination, against experimental widespread carcinomatosis induced in rats by intraperitoneal injection of 2×10^6 PROb colon carcinoma cells. Administration of the IL-2/butyrate combination resulted in cases of complete cure of carcinomatosis, with specific protection against PROb cells. The complete regression of tumour masses may be attributed, at least in part, to a butyrate-induced increase in immunogenicity of the cancer cells.

Thus, it may be advantageous to provide indigestible carbohydrates as an indirect source of butyrate to the large bowel. Recently, Campbell *et al.* (1997) evaluated in rats the effects of selected indigestible oligosaccharides on caecal and faecal SCFA concentration, pH, total large bowel wet weight and wall weight, and concentrations of intestinal microbiota. The duration of the study was 14 days. The sc-FOS-containing diet resulted in higher caecal butyrate concentrations compared with the control, or with cellulose or xylo-oligosaccharides diets.

41.3.7 *sc-FOS and protection/reduction of colonic tumours in animal models*

The protective effect of fibre against colon cancer is still questionable (Wasan & Goodlad 1996). Consequently, we have recently used rats (Perrin *et al.* 2000) and *Min* mice (Pierre *et al.* 1997) to investigate the effects of different types of fibre in both chemically induced and spontaneous cancer models. The relevance of animal models, as compared with human colon tumour studies, depends on the criteria considered (Pories *et al.* 1993).

Azoxymethane (AOM) -induced tumours are similar to human tumours in many histological, biochemical, immunological and cellular aspects, but many of the tumours do not follow the adenoma to carcinoma progression, frequently arising *de novo* from flat mucosa. This model permits the investigation of early stages of carcinogenesis, the end-point being a consensual precancerous marker, namely aberrant crypt foci. The mouse model (*Min* mice) is a model for both familial adenomatous polyposis and sporadic colon cancer. The *Min* mice are heterozygous for a non-sense mutation of the *Apc* gene, the murine homologue of APC. The *Min* mouse model adenoma are pertinent by their genetic origin, but they are more frequent in the small bowel than in the colon, as opposed to the human situation. These studies provided data on later stages of colon carcenogenesis, and the end-point was the number of detectable tumours.

In our experiments, the same diets have been tested in the two types of animal experiments: a poorly fermentable fibre, a retrograded high-amylose corn starch (RS_3), and the sc-FOS. The control diet was a low-fibre diet containing only 2% cellulose to maintain normal transit. BD IX rats ($n = 9$ per group) were fed one of the four diets during 44 days, and injected twice, at 1-week intervals with AOM (15 mg/kg). The rats were sacrificed at 1 month after the first injection. The SCFA concentrations were measured all along the large intestine, and the colon was studied to quantify the aberrant crypt foci. As a parallel experiment, *Min* mice aged 5–6 weeks ($n = 40$) were allocated randomly to one of the four diets. Each group was fed *ad*

libitum for 42 days, and then sacrificed. Gut tumours and small intestine lymphoid nodules were counted. Both RS and sc-FOS provided protection to the rats, reducing the number of aberrant crypt foci in conjunction with high butyrate production all along the large intestine. Similar effects on reduction of the number of aberrant crypt foci in CF1 mice treated with AOM and fed diets containing sc-FOS and exogenous bifidobacteria, were published by Koo and Rao (1991). By contrast, neither starch-free wheat bran, nor the control diet, modified the number of aberrant crypt foci in rats, only sc-FOS being efficient in preventing tumour occurrence and concomitant development of gut-associated lymphoid tissue.

Clearly, in both experiments, only butyrate-producing fibres (e.g. sc-FOS and RS) were protective against colon cancer. However, resistant starch was ineffective in the *Min* mice model, which corresponds to a more advanced stage of carcinogenesis. Although difficult to interpret on the basis of the present knowledge, the *Min* mice data suggest that fermentation is not sufficient to protect against carcinogenesis that has already been initiated. Although in our experiment we did not measure the bifidobacteria in *Min* mice, Howard *et al.* (1995) demonstrated that dietary supplementation with the same sc-FOS enhanced the population of bifidobacteria in mouse colon as early as 14 days. We hypothesise that an additional stimulus to the immune system by sc-FOS [confirmed by the gut-associated lymphoid tissue (GALT) development] via their bifidogenic effect could increase the efficiency of sc-FOS.

41.4 Conclusions

Fructo-oligosaccharides have aroused interest during the past decade, mostly because of their nutritional properties. To a large extent, fructans escape digestion in the human upper intestine and reach the colon where they are totally fermented, mostly to lactate and short-chain fatty acids (acetate, propionate, butyrate). The most important property of FOS is their ability specifically to stimulate bifidobacterial growth while suppressing the growth of some other bacterial species in the colon, such as *Clostridium perfringens*. The prebiotic effect and functional activity of fructans is dose-dependent and chain length-related. The demonstration of the health benefits of fructans is a clear challenge for the next decade.

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42 Galacto-oligosaccharides: Properties and Health Aspects

H.C. (Margriet) Schoterman

42.1 Introduction

Consumers are becoming increasingly aware of the influence of diet on health and quality of life. During the past few years, this has resulted in an increase in consumer demand for foods with a certain beneficial effect on the human body. One ingredient used for these foods is the prebiotic, defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, with subsequent improvement in host health (Gibson & Roberfroid 1995). Galacto-oligosaccharides, which in Europe are sold commercially as Elix'or® (Borculo Domo Ingredients, The Netherlands), belong to this class of food ingredients. Although within the literature, galacto-oligosaccharides are also referred to as transgalacto-oligosaccharides and transgalactosylated oligosaccharides, the first term will be used in this chapter.

42.2 Properties of galacto-oligosaccacharides

42.2.1 *Structure and occurrence*

Galacto-oligosaccharides are non-digestible carbohydrates structured as chains of galactose, and mostly have a glucose end-unit. They are produced from lactose by means of enzymatic conversion using the enzyme β-galactosidase, which has a transgalactosylation activity (Fig. 42.1). Commercially available galacto-oligosaccharides are mixtures of several oligosaccharides, lactose, glucose and a small amount of galactose. The composition of the galacto-oligosaccharide fraction varies in chain length and type of linkage between the monomer units (Sako *et al.* 1999). Galacto-oligosaccharides occur naturally in certain products with a dairy origin, such as human milk. Two which have been found in human milk also occur in commercial products; these are the trisaccharides, β(1→4)-galactosyllactose and β(1→6)-galactosyllactose (Yamashita & Kobata 1974; Anon. 1995).

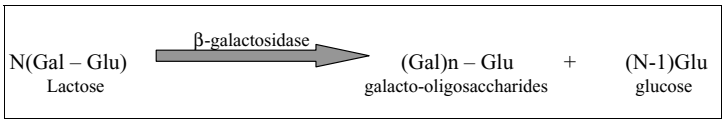

Fig. 42.1 Diagram of the production process of galacto-oligosaccharides. Gal = galactose; Glu = glucose; *n* = 1–7.

42.2.2 *Prebiotics and dietary fibre*

The key criteria for a food ingredient to be classified as a prebiotic, include: (1) not being hydrolysed or absorbed in the upper region of the gastrointestinal tract; and (2) stimulating the growth of potentially beneficial bacteria in the colon. Galacto-oligosaccharides fulfil these criteria, and are therefore identified as being prebiotic (Gibson & Roberfroid 1995; Salminen *et al.* 1998).

Galacto-oligosaccharides also belong to the group of non-digestible oligosaccharides (NDO), which can be regarded as soluble dietary fibres (Tomomatsu 1994; Lim *et al.* 1999; Wijnands *et al.* 1999). Authorities in several countries permit galacto-oligosaccharides to be labelled on foods as dietary fibre, although there is, as yet, no unequivocal definition of dietary fibre in Europe.

Dietary fibres can be classified into two categories: (1) soluble, viscous and fermentable; and (2) insoluble, non-viscous and non-fermentable. Galacto-oligosaccharides belong to the first category, because they are completely soluble and are fermented by specific bacteria present in the colon, resulting in the production of short-chain fatty acids (propionate, acetate and butyrate) (Lim *et al.* 1999).

42.2.3 *Heat and acid stability*

Galacto-oligosaccharides are very stable under conditions of high temperatures and low pH. Experiments in which galacto-oligosaccharides were treated at 160°C at neutral pH, 120°C at pH 3 and 100°C at pH 2 for 10 min, showed the galacto-oligosaccharides to remain completely stable (Sako *et al.* 1999). Other experiments have shown that galacto-oligosaccharides remain unchanged after treatments for 30 min at 120°C, 3 h at 100°C or 3 h at 80°C at pH-values of between 3 and 7. This high heat and acid stability make galacto-oligosaccharides especially suitable for inclusion in heat-treated products and/or acid products, such as fruit juices.

42.2.4 *Tolerance*

Human studies have shown that consumption of 15–20 g of galacto-oligosaccharides as part of the daily diet is well tolerated. van Dokkum (1995) concluded that the human tolerance of 15 g/day of galacto-oligosaccacharides could be described as ‘good’, as no detrimental side effects were seen. A study in humans showed that, compared with fructo-oligosaccharides, the consumption of galacto-oligosaccharides resulted in less breath hydrogen excretion. There was also a tendency towards fewer complaints of flatulence after consumption of galacto-oligosaccharides (Alles & Schoterman 1999).

42.2.5 *Energy value*

The energy value of non-digestible oligosaccharides, such as galacto-oligosaccharides, is relatively low because they are hardly hydrolysed and absorbed in the upper gastrointestinal tract. Part of the energy of non-digestible oligosaccharides is salvaged during fermentation and absorption of short-chain fatty acids in the colon. The energy value of galacto-oligosaccharides has been estimated as 1.0–2.0 kcal/g (Salminen *et al.* 1998; Sako *et al.* 1999).

42.3 Health aspects of galacto-oligosaccharides

While fermentation of ingested galacto-oligosaccharides increases the number of health-promoting bifidobacteria in the colon, several other health-related effects have also been reported, including increased absorption of calcium, relief of constipation and inhibition of the development of colon tumours.

42.3.1 *Stimulation growth of bifidobacteria*

The stimulatory effect of galacto-oligosaccharides on bifidobacteria growth has been demonstrated in several studies, both *in vitro* and *in vivo*.

In *in-vitro* studies, in which various bacteria appearing in the human colon were tested, galacto-oligosaccharides were found to be used mainly by several strains of bifidobacteria (Tanaka *et al.* 1983; Ohtsuka 1989; Dombou *et al.* 1991). The stimulatory effect on bifidobacteria growth was also seen *in vivo*. Bouhnik *et al*. (1997) studied the effect of the daily consumption of 10 g of galacto-oligosaccharides during 21 days in eight healthy volunteers. After 7, 14 and 21 days the faecal concentrations of bifidobacteria were increased in comparison with the first day (Fig. 42.2), but concentrations of enterobacteria were unchanged. The effects of daily administration of 2.5, 5.0 and 10.0 g of galacto-oligosaccharides on human faecal microflora were studied in 12 healthy volunteers (Ito *et al.* 1990). At the highest dose rate, the number of faecal bifidobacteria increased from $\log_{10}9.76$ (control) to $\log_{10}10.08$ bacteria per gram of faeces. A linear relationship was found between the galacto-oligosaccharide dose level and bifidobacterial count. In addition to the increase in bifidobacteria, there was also a trend towards higher numbers of lactobacilli, while faecal numbers of Bacteroidaceae and *Candida* spp. were decreased. In a later study by Ito *et al.* (1993a), an increase in both bifidobacteria and lactobacilli was seen after daily consumption of 15 g of galacto-oligosac-

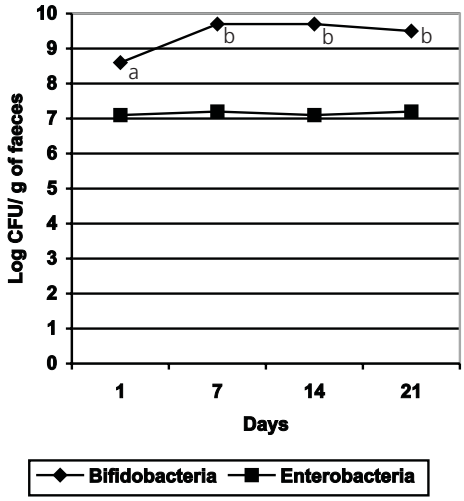

Fig. 42.2 Mean faecal concentrations of bifidobacteria and enterobacteria in eight healthy volunteers after ingestion of galacto-oligosaccharides for 21 days. Results are expressed as log colony-forming units (CFU)/g faeces. Values with different letters are significantly different, $P < 0.05$. (Data from Bouhnik *et al*. 1997.)

charides, while Ohtsuka *et al.* (1989) showed similar increases after daily galacto-oligosaccharide consumption of 8 g. Increases in bifidobacteria numbers were also found following the consumption of smaller quantities of galacto-oligosaccharides. Ishikawa *et al.* (1995) and Ito *et al.* (1993b) each showed that daily consumption of 2.5 g of galacto-oligosaccharides resulted in increased faecal bifidobacteria content in healthy volunteers who initially had relatively low numbers of these (indigenous) bacteria. Indeed, in one study (Tamai *et al.* 1992) in humans with a normal indigenous bifidobacteria content, an increase of bifidobacteria was seen after daily consumption of only 2.0 g of galacto-oligosaccharides.

42.3.2 *Stimulation of calcium absorption*

In addition to increasing the daily intake of calcium by, for example, calcium fortification of foods, it is possible to increase the absorption of dietary calcium by the consumption of galacto-oligosaccharides. By virtue of this enhancement of calcium absorption, galacto-oligosaccharides also appear to prevent bone loss (Chonan *et al.*, 1995).

While calcium is necessary for skeletal development, only a part of the dietary calcium intake is available for absorption, which occurs in both the small intestine and colon (van den Heuvel *et al*. 2000; Sako *et al.* 1999). The stimulatory effects of galacto-oligosaccharides on calcium absorption (and consequently prevention of bone loss) were demonstrated in a study with ovariectomised Wistar rats (a model for postmenopausal conditions) (Chonan *et al.* 1995). Feeding rats a diet containing 5% galacto-oligosaccharides over a 30-day period resulted in more efficiently absorbed calcium in comparison with the control diet, after both 8–10 and 18–20 days. The calcium content in the femur and ash weight of the tibia was increased after administration of galacto-oligosaccharides to sham-operated rats, in comparison with rats fed a control diet. When galacto-oligosaccharides were administered to ovariectomised rats, the bone ash weight (femur and tibia) and tibia calcium content were higher than after administration of the reference substance (Table 42.1). Chonan and Watanuki (1995) measured calcium absorption after administration of 5% and 10% galacto-oligosaccharides to male Wistar rats over a 10-day period, and found both treatments to increase calcium absorption.

Van den Heuvel *et al*. (2000) carried out a human study on the effect of a product rich in galacto-oligosaccharides (Elix'or®) on true calcium absorption in postmenopausal women. In this study, two treatments (2×10 g galacto-oligosaccharides per day, compared with a reference material) were given over 9 days. True calcium absorption was determined by means of a dual stable isotope technique. After consumption of the product rich in galacto-oligosaccharides, a relative increase in calcium absorption of 16% was observed, in comparison with the reference treatment. The increased calcium absorption was not accompanied by increased urinary calcium excretion, implying that galacto-oligosaccharides may also increase the uptake of calcium by the bones and/or inhibit bone resorption.

One hypothesis for the mechanism of enhanced calcium absorption is that the reduced pH of the colon contents caused by fermentation of galacto-oligosaccharides increases the concentration of the soluble, ionised form of calcium necessary for intestinal absorption (Chonan & Watanuki 1995; Chonan *et al.* 1995).

Several studies have also shown that galacto-oligosaccharides can stimulate the absorption of other minerals; for example, Chonan *et al.* (1996) and Yanahira *et al.* (1997) each showed increased magnesium absorption after administration of galacto-oligosaccharides to Wistar rats.

Table 42.1 Effects of dietary galacto-oligosaccharides (GOS) administration and ovariectomy on bone development in rats.

	Sham-operated		Ovariectomy		Two-way ANOVA		
	Control	GOS	Control	GOS	A	B	A×B
Femur (mg/100 g body weight)							
Dry weight	175.6 ± 10.4^{a}	182.3 ± 11.9^{a}	148.1 ± 10.4^{b}	156.3 ± 4.4^{b}	**	*	NS
Ash weight	103.5 ± 7.3^{a}	109.0 ± 8.0^{a}	81.2 ± 2.3^{b}	89.3 ± 3.1^{c}	**	**	NS
Calcium content	27.6 ± 1.9^{a}	30.1 ± 2.9^{b}	21.6 ± 1.1^{c}	24.0 ± 1.1^{c}	**	**	NS
Tibia (mg/100 g body weight)							
Dry weight	$138.7 \pm 17.8^{a,b}$	148.4 ± 10.2^{a}	116.9 ± 7.9^{c}	$129.6 \pm 3.7^{b,c}$	**	**	NS
Ash weight	$85.4 \pm 9.8^{a,b}$	93.5 ± 5.9^{c}	69.7 ± 3.9^{d}	77.8 ± 2.6^{b}	**	**	NS
Calcium content	$20.7 \pm 3.2^{a,b}$	22.5 ± 1.7^{a}	16.3 ± 1.5^{c}	19.6 ± 1.0^{b}	**	**	NS

Values are mean ± SD ($n = 9$). Means in the same row not sharing a common superscript letter are significantly different by Tukey's test ($P < 0.05$). A, ovariectomy; B, dietary GOS administration; ** and * significant effect ($P < 0.01$, $P < 0.05$).
NS, not significant.
Source: Chonan *et al*. 1995.

42.3.3 *Relief of constipation*

Several human studies have shown that consumption of galacto-oligosaccharides improves defaecation in subjects who are either constipated, or who show a tendency towards constipation.

In a study by Deguchi *et al.* (1997), 78 volunteers with constipation tendency consumed a beverage containing 2.5 or 5.0 g of galacto-oligosaccharides per day over a period of 1 week, while 50 more volunteers consumed 10.0 g/day. Following consumption of 5.0 or 10.0 g of galacto-oligosaccharides, the defaecation frequency increased and the faeces became softer. In all volunteers consuming 10.0 g/day galacto-oligosaccharides, an increase was seen in the number of days with defaecation each week. Teuri and Korpela (1998) investigated the effect of consuming 9 g/day galacto-oligosaccharides on the relief of constipation in elderly subjects. The defecation frequency increased after galacto-oligosaccharide treatment, compared with that in controls. No changes were seen in the use of laxatives, the consistency of faeces, and the ease of defaecation, although consumption of galacto-oligosaccharides tended to make defaecation easier. Shitara (1988) also demonstrated an improvement on constipation in elderly people given 8 g/day galacto-oligosaccharides for a 1-week period.

42.3.4 *Inhibition of colon carcinogenesis*

Galacto-oligosaccharides appear to play an inhibitory role in the development of colon cancer. Several studies have shown ingestion of galacto-oligosaccharides to result in a reduction of 'risk factors' for the development of these tumours. These factors include the levels of the bacterial enzymes, β-glucuronidase and nitroreductase, as well as secondary bile acids, ammonia and indole (Rowland & Tanaka 1993; Roberfroid *et al.* 1995; Sako *et al.* 1999; Wijnands *et al.* 1999). Furthermore, in animal studies a decrease in the induction of colo-

rectal tumours was seen after administration of large amounts of galacto-oligosaccharides (Wijnands *et al.* 1999; Wijnands & Woutersen 1999).

A reduction in the levels of bacterial β-glucuronidase and nitroreductase, and an increase in β-glucosidase, was seen in rats fed 5% galacto-oligosaccharides for 4 weeks (Rowland & Tanaka 1993). The level of conversion of the mutagen, 2-amino-3-methyl-3H-imidazo[4,5-f] quinoline (IQ), into its genotoxic metabolite was also decreased. In a human study it was shown that consumption of 15 g of galacto-oligosaccharides led to a reduction in the amount of ammonia, *p*-cresol and indole in faeces (Ito *et al.* 1993). Ishikawa *et al.* (1995) demonstrated a reduction in faecal secondary bile acids in humans after daily consumption of 2.5 g and 10 g galacto-oligosaccharides. Wijnands *et al.* (1999) compared the effects of diets with either a low (4.57–5.19 wt%) or high (22.55–24.51 wt%) amount of the non-fermentable fibre cellulose, or a low (8.30–9.54 wt%) or high (26.34–28.63 wt%) amount of the fermentable fibre galacto-oligosaccharides (combined with different levels of dietary fat) on the development of colorectal cancer. All rats were treated with 1,2-dimethylhydrazine to induce colorectal tumours. In general, the diet with a high cellulose content had either no effect or an enhancing effect on the formation of colorectal tumours, though the development of carcinomas was decreased. In contrast, the diet containing a high amount of galacto-oligosaccharides resulted in a decreased multiplicity of adenomas, carcinomas and total tumours (Fig. 42.3), and a decreased mean size of carcinomas and total tumours, regardless of the fat content. A trend was seen towards a decreased incidence of tumours. It was concluded that the diets containing galacto-oligosaccharides conferred a greater protection against colorectal cancer than those containing cellulose. In another study (Wijnands *et al*. 1999), the effects of a diet containing a low (5 wt%) or high (20 wt%) amount of galacto-oligosaccharides on the development of preneoplastic lesions and tumours in the colon and rectum of rats was studied. In rats fed a diet containing a high amount of galacto-oligosaccharides, a protective effect against the development of colorectal tumours was seen, and this protective effect was exerted mainly during the promotion phase of the carcinogenic process.

42.4 Target groups and applications

Galacto-oligosaccharides are especially suitable in food products for special target groups, such as infant nutrition, clinical nutrition and foods for elderly people. Certain groups of people may gain additional benefit from the consumption of galacto-oligosaccharides. Bottle-fed infants for example, may benefit from the bifidogenic effect because their microflora contains a low amount of bifidobacteria compared with breast-fed infants (Beerens *et al.* 1980; Benno *et al.* 1984; Sako *et al.* 1999). Consumption of galacto-oligosaccharides can also benefit the elderly in several ways, as they have lower counts of bifidobacteria in their microflora than do younger adults (Mitsuoka 1990), they often suffer from constipation, and their capacity to absorb calcium is lower than that of younger adults.

In addition to the application of galacto-oligosaccharides to food products for special target groups, these materials may also be added to traditional foods, such as dairy products, bakery products, beverages and supplements. Products that are made under high temperature and low pH conditions are especially suitable for inclusion of heat- and acid-stable galacto-oligosaccharides.

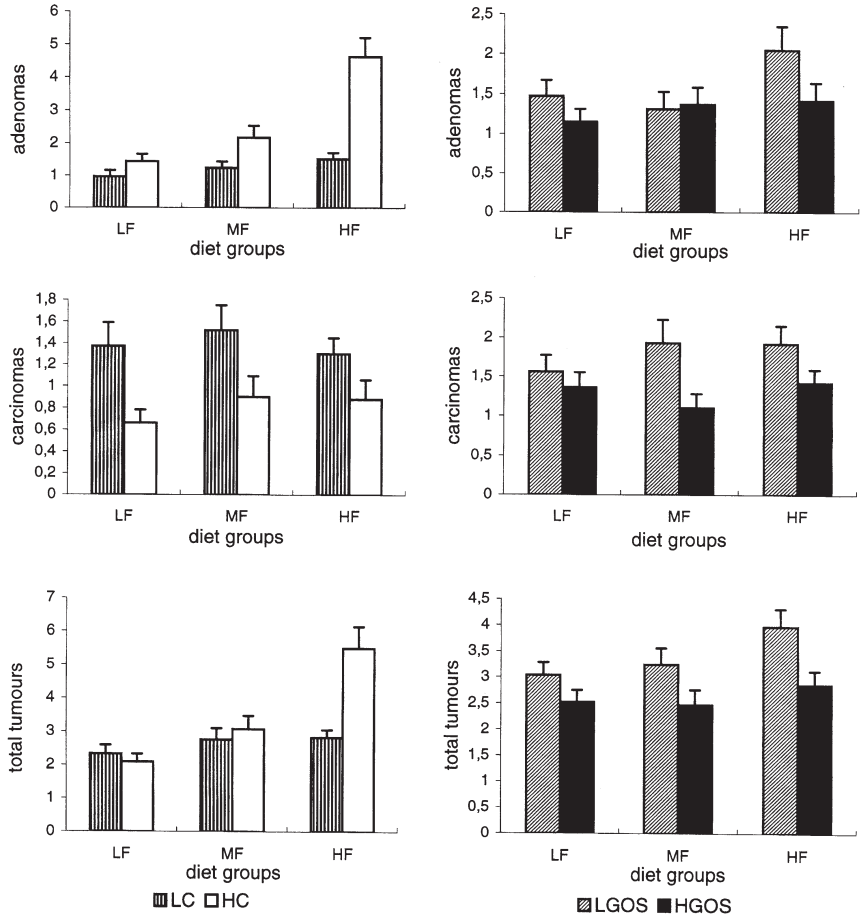

Fig. 42.3 Multiplicity of adenomas, carcinomas and total tumours, expressed as the mean number (± SEM) of tumours per tumour-bearing animal. The high-cellulose (HC) diets resulted in an increase of the multiplicity of adenomas ($P < 0.01$) and total tumours ($P < 0.01$), but in a decrease of the carcinoma multiplicity ($P < 0.01$). In the high-galacto-oligosaccharide (HGOS)-fed animals, the multiplicity of adenomas, carcinomas ($P < 0.01$) and total tumours ($P < 0.01$) was decreased. LF, MF and HF = low-, medium- and high-fat, respectively; LC = low-cellulose; LGOS = low-galacto-oligosaccharides. (Data from Wijnands *et al.* 1999.)

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43 Polydextrose: Analysis and Physiological Benefits

Stuart A.S. Craig

43.1 Introduction

Polydextrose (PDX) was invented at Pfizer Central Research during the late 1960s, and patented in 1973 (Rennhard 1973). It was originally developed as a reduced calorie (1 kcal/g) replacement for sugar, and partial replacer for fat, flour and starch. PDX is prepared by vacuum thermal polymerisation of glucose, using sorbitol and an approved food acid as catalyst. Random polymerisation and branching yield various types of glycosidic bonds in the structure (1,6 bonds predominate) (Rennhard 1973; Allingham 1982). A representative structure is shown in Fig. 43.1. Improved versions of PDX (Litesse®) have been patented that utilise ion exchange and hydrogenation, and provide even broader utility in foods (Borden *et al.* 1997; Guzek *et al.* 1997a, b).

The structural compactness and complexity of PDX prevents mammalian enzymes from hydrolysing the molecule. This imparts reduced caloric content, as the majority of PDX passes through the stomach and enters the large intestine, whereupon it behaves as a dietary fibre (Craig *et al.* 1998). This chapter discusses the physiological benefits and analytical measurement of PDX.

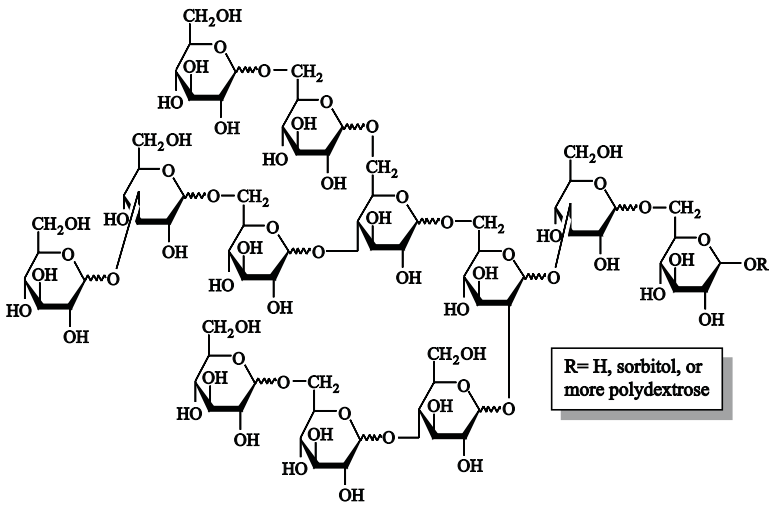

Fig. 43.1 Representative structure for polydextrose (PDX).

43.2 Structure of PDX

The representative structure of PDX (Fig. 43.1) was determined from a variety of analyses, including methylation, periodate oxidation, acetolysis, ^{13}C-NMR, chromatography and laser light scattering (Allingham 1982; Craig *et al.* 1996). The average degree of polymerisation (DP) is ~12 (weight average molecular weight of ~2000 Da), although the range of molecular weights is from 162 to ~20 000 Da. The International Union of Pure and Applied Chemistry (IUPAC) defines oligosaccharides as carbohydrates with a DP of between 3 and 9, and polysaccharides as DP ≥10 (Cummings & Englyst 1995). Therefore, PDX is most accurately defined as a polysaccharide. However, PDX does not analyse as a polysaccharide by a widely used technique – precipitation with aqueous (80%) ethanol. This is due to its highly branched structure and high water solubility, and this is therefore a significant issue with the determination of PDX as a dietary fibre. The aqueous ethanol step is part of the current Association of Official Analytical Chemists (AOAC) assay for dietary fibre; thus, PDX is not measured as dietary fibre.

43.3 Analysis of PDX

AOAC method 985.29 for determining total dietary fibre (TDF) in food is enzyme-gravimetric, and this is accepted in most countries as an official technique. It has been found that PDX provides no statistically significant TDF value by this method (Craig *et al.* 2000), although it has also been determined that PDX is not hydrolysed by the enzymes used in method 985.29, by analysing the aqueous ethanol supernatant (Craig *et al.* 2000). We have therefore developed a method for measuring PDX in foods that can be used as an adjunct to method 985.29 (and similar methods). This method has undergone a collaborative study, and was adopted by the AOAC as Official Methods^{SM} number 2000.11.

The first method developed to measure PDX in a food involved aqueous extraction of PDX, followed by a colorimetric assay (Dubois *et al.* 1956). The method uses acid hydrolysis to break the glycosidic bonds, followed by dehydration and derivatisation of the dextrose using phenol and sulphuric acid. The derivatised dextrose is measured spectrophotometrically at 490 nm. This method is cumbersome, and has only satisfactory precision. Moreover, the accuracy of this method can also be affected by other carbohydrates that may be present in a food system.

Several liquid chromatography (LC)-based methods have been published (Kobayashi *et al.* 1989; Arrigoni & Amado 1990; Noffsinger *et al.* 1990; Stumm & Baltes 1992). An assay currently used in Japan quantifies PDX as a fibre in foods (Kobayashi *et al.* 1989). As with the AOAC dietary fibre method, enzymes are used to degrade starch and maltodextrins in the food. The sample is then membrane-filtered, deionised and injected into the LC system (Ultron PS-80 N column, using refractive index detection). Noffsinger and co-workers (1990) used a sulphonated, polystyrene divinylbenzene (calcium form) column (Biorad Aminex HPX-87C) and refractive index detection, but no enzyme step. Arrigoni and Amado (1990) used a sulphonated polystyrene divinylbenzene (lead form) column (Biorad Aminex HPX-87P) and refractive index detection, but with a different enzyme. These methods work well for simple food systems (e.g. clear beverages), but the sample preparation does not remove many resistant oligomers or polymers, and this may lead to potential interference. In

addition, refractive index is a universal detection system that is non-specific to carbohydrates (including PDX).

Stumm and Baltes (1992) used an enzyme step followed by an anion exchange column (Dionex PA1), mobile phase gradient, and pulsed amperometric detection. This provides more selectivity for carbohydrates, but is a more complex technique. Our method (see below) is based on that of Stumm and Baltes, but uses an improved sample preparation step. PDX is extracted from food with hot water, and the extract is centrifuged. The supernatant then passes through a centrifugal ultrafilter to remove high-molecular weight interfering components. The filtrate is treated with an enzyme mix (isoamylase, amyloglucosidase and fructanase) to remove any oligosaccharide interference (mainly malto-oligomers and fructans). PDX standards undergo the same treatment. High-pressure anion exchange chromatography with electrochemical detection (HPAEC-ED) is used to detect and quantitate a high-molecular weight fraction of PDX. Internal validation of the method on various foods and PDX levels (Craig *et al.* 2000) demonstrated an average recovery of 95%, and average % relative standard deviation (RSD) of 3%. Calibration curves consistently gave a R^2 value of ≥ 0.998.

43.4 Physiological benefits of PDX

Several studies on PDX (human clinical, animal clinical and *in vitro*) have demonstrated physiological effects associated with dietary fibre (Polydextrose FAP 1978; Hamanaka 1987; Tomlin & Read 1988; Nakagawa *et al.* 1990; Endo *et al.* 1991; Oku *et al.* 1991; Wang & Gibson 1993; Achour *et al.* 1994; Harada *et al.* 1995). Upon reaching the lower intestine, PDX is partially fermented by colonic bacteria to short-chain fatty acids (SCFA). A qualitative measurement of SCFA produced from PDX gut fermentation was determined in a study using radiolabelled PDX (Polydextrose FAP 1978). The profile of acetate, propionate and butyrate was typical of that generated by dietary fibre. Another study measured the *in-vitro* production of SCFA from fermentation of 17 carbohydrates by slurries of mixed human faecal bacteria (Wang & Gibson 1993). PDX produced a molar ratio of acetate:propionate:butyrate of 61:25:14, which was the second highest proportion of propionate and the third highest proportion of butyrate of the carbohydrates studied. Butyrate is the preferred source of energy for colonocytes (Roediger 1980, 1982), being metabolised in preference to glucose and other substrates. SCFA were found to stimulate the proliferation of normal human caecal colonocytes (Scheppach *et al.* 1991), with butyrate being more effective than either propionate or acetate. Butyrate has also been shown to slow the rate of cancer cell proliferation and to promote the expression of differentiation markers *in vitro* (Kim *et al.* 1980; Whitehead *et al*. 1986).

PDX ingestion leads to a lower faecal pH, increased faecal bulking, reduced transit time and softer stools (Polydextrose FAP 1978; Nakagawa *et al.* 1990; Endo *et al.* 1991; Oku *et al.* 1991; Wang & Gibson 1993; Achour *et al.* 1994; Harada *et al.* 1995). Intestinal infusion of PDX (Polydextrose FAP 1978) in humans resulted in a pH drop from 7.24 ± 0.45 to 6.44 ± 0.35 after 150 min ($P < 0.05$). Endo *et al*. (1991) found a similar drop in pH in another human trial. Oku *et al*. (1991) fed PDX to rats (3% of diet), and found an increased faecal volume and weight, decreased transit time, and increased faecal moisture content. Nakagawa *et al*. (1990) found that PDX fed to women led to softer stools, while Harada *et al*. (1995) showed PDX ingestion to stimulate a more rapid maturation of the gastrointestinal tract in weanling rats. Wang and Gibson (1993) measured the growth of colonic bacteria in batch fer-

menters with various carbohydrates added, and found that over 12 h, PDX led to an increase in total bacteria that was similar to that achieved with other carbohydrates (including pectin, starch, inulin, oligofructose and fructose). This is an important finding because bacteria form the majority of the weight of faeces.

The intestinal microflora are modulated by PDX, with an increase of beneficial bacteria (e.g. *Lactobacillus and Bifidobacterium*) and a decrease in detrimental species (e.g. *Clostridium*) (Endo *et al.* 1991). In addition, PDX fermentation reduced the concentration of certain putrefactive/carcinogenic substances (e.g. indole and *p*-cresol) in the colon (Endo *et al.* 1991). PDX also aids in blood glucose homeostasis as a result of its low glycaemic index (15% compared with glucose at 100%) (Polydextrose FAP 1978).

43.5 Conclusions

PDX is a polysaccharide that is not measured as dietary fibre in foods by AOAC method 985.21 (and similar methods) due to its solubility in aqueous ethanol. We have developed a rugged method for measuring PDX in foods (AOAC method 2000.11) to be used as an adjunct to 985.29 (and similar methods), in foods where PDX is thought to be present. PDX is resistant to digestion in the small intestine, but is partially fermented in the large intestine by colonic microflora. This leads to a variety of beneficial physiological effects associated with dietary fibre.

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44 Fibersol-2: a Soluble, Non-digestible, Starch-derived Dietary Fibre

Kazuhiro Ohkuma and Shigeru Wakabayashi

44.1 Introduction

Starch has been widely consumed by humans as an inexpensive and stable source of available carbohydrate. Starch, in the native form, starch hydrolysates or modified starches have been utilised in various ways due to its excellent digestibility.

In recent years, indigestible species or components have been found in starches, and these starches are called 'resistant starches' (Englyst & Cummings 1987). Resistant starches have been widely studied and shown to have physiological functions similar to those of dietary fibres.

Similarly, we have found the same types of indigestible components in starch hydrolysates, such as dextrin, maltodextrin and corn syrup. Starch hydrolysates containing the indigestible components are termed 'resistant maltodextrins'. One such material, which is produced by a combination of hydrolysis and transglucosidation reactions (that occur during hydrolysis), has physiological attributes resembling those of dietary fibre. The physical characteristics of this material make it suitable for use in various food applications.

In this chapter we will describe the properties of one of these resistant maltodextrin materials, namely Fibersol-2®.

44.2 Production method and basic characteristics of Fibersol-2

Fibersol-2 is produced by a combination of heat and enzymatic treatment of cornstarch, as detailed in US Patent Nos. 5620873 and 5358729. In the first reaction, cornstarch is heated with a small amount of hydrochloric acid under low-moisture conditions. During this reaction, the cornstarch is hydrolysed by transglucosidation. In the second reaction, the above-obtained solution is hydrolysed by an amylase. The material is then refined to separate out impurities, analysed to ensure that the dextrose equivalent (DE) is below 20, and is then powdered by spray-drying. In Japan, this material is of the type known as an indigestible dextrin, and simultaneously meets the US GRAS (generally recognised as safe) requirements as set forth in 21 CFR.184-1444 (Maltodextrin).

An average molecular weight of Fibersol-2 is 2000 Da, and the proposed structural composition is shown in Fig. 44.1. Fibersol-2 is composed not only of $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glucosidic bonds, as are present in the native starch, but also contains $1\rightarrow2$ and $1\rightarrow3$ linkages and levoglucosan. Due to these structural characteristics, Fibersol-2 contains well-developed, branched particles that are partially hydrolysed by human digestive enzymes.

The product specifications of Fibersol-2, and the analytical methods used to measure this material, are shown in Table 44.1. Fibersol-2 is a white powder and contains about 90% of

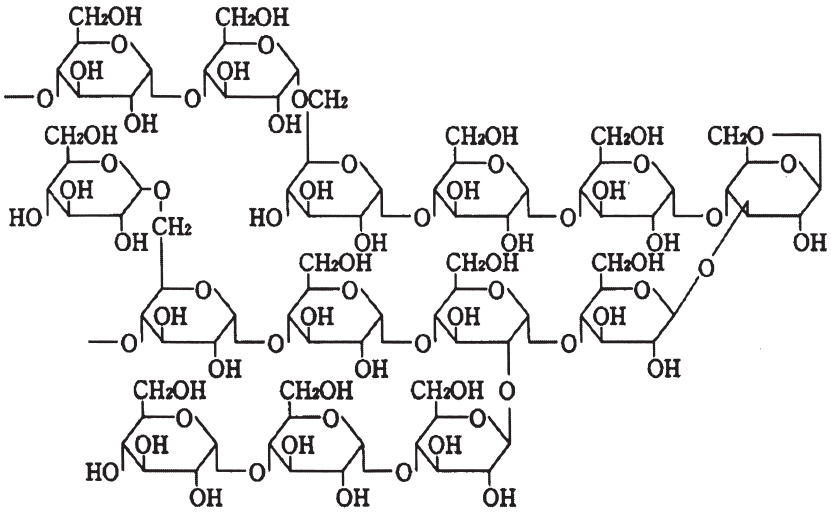

Fig. 44.1 Estimated structural formula of Fibersol-2.

Table 44.1 Product specifications of Fibersol-2.

Property	Specification	Test method
Appearance	White, free-flowing powder	Sensory test
Taste/odour	Slightly sweet/odourless	Sensory test
Solution	Clear	Sensory test
Moisture	5% maximum	JAS method
Total dietary fibre	85% minimum	Enzyme-HPLC method
Dextrose equivalent	8.0–12.0	WS method
pH	4–6 in 10% solution	pH meter
Ash	0.2% maximum	Japanese Standards for Food Additives
Arsenic	1 p.p.m. maximum	
Heavy metals	5 p.p.m. maximum	
Microbiological		
Standard plate count	300/gram, maximum	Japanese Food Sanitation Law
Yeast and mould	100/gram, maximum	
Salmonella	Negative/25 g	
Coliforms	Negative/g	

indigestible components (as we claim to be dietary fibre). The enzyme-high-performance liquid chromatography (HPLC) method (Table 44.1) has been validated by the Japanese Government as an official analytical method for determining total dietary fibre, including low-molecular weight soluble dietary fibre (Ohkuma *et al.* 1990).

Fibersol-2 has various and unique physical characteristics. Its viscosity is lower than that of a conventional maltodextrin, although both have the same DE value. A solution of Fibersol-2 is very clear and stable, and does not become cloudy or show signs of any precipitation (retrogradation) when kept for long periods of time. It also has very good anti-acid

properties (in contrast to sugars), and can be cooked and sterilised at high temperature in food applications due to its stability in heat processes.

44.3 Safety of Fibersol-2

Neither acute toxicity (LD_{50} in rats >20 g/kg) nor mutagenicity have been found with Fibersol-2 (Wakabayashi *et al.* 1992a). According to a long-term administration study in rats, Fibersol-2 scarcely affects animal growth, weight of internal organs or any blood biochemical parameters. Indigestible saccharides, including Fibersol-2, have been shown to cause some diarrhoea when taken in excessive quantities, although we have the dose required for this (ED_{50}) to be >1.0 g/kg body weight. It is considered that the higher ED_{50} value of Fibersol-2 compared with other indigestible saccharides (e.g. sugar alcohols) is due to its higher molecular weight and lower osmotic pressure (Satouchi *et al.* 1993).

44.4 Internal movement, energy value and physiological functions of Fibersol-2

Fibersol-2 escapes digestion and absorption in the upper gastrointestinal tract, but when it reaches the large intestine it is partly fermented by bacteria, producing short-chain fatty acids (SCFA). In a previous *in-vitro* study, it was shown that ~10% of Fibersol-2 was degraded by artificial gastric juice, amylase and intestinal mucosa enzymes. Based on the results of a single administration test in rats, the faecal excretion rate of Fibersol-2 was 38% (Wakabayashi *et al*. 1991). Thus, it is estimated that ~90% of the administered Fibersol-2 reaches the large intestine, and half of that is metabolised by intestinal bacteria; the remaining 40% is excreted in the faeces, unused. By contrast, studies on the growth rates of rats fed Fibersol-2 showed that <10% of dextrose is contributing net metabolisable energy. Fibersol-2 has an energy value of 0.5 kcal/g (Tsuji & Gordon 1998).

Based on the above findings, the physiological functions of Fibersol-2 can be separated into those occurring in the upper digestive tract, and those occurring in the lower digestive tract. Fibersol-2 itself affects the absorption rate of carbohydrate in the human body (Fig. 44.2), and this in turn moderates postprandial blood glucose levels. Furthermore, in terms of the indirect effects of Fibersol-2 in humans, the metabolic products of Fibersol-2 (e.g. SCFA) might be expected to improve the intestinal microflora, intestinal regularity and the immune function. SCFA produced in the large intestine are also expected to stimulate bowel movement.

44.4.1 *Moderating effect of postprandial blood glucose levels*

The results of loading tests in rats using various saccharides (Wakabayashi *et al.* 1993, 1995) are shown in Fig. 44.3. The blood glucose levels and insulin secretion of 8-week-old male SD strain rats were monitored for 2 h after oral administration of 1.5 g/kg body weight of sugars, with or without 0.15 g/kg body weight of Fibersol-2. Co-administration of Fibersol-2 led to a lowering of blood glucose levels (to 70% of peak value) found after sucrose or maltose loading. In addition, Fibersol-2 lowered insulin secretion to 63–70% of those of the sucrose-, maltose- and maltodextrin-loaded groups. Thus, the blood glucose-moderating effect of

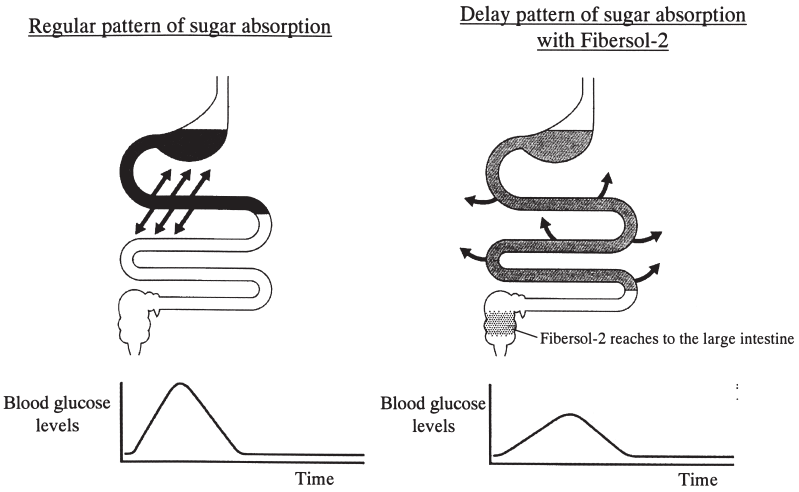

Fig. 44.2 Internal movement of Fibersol-2.

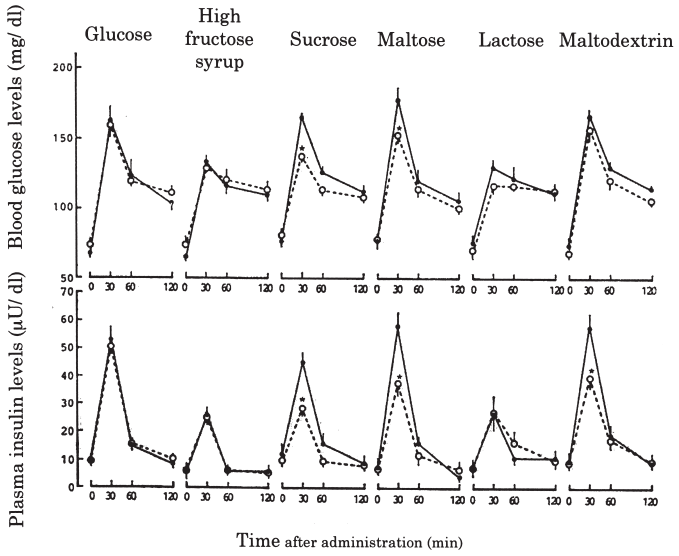

Fig. 44.3 Effect of Fibersol-2 on various sugar tolerance in rats. Changes in plasma glucose (upper panel) and insulin (lower panel) levels after administration of various sugars (1.5 g/kg body weight) with (○) or without (●) Fibersol-2 (0.15 g/kg body weight) in male Sprague-Dawley rats. *$P<0.05$.

Fibersol-2 is specific to disaccharides and starch. This led us to propose that Fibersol-2 inhibits the disaccharidase-mediated hydrolysis of sugars, although in an *in-vitro* study it did not inhibit sucrase or maltase activities of enzymes obtained from the small intestine mucosa of rats (Wakabayashi 1992; Wakabayashi *et al.* 1993). Furthermore, in sucrose and glucose digestion and absorption studies using an everted intestinal sac of rats (Wakabayashi 1992) and in an *in-situ* perfusion study (Wakabayashi *et al.* 1993), it was shown that Fibersol-2 most likely did not affect either the digestion or absorption of monosaccharides. Fibersol-2 ap-

pears selectively to inhibit the absorption of glucose generated by the digestion of di-, oligo- and polysaccharides. From these results, it may be considered that the effect of Fibersol-2 on blood glucose levels differs from that of viscous soluble dietary fibres such as pectin or guar gum, the latter compounds inhibiting physical absorption of nutrients in the digestive tract by the formation of a gel. Fibersol-2 appears to act specifically on sugar absorption in the small intestine by reversibly blocking the cooperative disaccharidase-related transport system, i.e. the 'gate' located on the surface of the small intestines. This 'gate' is the mechanism by which glucose generated from disaccharides is selectively transported (Malathi *et al.* 1973; Ramaswamy *et al.* 1974, 1976). This hypothesis has been discussed previously (Wakabayashi 1992; Wakabayashi *et al.* 1993, 1995).

The ingestion of Fibersol-2 also affects postprandial glucose levels (Tokunaga & Matsuoka 1999). Changes in postprandial blood glucose levels were monitored in 40 healthy humans (32 males, 8 females; average age 37 years; average height 167 cm; average body weight 65 kg; average body mass index (BMI) 23.1) by taking a fixed menu of Kitsune-udon (Japanese Udon noodle with a fried bean curd) and rice with topping (protein, 16 g; fat, 9 g; sugar, 105 g; total caloric value 580 kcal) either with green tea or a beverage containing 5.0 g of Fibersol-2. Changes in the mean blood glucose level over a 2-h period after the meal are shown in Fig. 44.4. Postprandial blood glucose levels for subjects receiving green tea plus Fibersol-2 remained low when compared with those of subjects ingesting only the green tea, despite the two groups having ingested the same fixed menu.

44.4.2 *Effect on prevention of obesity*

The animal investigations and clinical studies described above show that the co-ingestion of Fibersol-2 with saccharides (e.g. sucrose or corn syrup) can moderate increases in postprandial blood glucose levels. Thus, continuous ingestion of Fibersol-2 has the effect of correcting obesity, which is considered to be a 'trigger' for many types of diseases in adults (Fujioka *et al.* 1987).

Five-week-old male Sprague-Dawley (SD) rats were given either a standard diet, a high-sucrose diet (containing 65% sucrose) or the same high-sucrose diet with Fibersol-2 (5% of

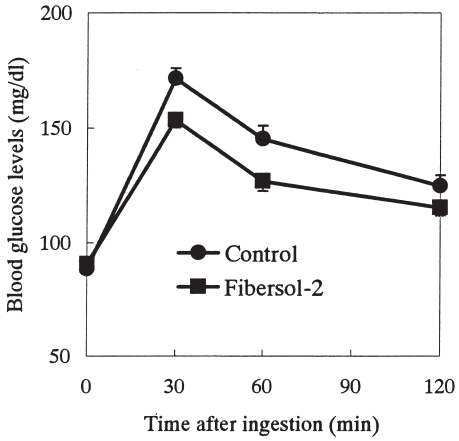

Fig. 44.4 Effect of Fibersol-2 on postprandial blood glucose levels in healthy human subjects ($n = 40$).

the diet), for 8 weeks. The body weight and proportion of body fat before and after ingestion are shown in Fig. 44.5. Although the body weight of rats fed the high-sucrose diet did not increase substantially, their body fat content rose significantly. However, no difference was seen in either body weight or body fat content between rats fed the high-sucrose diet + Fibersol-2 and those fed a standard diet. It appears that addition of Fibersol-2 to the high-sucrose diet moderated the increase in postprandial glucose levels and reduced the insulin requirement, thus preventing the fat accumulation seen as a marker of obesity (Wakabayashi *et al.* 1992b, 1995).

The same effect was found in 110-day-old broiler chickens (Watanabe *et al*. 1993), the yield (ratio of non-fat meat to total body weight) of the broilers being improved when the animals were fed a diet containing 5% Fibersol-2, as well as lowering the body fat content. There was a significant reduction in the fat content of the liver (from 27% to 17%), and also of fat accumulation in internal organs. These effects on the correction of obesity have been confirmed in dogs and cats with a high degree of obesity (Hosoido 1993) by feeding a pet food containing 1.2–1.3 g/kg body weight of Fibersol-2 for 1 month. Ingestion of Fibersol-2 was found to reduce neither the animal's body weight nor degree of obesity when both their original degree of obesity and body weight were in the normal range (Fig. 44.6). Moreover, these data confirm that Fibersol-2 can be ingested safely, without showing the problems associated with 'slimming drugs'.

From these studies it was clear that accumulated body fat (obesity) of various animal species could be reduced with Fibersol-2 by moderating postprandial blood glucose levels, and by lowering insulin secretion. In current clinical evaluations, body fat content is used as an index of the effect of Fibersol-2 in the prevention and treatment of obesity (Tokunaga & Matsuoka 1999).

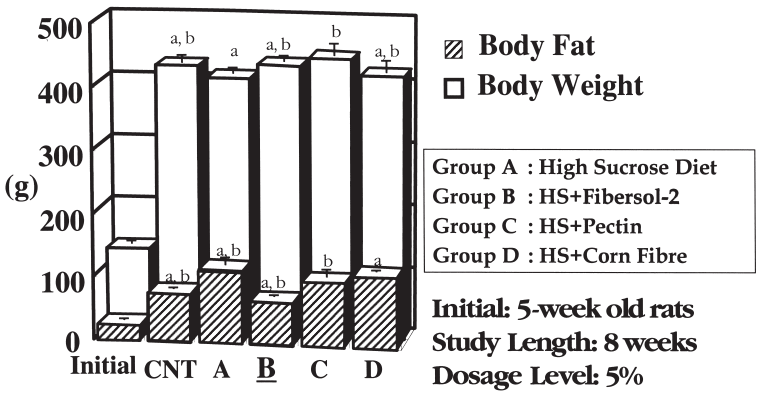

Fig. 44.5 Effect of Fibersol-2 on body weight and whole body fat accumulation in rats. Values not sharing a common letter are significantly different from each other.

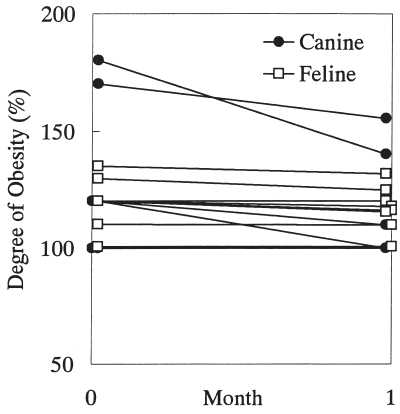

Fig. 44.6 The prevention of obesity by Fibersol-2 in canine and feline species.

44.4.3 *Clinical improvement of sugar and fat metabolism*

Improvements in sugar and fat metabolism after ingestion of Fibersol-2 over an 8- to 12-week period have been reported by several authors (Matsuoka *et al*. 1992; Fujiwara *et al.* 1993; Hosoido 1993). One study involved 10 healthy adults and 10 type 2 diabetes mellitus (type 2-DM) patients. These subjects ingested 10–20 g of Fibersol-2 after each meal (total 30–60 g/day), and levels of fasting blood glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides (triacylglycerol) were monitored. The results of the study are shown in Table 44.2. Fasting blood glucose levels of healthy adults who ingested Fibersol-2 remained in the normal range, but there was a significant improvement in blood glucose levels in type2-DM patients who ingested 20 g Fibersol-2. By week 12, the average blood glucose levels for five of these patients had improved to 103 mg/dl – within the normal range. Total serum cholesterol levels were significantly lowered in healthy adults, as well as in type 2-DM patients who ingested 20 g Fibersol-2. Although serum total cholesterol levels in type2-DM patients who ingested only 10 g Fibersol-2 tended to be lower than in controls, the reduction was not significant. It may be concluded that the effective dose of Fibersol-2 for Type2-DM patients is greater than that for healthy adults.

There are two types of serum cholesterol. The first is HDL-cholesterol, which salvages free cholesterol from distal tissues. The other is low-density lipoprotein (LDL)-cholesterol, which releases cholesterol esters through the receptor. Thus, depending on their roles, HDL-cholesterol is referred to as ‘good’, and LDL-cholesterol is ‘bad’. The simultaneous lowering of total cholesterol levels and HDL-cholesterol levels is believed to be problematic. However, it has been shown that Fibersol-2 lowered total cholesterol levels by lowering LDL-cholesterol levels, without a change in HDL-cholesterol levels. Furthermore, ingestion of Fibersol-2 lowered serum triglyceride levels in both healthy (control) adults and type2-DM patients. It is known that changes in serum triglyceride levels are related to carbohydrate metabolism; thus, lowering of these levels by Fibersol-2 should be considered as an improvement effect on carbohydrate metabolism (Matsuoka *et al*. 1992; Nomura *et al.* 1992; Fujiwara & Matsuoka 1993).

Table 44.2 Lowering effects of Fibersol-2 on serum levels of glucose (fasting), cholesterol and triacylglycerol.

	Time of treatment (weeks)			
	Start	4	8	12
Fasting blood glucose (mg/dl)				
Healthy (30 g/day; $n = 10$)	95 ± 3	97 ± 2	95 ± 5	–
Type 2 DM (30 g/day; $n = 5$)	213 ± 34	214 ± 33	220 ± 38	208 ± 36
Type 2 DM (60 g/day; $n = 5$)	147 ± 17	107 ± 7*	147 ± 21	103 ± 7*
Total cholesterol (mg/dl)				
Healthy (30 g/day; $n = 10$)	226 ± 10	214 ± 10	199 ± 10*	–
Type 2 DM (30 g/day; $n = 5$)	230 ± 25	227 ± 22	220 ± 25	217 ± 19
Type 2 DM (60 g/day; $n = 5$)	265 ± 10	209 ± 22*	205 ± 10*	209 ± 9*
HDL-cholesterol (mg/dl)				
Healthy (30 g/day; $n = 10$)	47 ± 4	45 ± 4	49 ± 4	–
Type 2 DM (30 g/day; $n = 5$)	40 ± 4	41 ± 4	42 ± 5	44 ± 4
Type 2 DM (60 g/day; $n = 5$)	49 ± 2	47 ± 3	49 ± 3	47 ± 3
Triacylglycerol (mg/dl)				
Healthy (30 g/day; $n = 10$)	242 ± 64	197 ± 34	178 ± 41*	–
Type 2 DM (30 g/day; $n = 5$)	285 ± 60	230 ± 53*	244 ± 45	189 ± 40*
Type 2 DM (60 g/day; $n = 5$)	243 ± 34	134 ± 24*	148 ± 11*	176 ± 42

*$P < 0.05$. DM, diabetes mellitus; HDL, high-density lipoprotein.

44.5 Maintenance of digestive tract function by products of intestinal fermentation

The amount of short-chain fatty acids (SCFA) generated by incubating the caecal contents of rats with various types of indigestible saccharides under anaerobic conditions for 24 h (Kishimoto *et al.* 1995) is shown in Fig. 44.7. Both the proportions of the generated amount of three SCFA (acetic, propionic, butyric) and the total amount varied depending on the indigestible saccharide studied. In the case of Fibersol-2, propionic acid was produced in the largest amounts. The proportions of these SCFA generated from Fibersol-2 are similar to those generated from glucose under anaerobic conditions for 24 h. This outcome is interesting, since the base unit of Fibersol-2 is glucose.

Reports exist of the promotional effects of SCFA on physiological function of the digestive tract, including effects on the large intestinal mucosa, the exocrine pancreas and mucosal bloodstream, and the promotion of moisture absorption and inhibition of cholesterol synthesis. Furthermore, it is reported that SCFA are useful in maintaining the composition and function of the small intestine mucosa. A change in height of small intestine mucosal membrane microvilli of rats fed an enteral nutrition product for 2 weeks is shown in Fig. 44.8. In rats fed only the enteral nutritional product, the microvilli height was significantly shortened, compared with that in control rats fed a basal diet. However, the microvilli height was maintained by adding Fibersol-2 to the liquid diet at a level of 1.4%.

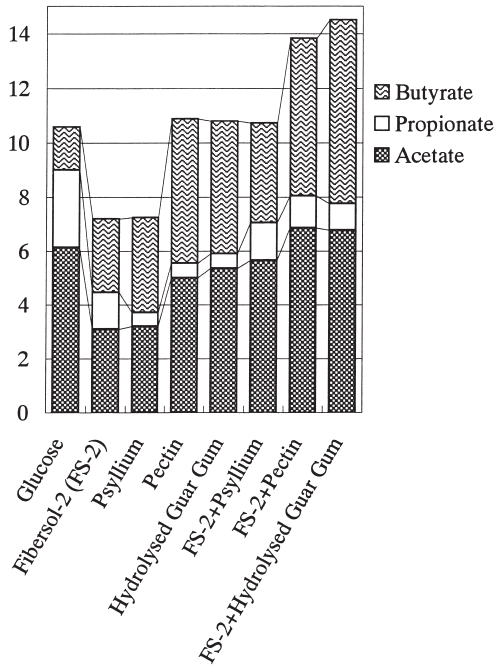

Fig. 44.7 Fermentability of various dietary fibres.

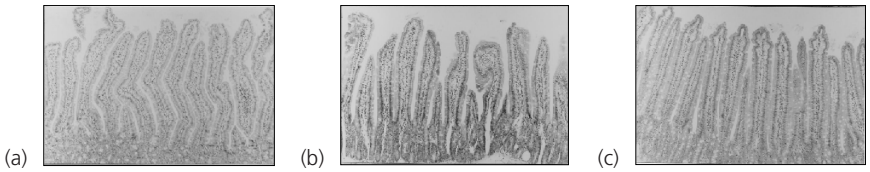

Fig. 44.8 Effect of Fibersol-2 on the maintenance of composition of the small intestinal mucosal microvilli of rats: (a) control (kept with stock diet); (b) enteral formula (no fibre); and (c) enteral formula with 1.4% Fibersol-2.

44.5.1 *Effect on bowel regularity*

The effect of Fibersol-2 on bowel regularity was confirmed by an ingestion study in 20 males and females with a tendency towards constipation (faecal frequency fewer than three times per week) (Satouchi *et al.* 1993). By ingesting 3.75 g of Fibersol-2 per day for 5 days, weekly faecal frequencies were improved from an average 2.6 before the study to 4.0 times during the study. Average faecal volume (expressed in terms of the volume of an egg) was also increased, from 6.0 before the study to 11.3 during the study. The proportion of bifidobacteria in the intestinal microflora was also significantly increased (Fig. 44.9).

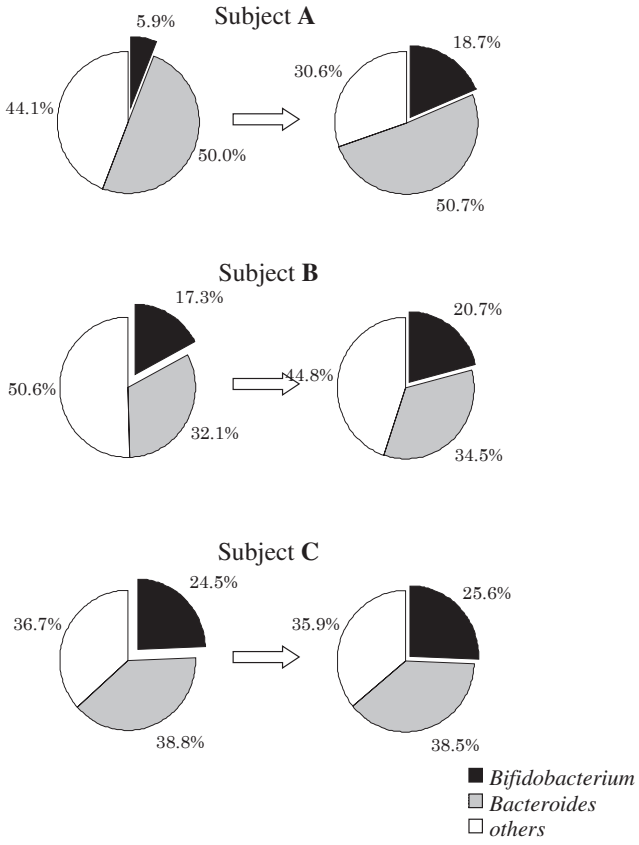

Fig. 44.9 Effect of Fibersol-2 on the proportion of *Bifidobacterium* among the intestinal microflora.

44.6 Food applications of Fibersol-2

44.6.1 *A dietary fibre source, and ingredient of functional food products*

Until recently, many of the dietary fibre sources marketed were insoluble dietary fibres. However, most of those marketed as soluble dietary fibres are made from natural gum-based materials; these have a high viscosity and can lead to problems in food processing and an unpleasant mouthfeel, due largely to the fibre's water-absorbing properties. Fibersol-2 is a very user-friendly dietary fibre with a good mouthfeel; it can be added easily to any type of food, in the same manner as sugar or salt. Dietary fibre-added products can be divided into two groups: processed food products, and dietary supplements.

Processed food products are made with ingredients containing dietary fibre such as cereals, vegetables and fruits. Fibersol-2 can be added to these as a source of dietary fibre to fortify them and to enable the producers to make a dietary fibre fortification claim such as 'Rich in fibre', or 'Good source of fibre'. The dietary fibre fortification claim has been employed in Japan and many other countries, and used for processed food products including fruit beverages, vegetable beverages, soups, cereals and breads.

Dietary supplement products are used to supplement the nutrients insufficiently provided in a normal diet, for example dietary fibre, calcium and vitamins. Dietary fibre supplement products are supplied in a wide variety of forms, such as tablets, powders (e.g. medical products), liquids (including carbonated beverages) and regular food product forms such as cookies or crackers.

Products with the physiological functions of Fibersol-2 have been introduced into the marketplace. At present in Japan there is an approval programme called 'Foods for Specified Health Use' (FOSHU). FOSHU products are certified through application to the Ministry of Health and Welfare in a similar manner to medical drug applications. Supporting data of safety, stability, physicochemical properties and test methods must be provided. FOSHU products, which must contain the approved ingredients and must provide medically and nutritionally effective physiological functions with an effective intake amount, can be marketed with health claims such as 'bowel regularity', 'blood glucose control effect', 'lowering cholesterol effect' and 'blood pressure-lowering effect'. Presently, FOSHU products containing Fibersol-2 as the effective ingredient, in the forms of beverages, powdered beverages, cookies and sausages have been approved, and are marketed.

Fibersol-2 has also been used in diet products such as concentrated liquid foods. Until recently, the concepts for enteral nutrition products have been 'high digestibility' and 'low precipitate'. However, it is now known that the long-term administration of enteral nutritional products often cause diarrhoea or constipation because of the diminishing digestive enzyme activity, shrinking of the mucosal membrane in the small intestine or changing intestinal microflora, and the effects of recovery of the digestive tracts after surgical procedures. Dietary fibres are an ideal ingredient for enteral nutritional products. As described earlier, Fibersol-2 can increase the population of useful intestinal bacteria. It can also be easily used in tube-feeding products because of its solubility, highly refined quality and low viscosity, and is an optimum ingredient for inclusion in enteral nutritional products. There is a thought that, because of the insulin secretion-sparing effect, Fibersol-2 could be used in sports drinks designed to sustain energy. Conventionally, maltodextrins have been used in sports drink applications as a carbohydrate source to prevent the lowering of blood glucose levels during activity. However, the maltodextrins, as do other digestible saccharides, promote additional secretion of insulin and interrupts the metabolism of fat – an important energy source in the body. The addition of Fibersol-2 to sports drinks containing maltodextrins may help to maintain stamina during exercise by maintaining blood glucose levels, without the additional secretion of insulin, while encouraging the effective metabolism of fat.

44.6.2 *Fibersol-2 in low-calorie products*

Due to its relatively low average molecular weight (2000 Da), low viscosity and low calorie value (0.5 kcal/g), Fibersol-2 can be used as a bulking agent (like polydextrose) for sweeteners such as aspartame, sucralose or acesulfame K, or as an essential ingredient for formulating low-calorie foods. Fibersol-2 has less sweetness and heaviness in the mouth, even at high concentrations – a property which has been used in the formulation of a low-fat ice cream (Table 44.3). By replacing all the sugar and most of the butter with Fibersol-2, sorbitol and aspartame, a new ice cream can be produced which has the same taste as conventional ice cream. Fibersol-2 not only serves as a bulking agent for aspartame, but also as a fat replacer; in this respect it may be used in various types of dairy product such as frozen desserts, yoghurts and soups, where the above-mentioned properties are desirable.

Table 44.3 Formulation of a low-fat and low-calorie ice chocolate cream with Fibersol-2.

Formulation	Control	Fibersol-2
Butter (g)	9.0	1.5
Skim milk powder (g)	10.0	10.0
Fibersol-2 (g)*	–	12.8
Solbitol (g)	–	6.0
Aspartame (g)	–	0.04
Sugar (g)	12.5	–
Emulsifier (g)	0.7	0.7
Cocoa powder (g)	0.3	0.3
Water (g)	67.5	68.66
Kilo-calories/100 g	155	75
Calorie reduction		51.6%
Fat reduction		83.3%
Sensory test	← No significant difference →	

*0.5 kcal/g is used for the caloric value of Fibersol-2.

It is impossible to make low-calorie or low-fat foods using only Fibersol-2. However, formulating Fibersol-2 together with other low-calorie and/or sugarless ingredients into new or currently available products can develop 'safer' and 'more delicious' foods that meet consumers' demands.

44.7 Measuring total dietary fibre in foods containing Fibersol-2

Earlier, we have described the physical and physiological functions of Fibersol-2 as a dietary fibre. In order for Fibersol-2 to be accepted as a dietary fibre, we have also had to develop a reliable and accurate method for its measurement in foods. The current AOAC method (Prosky *et al.* 1992, 1995) for total dietary fibre measurement does not accurately determine the dietary fibre values for foods containing Fibersol-2. The reason for this is that the low-molecular weight soluble dietary fibre components in Fibersol-2 do not precipitate in 78% ethanol in the standard AOAC format, that is Fibersol-2 is not quantified as dietary fibre by the current AOAC method.

Consequently, we developed a new method for the determination of the dietary fibre content of samples containing Fibersol-2, and decided to demonstrate the accuracy and precision of the method through conducting an AOAC collaborative study. The method consists of two main steps (Fig. 44.10). First, insoluble dietary fibre and high-molecular weight soluble dietary fibre are determined by the classical AOAC method (Prosky *et al.* 1992, 1995). Second, the filtrate from the AOAC method is treated with ion-exchange resins to remove salts and proteins, after which the low-molecular weight soluble dietary fibre is determined from the ratio of the peak area of a part of Fibersol-2 in the HPLC chromatograms of the above filtrates. Finally, total dietary fibre is calculated by summing the value from the AOAC method and that from the HPLC procedure. The protocol of this combined method has been approved as an AOAC collaborative study protocol, and an AOAC collaborative study has

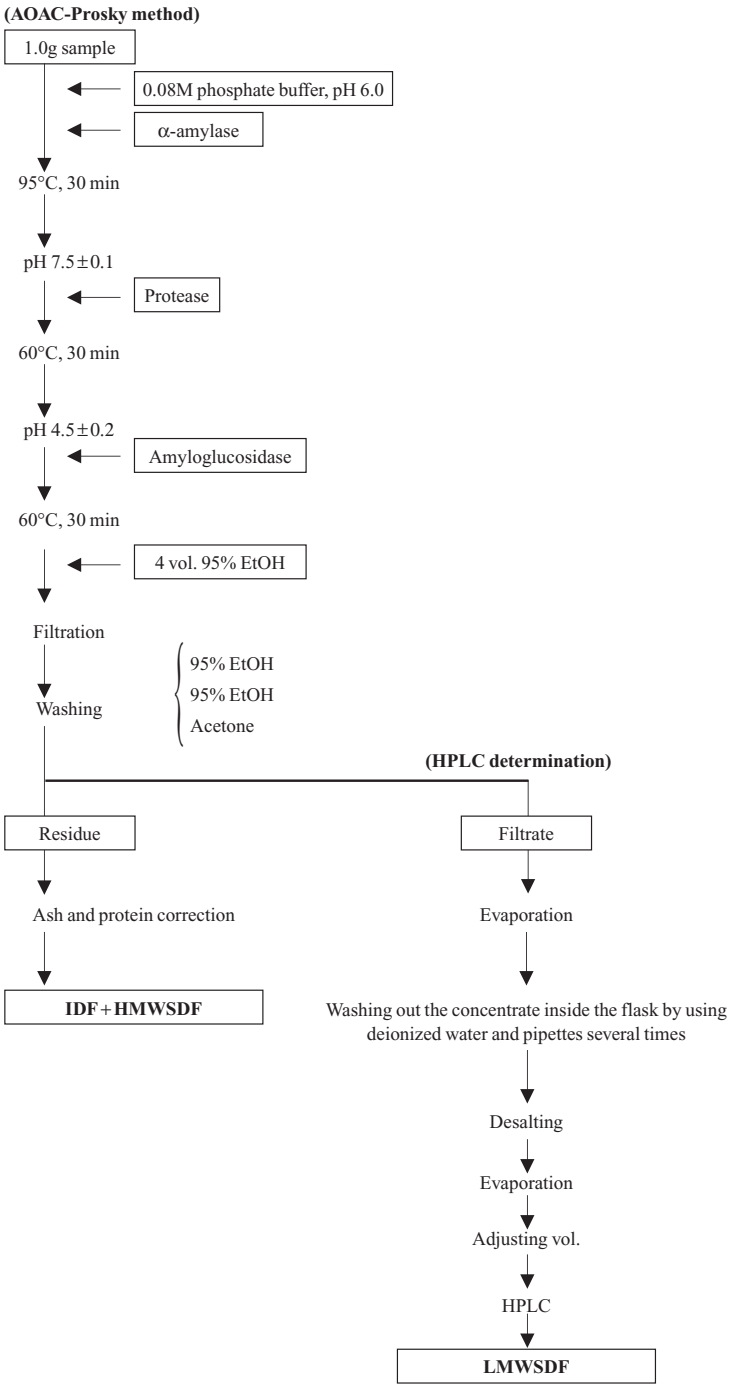

Fig. 44.10 Flowchart of the combination of AOAC-Prosky method and HPLC determination.

been conducted and resulted in accurate and repeatable results (Ohkuma & Gordon 1999). This method will be accepted as an AOAC official method in the near future.

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