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Editors: Liangli (Lucy) Yu, Rong Tsao and Fereidoon Shahidi

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# **Cereals and Pulses**

## **Nutraceutical Properties and Health Benefits**

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# 1 Cereals and pulses – an overview

Rong Tsao, Liangli (Lucy) Yu, and Fereidoon Shahidi

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## 1.1 Introduction

For thousands of years grains and pulses have been produced and consumed as staple foods. Bread, noodles, porridge, breakfast cereals, and other forms of food made from wheat, oats, barley, rice, corn, lentils, chickpeas, and soybean (and other dried seeds) are found in all cultures and cuisines around the world. Many of these foods continue to be home prepared, however, large amounts of the staple foods today, particularly in the industrialized countries, are also made commercially. The global market for breakfast cereals alone was \$24.5 billion in 2008, and it is estimated to grow by roughly 17.1% to a total value of \$28.7 billion by 2013 (Datamonitor, 2009).

The same report also showed that ready-to-eat cereals dominated, having an 87.8% share of the global breakfast cereals market. America leads the global breakfast cereals market, accounting for 64.9% of the market's value, according to the same report. However, processing may reduce the health benefits of food and this depends entirely on the form in which the products are consumed. Most of the wheat-based foods, including bread, noodles and pasta, and cookies, are made from bleached white flour (60% extraction). What is more important is that the 40% removed grain, mainly the bran and the germ, contains the majority of the health beneficial components.

Cereal grains and leguminous seeds contain myriad components that are important and essential to human health. The macro-nutrients, carbohydrates, proteins, and fats serve as a rich source of energy and contain many essential nutrients such as vitamins, amino acids, and fatty acids. However, in recent years, some of these and other minor components have been found to play important roles beyond satisfying basic nutritional requirements. Studies have shown that dietary fibers and certain phytochemicals can be key to health maintenance and disease risk reduction. Intakes of dietary fibers and phytochemicals have been associated with reduced risk of cancer, cardiovascular disease, diabetes, chronic inflammation, neural degeneration, and other chronic ailments and illnesses. These bioactives are, therefore, good candidates as ingredients for nutraceuticals and functional foods.

Meanwhile, many factors can affect the composition and the potential health benefits of foods rich in these bioactives throughout the value chain. Genetics, growing and storage conditions, post-harvest treatments, food formulation, and processing can all affect the content of these bioactives in cereal- and pulse-based food ingredients, and related foods and food supplements, ultimately affecting human health and wellness. This monograph focuses on the chemical and nutraceutical compositions, and potential health beneficial properties, of commonly consumed cereals and legumes. The effects of growing conditions, post-harvest treatments, and food processing and formulation on nutraceutical properties of the cereals and legumes are also covered. In addition, the mechanisms involved in rendering the beneficial effects of cereal and legume components are discussed.

### 1.2 Chemistry and nutraceutical compositions

Intact kernels of grains or seeds contain three major parts: germ/embryo, endosperm/cotyledon, and bran/seedcoat. It is also important to know that most of the nutrients, including dietary fibers and polyphenols, are found in the germ and bran or seedcoat, therefore to receive maximum health benefits, food products made from whole grains or pulses are preferable. Refined grains and pulses often have the germ and the bran or seedcoat removed, thus important dietary fibers, vitamins, minerals, and bioactive phytochemicals are lost; although in countries such as the US and Canada manufacturers are required to enrich white flour with several vitamins and iron.

Phytochemicals are plant-originated secondary metabolites that possess various biological activities. These natural products can be categorized into different chemical classes (Liu, 2004; Tsao, 2010). Cereal grains and pulses are a rich source of bioactive phytochemicals. While polyphenols and carotenoids are perhaps the most studied phytochemicals, particularly for their antioxidant activities, other groups such as phytosterols and saponins are major contributors to the health benefits of cereal grains and pulses.

Food compositions can be altered by targeted breeding. Grains and pulse crops can produce significantly more or less of certain components, for example, soybeans with low, medium, and high isoflavone contents have been developed and formulated into functional soy-breads that contain different levels of naturally occurring isoflavones (Shao *et al.*, 2009). Environmental factors such as growing season, soil type, temperature, and agronomic practices (organic vs. conventional) have also been found to significantly affect the phytochemical compositions (Zhou *et al.*, 2005). Phytochemicals such as polyphenols and carotenoids are relatively unstable under high temperature, thus food processing, such as production of breakfast cereals, can lead to loss of important bioactive compounds that are key to human health (Slavin *et al.*, 2000; Muzhingi *et al.*, 2008).

### 1.3 Potential health beneficial effects

Dietary fibers and phytochemicals are important components of a healthy diet. Dietary fibers, particularly soluble fibers such as  $\beta$ -glucans from barley and oats, have been found to significantly reduce the total and LDL (low-density lipoprotein) cholesterol levels (Brown *et al.*, 1999; Chapters 2 and 3 in this volume); and the effect was discovered to be related to

the physicochemical properties such as the molecular weight of  $\beta$ -glucan (Wolever *et al.*, 2010). Dietary fiber of rice bran also reduces LDL cholesterol, as discussed in Chapter 5. On the other hand, results of epidemiological studies of dietary fiber and cancer risks have not been consistent. For example, examining the consumption of dietary fiber and the risk of colorectal cancer, a recent Japanese study found that total, soluble, and insoluble dietary fibers were not measurably associated with overall risk or subsite-specific risk of colorectal cancer (Uchida *et al.*, 2010). However, the same study suggested a decreased risk of distal colorectal cancer associated with rice consumption. Nevertheless, other studies have indeed shown a positive correlation between the consumption of dietary fibers and cancer risks. Howe *et al.* (1992) showed convincingly that intake of fiber-rich foods was inversely related to risk of cancers of both the colon and rectum. Among the 13 case-control studies, 12 showed significant correlation between dietary fiber intake and the decrease of risk of both left- and right-sided colon and rectal cancers, for men and women, and for different age groups, but no associations were seen for the intakes of vitamin C and  $\beta$ -carotene (Howe *et al.*, 1992). A more recent study concluded similarly that the intake of dietary fiber was inversely associated with colorectal cancer risk. The authors also suggested that methodological differences (i.e. study design, dietary assessment instruments, definition of fiber) may account for the lack of convincing evidence for the inverse association between fiber intake and colorectal cancer risk in some previous studies (Dahm *et al.*, 2010). Dietary fibers from pulse crops may also contribute to the reduction of LDL cholesterol and the risk of cancer, however, more research needs to be done in this area. Dietary fibers from other food crops including psyllium and sorghum are also known for similar health benefits (Chapters 11 and 12, respectively). Other forms of carbohydrates, such as resistant starch in corn (Chapter 7), also play important roles in alleviating health risks. The health properties of dietary fiber preparations and the potential molecular mechanisms involved in their beneficial actions are summarized in Chapter 18. In general, psyllium, oats, barley, and several edible legumes are important dietary sources of soluble fibers, while bran of wheat and corn are good sources of insoluble fiber. Soluble fibers may absorb moisture in the GI (gastrointestinal tract) track and form viscous fluid or gel, which may trap lipid and bile acids reducing their bioavailability and total energy intake. They may also be fermented in the large intestine and form short chain fatty acids, which can reduce the local pH and enhance the movement of intestinal contents. Such effects may lead to reduced absorption of energy and toxins, as well as changes of the microorganism profile in the large intestine. High intake of dietary fibers may reduce the risk of several human chronic diseases, such as cardiovascular diseases, diabetes, and colon cancer (Chapter 18).

While dietary fibers are an important ingredient contributing to the health benefits of cereal grains and pulses, ample evidence exists that phytochemicals may play greater roles. Many different classes of phytochemicals have been identified and their specific bioactivities reported. The major phytochemicals that have shown health benefits include various phenolic compounds, carotenoids, saponins, and phytosterols. Many of these secondary metabolites provide chemical defense against invading insects or microorganisms, or participate in wound healing in the plants.

Phenolic compounds, including the phenolic acids and flavonoids are responsible for the total antioxidant activity of cereals and pulses (Chapter 19). The majority of the phenolics are found in the bran or seed coat of the grains, therefore, consumption of whole grain and intact seed-based foods is of greater benefit. Diets rich in phenolics have been linked to the reduction of several chronic diseases, particularly those caused by

oxidative stress such as cancer, cardiovascular diseases, diabetes, and inflammatory illnesses. However, additional roles of phenolic compounds, particularly flavonoids, have been identified in recent years. In addition to the direct antioxidant activities, flavonoids, for example, have been shown to modulate cell signaling pathways at physiological concentrations way below those required to impact cellular antioxidant activities. Modulation of cell signaling pathways by flavonoids could help prevent cancer by stimulating phase II detoxification enzyme activity and by inhibiting proliferation and inducing apoptosis. Inhibition of biomarkers such as NFkB of inflammation and increase of the endothelial nitric oxide synthase (eNOS) activity may help prevent cardiovascular diseases.

The antioxidant activity of the phenolics has been associated with many chronic diseases. Cinnamic acid and its derivatives, particularly ferulic acid, are the main phenolic acids in cereal grains (Zhou *et al.*, 2005). Phenolic acids are found mostly in the bran, and the majority may exist in conjugated and bound forms (Liyana-Pathirana and Shahidi, 2006; Kim *et al.*, 2006; Chandrasekara and Shahidi, 2010; Chapter 19). Cereal grains are also the major dietary source of lignans, a group of polyphenols that play important roles in human health, particularly as precursors of mammalian lignans. These compounds are mostly found in the bound form, thus are not normally extractable by organic solvents (Chapters 6 and 9). Flavonoids, mainly flavones and flavonols, have been found in cereal grains. Apigenin, kempferol, and quercetin glycosides are major flavonoids, however, anthocyanins contribute significantly to the total flavonoid content in dark colored grains such as purple corn (Chapter 7). Catechins have also been identified in grains such as buckwheat (Chapter 10). Sorghum and millet, with their unique drought-resistance and high level of polyphenols, are considered important to combat the continuously increasing health problems of obesity, diabetes, cardiovascular diseases, and cancer. Sorghum is unique among cereals with its high content of condensed tannins that are oligomeric and polymeric flavonoids. These proanthocyanins are strong antioxidants and considered key for lowering risks of several chronic diseases (Chapter 12). Seed coat of pulses is also a good source of flavonoids (Chapters 14 and 15). Isoflavones, a subgroup of flavonoids, are only found in soybean and other legumes. Isoflavones and lignans are phytoestrogens, therefore, in addition to other biological activities, their role in hormone-related diseases such as breast cancer and osteoporosis is also important (Chapters 17). Other legumes such as pulse crops have been studied in recent years and efforts have been made to determine the bioactives and their relationship with health benefits (Chapters 14 and 15).

Carotenoids are critical to the photosynthesis of plants, however, many of these compounds, such as  $\beta$ -carotene, are also important to humans as vitamin A precursors. In cereals and pulses, while many carotenoids have been identified, zeaxanthin and lutein are worthy of special mention. These two non-vitamin A precursors, found mainly in corn and other cereal grains such as wheat, are not only strong antioxidants, but can also inhibit cancer cell proliferation and prevent cell mutation. These compounds are especially critical to the health of the eye (Chapter 7).

Other phytochemicals, such as phytates, saponins, and phytosterols, have also been found to contribute to the potential health benefits of cereals and pulses. Policosanols and lactams are unique bioactives found in a minor cereal crop adley, among other commonly found phytochemicals that showed various health benefits (Chapter 8). *D-chiro*-inositol and fagopyritols in buckwheat have also been found to benefit diabetics (Chapter 10). However, it is generally understood that these and all other above-discussed active components play an

assorted role in various health problems, as pointed out in Chapter 18. Their composition and specific roles can be found in different chapters of this book. It is our hope that this book offers a focused discussion of cereals and pulses as important contributors to health, and how we can improve the quantity and quality of their functional components in the diet throughout the value-chain, i.e. breeding, production, postharvest storage, and food processing. Reviews on the chemistry, biochemistry, and mechanisms of action of the bioactives, including dietary fibers and the various phytochemicals, will also provide insights into future research.

## References

- Brown, L., Rosner, B., Willett, W.W., and Sacks, F.M. (1999) Cholesterol-lowering effects of dietary fiber: A meta-analysis. *American Journal of Clinical Nutrition*, **69**: 30–42.
- Chandrasekara, A. and Shahidi, F. (2010) The content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry*, **58**: 6706–6714.
- Dahm, C.C., Keogh, R.H., Spencer, E.A., Greenwood, D.C., Key, T.J., Fentiman, I.S., Shipley, M.J., Brunner, E.J., Cade, J.E., Burley, V.J., Mishra, G., Stephen, A.M., Kuh, D., White, I.R., Luben, R., Lentjes, M.A., Khaw, K.T., and Rodwell Bingham, S.A. (2010) Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. *Journal of the National Cancer Institute*, **102**: 614–626.
- Datamonitor. 2009. Breakfast Cereals: Global Industry Guide, [http://www.fastmr.com/prod/47026\\_breakfast\\_cereals\\_global\\_industry\\_guide.aspx](http://www.fastmr.com/prod/47026_breakfast_cereals_global_industry_guide.aspx). Howe, G.R., Benito, E., Castelleto, R., Cornée, J., Estève, J., Gallagher, R.P., Iscovich, J.M., Deng-ao, J., Kaaks, R., Kune, G.A., Kune, S., L'bbe, K.A., Lee, H.P., Lee, M., Miller, A.B., Peters, R.K., Potter, J.D., Riboli, E., Slattery, M.L., Trichopoulos, D., Tuyns, A., Tzonou, A., Wittermore, A.S., Wu-Williams, A.H., and Shu, Z. (1992) Dietary intake of fiber and decreased risk of cancers of the colon and rectum: from the combined analysis of 13 case-control studies. *Journal of the National Cancer Institute*, **84**: 1887–1896.
- Kim, K.H., Tsao, R., Yang, R., and Cui, S.W. (2006) Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effects of hydrolysis conditions, *Food Chemistry* **95**: 466–473.
- Liu, R.H. (2004) Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal of Nutrition*, **134**: 3479S–3485S.
- Liyana-Pathirana, C.M. and Shahidi, F. (2006) Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry*, **54**: 1256–1264.
- Muzhingi, T., Yeum, K.J., Russell, R.M., Johnson, E.J., Qin, J., and Tang, G. (2008) Determination of carotenoids in yellow maize, the effects of saponification and food preparations. *International Journal of Vitamin Nutrition Research*, **78**: 112–120.
- Shao, S., Duncan, A.M., Yang, R., Marcone, M.F., Rajcan, R., and Tsao, R. (2009) Tracking isoflavones: From soybean to soy flour, soy protein 3 isolates to functional soy bread. *Journal of Functional Foods*, **1**: 119–127.
- Slavin, J.L., Jacobs, D., and Marquart, L. (2000) Grain processing and nutrition. *Critical Reviews in Food Science Nutrition*, **40**: 309–326.
- Tsao, R. (2010) Chemistry and biochemistry of dietary polyphenols. *Nutrients*, **2**: 1231–1246.
- Uchida, K., Kono, S., Yin, G., Toyomura, K., Nagano, J., Mizoue, T., Mibu, R., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Maekawa, T., Yasunami, Y., Takenaka, K., Ichimiya, H., and Terasaka, R. (2010) Dietary fiber, source foods and colorectal cancer risk: the Fukuoka Colorectal Cancer Study. *Scandinavian Journal of Gastroenterology*, **45**: 1223–1231.
- Wolever, T.M., Tosh, S.M., Gibbs, A.L., Brand-Miller, J., Duncan, A.M., Hart, V., Lamarche, B., Thomson, B.A., Duss, R., and Wood, P.J. (2010) Physicochemical properties of oat  $\beta$ -glucan influence its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial. *American Journal of Clinical Nutrition*, **92**: 723–732.
- Zhou, K., Yin, J.J., and Yu, L. (2005) Phenolic acid, tocopherol and carotenoid compositions, and antioxidant functions of hard red winter wheat bran. *Journal of Agricultural and Food Chemistry*, **53**: 3916–3922.



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## 2 Effects of barley consumption on cardiovascular and diabetic risk

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### 2.1 Introduction

Barley is one of the most important cereal crops cultivated in the world. In history, it has been consumed mainly as a staple food, whereas in modern times it is mostly used in the beer industry. In the human diet, barley not only provides protein and carbohydrate, but is also a good source of dietary fibre. There is growing evidence supporting a role for dietary fibre in lowering plasma cholesterol, improving lipid metabolism, and reducing glycemic index (Li *et al.*, 2003; Behall *et al.*, 2004, 2005, 2006). Therefore, consumption of barley whole grain and/or barley-based foods has been associated with lower risks of cardiovascular diseases, diabetes, and some types of cancer (Pins and Kaur, 2006; Ames and Rhymer, 2008).

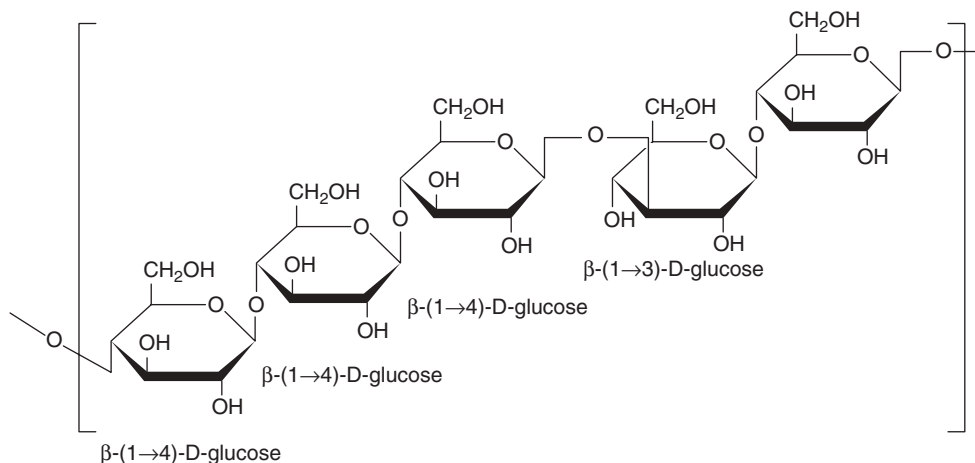
In recent years, the health benefits provided by barley have led to an increased focus on the study of nutraceutical components in barley. The (1 → 3, 1 → 4)- $\beta$ -D-glucans, commonly known as  $\beta$ -glucans, are the major constituents in barley dietary fibre. They are structural components in barley endosperm cell walls. The molecular structures and physicochemical properties of  $\beta$ -glucans in barley have been characterized by Izydorczyk and Dexter (2008) and their effects on cardiovascular and diabetic risks have been reviewed by Pins and Kaur (2006). In addition to  $\beta$ -glucans, the other health promoting compounds mostly studied in barley are phenolic compounds. They are reported to play an important role in the prevention of and protection from oxidation-induced diseases.

Therefore, this chapter provides a review of the major nutraceutical components in barley grain and their roles in the maintenance of human health. Special attention will be paid to  $\beta$ -glucans.

### 2.2 Barley $\beta$ -glucan and risk of cardiovascular diseases, diabetes and colon carcinogenesis

#### 2.2.1 Chemical structure of barley $\beta$ -glucan

Barley  $\beta$ -glucans are linear homopolysaccharides of D-glucopyranosyl residues linked mostly via two or three consecutive  $\beta$ -(1 → 4) linkages that are separated by a single  $\beta$ -(1 → 3) linkage



**Figure 2.1** General structure of barley β-glucans.

(Figure 2.1). Despite sharing the same general molecular structure, β-glucans from different genera of barley are varied in the length and branching. The ratios of β-(1→4)/β-(1→3) linkages are also different.

## 2.2.2 β-glucan content in barley grain

Barley grain contains remarkable β-glucan content ranging from 2.5 to 11.3% (Izydorczyk and Dexter, 2008). Izydorczyk and Dexter summarized the β-glucan contents in different genotypes of hulled and hullless barley, which are given in Table 2.1. It appears that barley genotypes with waxy or high amylose starch contain higher content of β-glucans than those with normal starch. There is no significant difference in total β-glucan content between hulled and hull-less barley types.

In addition to the genetic factor, the concentration of β-glucan in barley is also influenced by environmental factors (Andersson *et al.*, 1999). The total content of β-glucans in barley generally increases when it is grown in hot and dry conditions (Morgan and Riggs, 1981).

## 2.2.3 Barley β-glucan in the prevention and management of cardiovascular diseases

Cardiovascular disease (CVD), primarily in the form of heart disease and stroke, is the Nation's leading killer for both men and women among all racial and ethnic groups. The major risk factors associated with CVD include high levels of total cholesterol and low-density lipoprotein (LDL) cholesterol (Ames and Rhymer, 2008). Consumption of barley β-glucan enriched foods has been associated with the reduced incidence of CVD due to the ability of β-glucan to lower serum cholesterol and LDL cholesterol levels.

McIntosh *et al.* (1991) reported that when 21 patients with light hypercholesterolemia were given 8 g of β-glucan through a barley diet (170 g/d) for four weeks, the total cholesterol

**Table 2.1** Beta-glucan contents in different genotypes of hulled and hull-less barley

Barley genotype	Total beta-glucan	Water-soluble beta-glucan
Hulled, normal starch		
AC Metcalfe	4.5	1.2
CDC Kendall	4.4	1.3
Legacy	5.1	1.2
Xena	4.9	1.4
Hull-less, normal starch		
CDC McGwire	4.7	1.3
AC Millhouse	4.5	1.2
CDC Dawn	3.9	1.2
AC Bacon	4.0	2.0
Falcon	3.6	1.6
Hull-less, waxy starch		
CDC Alamo	7.5	2.8
CDC Rattan	7.5	2.9
CDC Fibar	9.7	3.5
CDC Candle	6.9	2.5
Enduro	6.8	2.4
Hull-less, high amylose starch		
SH99250	8.9	2.0
SB94893	8.9	1.5

Source: Reproduced from Izydorczyk and Dexter, copyright 2008, with permission of Elsevier.

and LDL levels were reduced by 6% and 7%, respectively. More recently, Behall *et al.* (2004) conducted two studies on the effect of barley  $\beta$ -glucans among hypercholesterolemic patients. In one of their studies, 7 men and 18 women were supplemented with a diet containing 0, 3 or 6 g/day of barley  $\beta$ -glucan with equivalent levels of total dietary fiber for 15 weeks. The total cholesterol was decreased by 5% and 6% when the diet contained 3 g or 6 g barley  $\beta$ -glucan/day, respectively, and LDL cholesterol level was reduced by 10% and 13%. In another study (Behall *et al.*, 2004), a five-week diet with low, medium, and high-soluble barley fiber reduced total cholesterol by 14%, 17%, and 20%, respectively, and LDL cholesterol by 17%, 17%, and 24%, respectively. Based on these human clinical trials, the US Food and Drug Administration (FDA) released an announcement that daily intake of 3 g of soluble  $\beta$ -glucan from barley or certain dry milled barley products would decrease plasma total cholesterol level by 5–8% (FDA, 2005).

The mechanism by which barley  $\beta$ -glucan lowers serum cholesterol has not been completely understood. However, two possible mechanisms were proposed including the delayed intestinal absorption of glucose and lipids and the inhibition of absorption and re-absorption of cholesterol and bile acids (Anderson *et al.*, 1984, 1988; Wilson *et al.*, 2004).

## 2.2.4 Barley $\beta$ -glucan in the treatment of diabetes

Diabetes is a chronic disease characterized by high blood glucose level (hyperglycemia). There are three type of diabetes; type 1 is caused by T-cell mediated autoimmune destruction of islet insulin-secreting  $\beta$ -cells (Rother, 2007), type 2 is characterized by the resistance to

insulin with both hyperglycemia and hyperinsulinemia followed by the deficiency of insulin, and type 3 has both decreased insulin and increased resistance to insulin (Carpenter, 2007; Schinner *et al.*, 2005). Among the three types of diabetes, type 2 accounts for 90% of the total incidences of diabetes. The major causes of type 2 diabetes involve lifestyle factors as well as genetics.

Many studies have shown that  $\beta$ -glucan in barley is helpful in controlling blood glucose and insulin response. Pick *et al.* (1998) studied the long-term effects of incorporating high  $\beta$ -glucan content ( $\beta$ -glucan = ~7%), waxy, hulless barley bread products in every day diets of non-insulin-dependent diabetic (type 2) subjects. The results showed that mean blood glycemic response area (AUC) was lower (NS) and insulin response area was higher ( $P \leq 0.05$ ) when subjects were fed barley bread as opposed to refined wheat flour bread. Incorporating barley bread products (5 g/d  $\beta$ -glucan) into the diet of the type 2 diabetics improved their glycemic response and increased insulinemic response leading to some subjects reducing their dose of oral hypoglycemics. In another study, the effect of barley breakfast cereal made by a hulless barley on blood glucose and insulin response was reported (Rendell *et al.*, 2005). In their study, Prowashonupana, a barley variety with low starch, high fiber, high protein, and relatively high concentration of free sugars, containing 3 times as much  $\beta$ -glucan as other standard hulless barley, was used. The results showed that the post-prandial glycemic index was reduced for Prowash compared to commercial liquid meal replacer (LMR) or oatmeal for both diabetics and non diabetics. In the non-diabetic subjects, the maximal rise in glucose from baseline was  $26.3 \pm 3.9$  mg/dL after LMR,  $41.3 \pm 3.9$  mg/dL after oatmeal, and  $6.4 \pm 2.7$  mg/dL after Prowash ( $p < 0.01$ ). The maximal increase in glucose in the diabetic patients was  $69.9 \pm 4.5$  mg/dL after LMR,  $80.8 \pm 8.8$  mg/dL after oatmeal, and  $28.4 \pm 3.5$  mg/dL after Prowash ( $p < 0.01$ ). The maximal increase in insulin post-LMR was  $33.9 \pm 3.6$  mIU/mL in the diabetic patients and  $54.0 \pm 9.8$  mIU/mL in the non-diabetic controls. Oatmeal elicited a maximal insulin increase of  $29.9 \pm 4.2$  mIU/mL in the control subjects and  $21.4 \pm 2.5$  mIU/mL in the diabetic patients. In contrast, the maximal insulin increase after Prowash was  $8.6 \pm 1.5$  mIU/mL in the non-diabetic controls and  $6.8 \pm 1.2$  mIU/mL in the diabetic patients ( $p < 0.01$ ). The reduced post-prandial glucose levels in the subjects consuming Prowash were similar to those observed in diabetic patients treated with alpha-glucosidase treatment. In insulin-dependent type 1 diabetics, lower post-prandial blood glucose permits equivalent reduction in insulin doses. For many individuals, a food product is highly preferable compared to pharmacological agents. Behall *et al.* (2005) also reported that when barley Prowashonupana and oatmeal were given to ten women with average age of 50 years old and body mass index of 30 (overweight), peak glucose and insulin levels were significantly lower after barley meals than oat, suggesting that high  $\beta$ -glucan was the key factor in those reductions. In another report, the breakfast made by  $\beta$ -glucan-enriched barley exhibited favorable responses on glucose metabolism, and particularly on insulinemic responses compared with whole grain wheat breakfast in a group of ten healthy volunteers (five males, age  $25.4 \pm 0.5$  yrs, BMI  $22.6 \pm 0.7$  kg/m<sup>2</sup>) (Casiraghi *et al.*, 2006).

According to the above literature, it is believed that daily intake of barley  $\beta$ -glucans helps lower blood glucose and that barley  $\beta$ -glucans can be used in the treatment of diabetic patients. However, a detailed mechanism of action of  $\beta$ -glucans is still to be established. A possible theory is that  $\beta$ -glucans increase the viscosity of the intestinal contents resulting in the reduced postprandial insulin and glucose levels. Over time, lower ambient insulin levels improve cellular insulin sensitivity, causing improved glucose metabolism (Pins and Kaur, 2006).

### 2.2.5 $\beta$ -glucan and chemoprevention of colon carcinogenesis

The chemoprevention of colon carcinogenesis by  $\beta$ -glucans has been mostly studied *in vitro*. Kim *et al.* (2009) reported that bacterial  $\beta$ -glucans induced 1) the apoptosis of colon cancer SNU-C4 cells and 2) changes of cell morphology and of the expression of apoptotic genes. The same  $\beta$ -glucans also increased the activity of caspase-3 enzyme. In other reports,  $\beta$ -glucans inhibited the activity of isozymes of cytochrome family (phase I enzyme) involved in the first activation of carcinogens (Hashimoto *et al.*, 2002; Okamoto *et al.*, 2004). Barley  $\beta$ -glucans may have protective effects against damage induced by methyl methanesulfonate (MMS) in the CHO-K1 cell line (deficient in drug metabolism) and 2-aminoanthracene (2AA) in the HTC cell line (proficient in drug metabolism) (Oliveira *et al.*, 2006). The antitumor activity and immune responses of  $\beta$ -glucans depend on their molecular structures and physical properties. There is need for elaborate clinical trials to assess the effectiveness of purified  $\beta$ -glucans among cancer patients.

Several studies have shown that  $\beta$ -glucan extracts from barley can prevent chemical agent-induced tumor. Earlier studies have shown that barley bran could reduce 1,2-dimethylhydrazine (DMH) intestinal tumor incidence and burden (McIntosh, 1996). Co-administration with  $\beta$ -glucan from barley enhanced efficacy of photodynamic therapy in treatment of Lewis lung carcinoma (Akramiene *et al.*, 2009). Pre-incubated barley  $\beta$ -glucan reduced the DNA damage in CHK-K1 cell line when induced by methyl methanesulfonate (MMS) (Oliveira *et al.*, 2006). The protection of barley  $\beta$ -glucan against 2AA (2-aminoanthracene) and MMS was also observed in CHO-K1 (Angeli *et al.*, 2006).

The mechanism involved in antitumor activity may be attributed to the antioxidant properties of  $\beta$ -glucans, thereby preventing oxidant injury by reactive oxygen species, which lead to formation of cancers. Moreover,  $\beta$ -glucans showed protection effects against genotoxicity and cytotoxicity induced by chemotherapy drugs such as cyclophosphamide, abriamycin, and cisplatin (Tohamy *et al.*, 2003). This protective effect may be due to the ability to trap free radicals or other reactive species during metabolism of the drugs.

## 2.3 Other nutraceutical components and properties in barley

In addition to  $\beta$ -glucans, barley grain also contains a wide range of phenolic compounds. According to Madhujith and Shahidi (2007) and Bellido and Beta (2009), phenolic extracts from barley showed high antioxidant activity. By using the extracts from barley, the cell proliferation of Caco-2 colon cancer cell was inhibited 29.3–51.2% and 9.3–15.9% at 0.5 and 0.05 mg/ml, respectively (Madhujith and Shahidi, 2007). Therefore, the full characterization of phenolic compounds in barley is important.

### 2.3.1 Phenolic acids in barley grain

Phenolic acids can be classified as hydroxylated derivatives of benzoic and cinnamic acids. Hydroxycinnamic acids are more common than hydroxybenzoic acids and mainly consist of *p*-coumaric, caffeic, ferulic, and sinapic acids (Manach *et al.*, 2004). Ferulic acid is the most abundant phenolic acid in cereal grains. As one of the cinnamic acid derivatives, ferulic acid

**Table 2.2** Ferulic, *p*-coumaric, and vanillic acids content in various barley varieties

Barley genotype	Ferulic acid (ug/g) <sup>1</sup>	<i>p</i> -Coumaric acid (ug/g) <sup>1</sup>	Vanillic acid (ug/g) <sup>1</sup>
Peru 3	650.3 ± 28.65	24.5 ± 7.82	42.5 ± 22.99
Peru 5	838.9 ± 62.61	11.9 ± 4.79	58.8 ± 4.59
Peru 16	745.6 ± 100.44	53.9 ± 8.39	66.8 ± 4.99
Peru 35	877.1 ± 108.06	22.2 ± 3.35	50.6 ± 9.86
Peru 45	797.1 ± 20.61	21.8 ± 5.14	45.5 ± 1.03
Ex 116	589.8 ± 0.25	39.0 ± 2.00	28.9 ± 2.34
Ex 127	749.4 ± 218.60	16.6 ± 5.03	66.6 ± 32.54
CI 4325	706.8 ± 58.59	70.2 ± 29.11	65.6 ± 0.15
Ex82 x CI 19973	642.9 ± 66.44	15.4 ± 4.30	99.6 ± 51.60
Ex87 x CI 19973	723.3 ± 21.51	14.5 ± 2.78	94.7 ± 12.11
Ex 83	675.1 ± 64.47	37.0 ± 10.14	45.5 ± 16.23
CI 1370	704.9 ± 116.63	9.4 ± 2.01	75.7 ± 38.97
CI 4374	693.9 ± 101.05	14.5 ± 2.59	77.4 ± 28.79
CI 9977	607.4 ± 85.91	9.6 ± 1.22	58.1 ± 16.33
CI 10151	848.6 ± 22.24	10.1 ± 1.12	93.1 ± 44.27
CI 2318	978.7 ± 133.17	11.1 ± 0.75	79.1 ± 26.92
CI 1248	732.5 ± 46.05	12.2 ± 1.05	62.2 ± 10.07
CI 2230	703.1 ± 94.68	10.3 ± 0.13	78.4 ± 16.67
CI 4013	908.3 ± 58.54	11.7 ± 0.35	62.8 ± 3.98
Hokuto Hadaka	745.5 ± 91.68	12.3 ± 0.96	69.0 ± 6.10
Shiga Waseh	863.3 ± 1.31	20.9 ± 0.49	81.7 ± 1.35

<sup>1</sup>Mean ± SD.

shows high antioxidant because of CH = CH-COOH group. Several studies have shown that ferulic acid have anti-inflammatory (Sakai *et al.*, 1999), skin photoprotection, and tumor inhibition (Priefert *et al.*, 2001; Murakami *et al.*, 2002) properties. Ferulic acid also exhibits potential chemopreventive effect on oral (Mori *et al.*, 1999) and large bowel carcinogenesis (Kawabata *et al.*, 2000). It may protect against coronary heart disease because of the antioxidant and cholesterol-lowering activities (Sakamoto *et al.*, 1987). Therefore, the high content of ferulic acid in outer layers of cereal kernels may be associated with the health benefits of whole grains.

Phenolic acids in barley have been studied extensively (Holtekjlen *et al.*, 2006; Quinde *et al.*, 2006; Hernanz *et al.*, 2001). As with other cereal grains, phenolic acids in barley occur in free, conjugated (soluble) and bound (insoluble) forms with their concentrations in the order of bound > conjugated > free. The bound form makes up more than 60% of the total phenolic acid (Adom and Liu, 2002; Li *et al.*, 2008). Nine phenolic acids have been detected in barley flour, including caffeic acid, ferulic acid, sinapic acid, protocatechuic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, and ferulic acid dehydrodimers (Mattila *et al.*, 2005). Ferulic acid (FA) is the major phenolic acid constituting 90% of the total, while *p*-coumaric and vanillic are minor acids in barley grain (Table 2.2). A wide range in total phenolic acid content (254–675 µg/g) was observed across different barley samples analyzed under the European HEALTHGRAIN program. Bound phenolic acids make up approximately 73% of the total phenolic acid content with concentrations ranging from 133 to 523 µg/g. Soluble conjugated phenolic acids comprised approximately 25% of the total phenolic acid content with levels ranging from 86 to 198 µg/g. Free phenolic acids comprised only a very small proportion (<3%) ranging from 4.6 to 23 µg/g. The free phenolic acids comprised ferulic (27%), vanillic (28%), syringic (17%), and *p*-coumaric acid (22%). Ferulic

acid is the major bound phenolic acid with the concentrations ranging between 149 and 413  $\mu\text{g/g}$  and comprising 68% of the total fraction. *p*-Coumaric acid is the second most abundant phenolic acid in barley ranging from 5.25 to 115.5  $\mu\text{g/g}$  (Andersson *et al.*, 2008). In another report (Holtekjlen *et al.*, 2006), total phenolic acids in barley ranged from 604 to 1346  $\mu\text{g/g}$  including ferulic dehydrodimers, and the content of ferulic acid and *p*-coumaric acid ranged from 403 to 723  $\mu\text{g/g}$  and 15 to 374  $\mu\text{g/g}$ , respectively. The ferulic acid and *p*-coumaric acid contents in hulled varieties are considered higher than in hullless barley. Quinde *et al.* (2006) also reported that the total phenolic acid and ferulic acid content in hulled barley were higher than in hullless barley (Quinde *et al.*, 2006). Phenolic acids in three wild genotypes of barley, Odens Aker, Abed Archer, and Rostov, were studied in Dr Beta's laboratory. The results showed that the total phenolic acid content ranged from 784 to 1131  $\mu\text{g/g}$ , and hullless variety Odense Aker had the lowest content of 784  $\mu\text{g/g}$ . Ferulic acid and *p*-coumaric acid content ranged from 589 to 666  $\mu\text{g/g}$  and 17.9 to 348  $\mu\text{g/g}$ . Two hulled barleys, Abed Archer and Rostov, had significantly higher *p*-coumaric acid of 341.8 and 331.4  $\mu\text{g/g}$ , results consistent with those reported by Holtekjlen *et al.* (2006).

Besides the monomers of phenolic acids, ferulic dehydrodimers have been reported in barley after alkali hydrolysis. In the cell walls, diferulic acids (DiFA) can be formed via a radical oxidative mechanism or intracellular peroxidases (Andreasen *et al.*, 2000) and provide cross-linking between the cell wall polymers (Fry *et al.*, 2000). The esterified and cross-linked diferulic acids protect the cell wall polysaccharides from degradation by enzymes and control the mechanical properties of the cell wall (Grabber *et al.*, 1998). Cano *et al.* (2002) reported that the superoxide scavenging capacity of ferulic acid was enhanced by dimerization. To date, six common ferulic acids have been found in plants. They are 8-*O*-4 diFA, 8,5-diFA open form, 8,5-diFA dehydrobenzofuran form, 5,5-diFA, 8,8-diFA noncyclic form, and 4-*O*-5-diFA; with the first four having been reported in cereal grains (Hernanz *et al.*, 2001). The extent to which bound phenolics are bioavailable has not been well-established as cell wall materials are challenging to digest. However, Andreasen *et al.* (2001) have shown that both human and rat colonic microflora can release diferulic acids from dietary cereal bran, and that esterified phenolics can be cleaved by esterase from the human gut (Andreasen *et al.*, 2006). The results imply that some bound phytochemicals may be released and absorbed in the intestine, thereby contributing to human health improvement attributable to whole grains. The DiFA in barley ranged from 158 to 261  $\mu\text{g/g}$ , with 8,5'-diFA (50–88  $\mu\text{g/g}$ ) and 4-*O*-5'-diFA (72–114  $\mu\text{g/g}$ ) as the major diferulates (Holtekjlen *et al.*, 2006).

### 2.3.2 Flavonoids in barley grain

Flavonoids are a large group of phenolics sharing a C6-C3-C6 skeleton. In cereal grains, the most common flavonoids are flavones, flavonols, flavanone, flavanols, flavan-3-ols, and anthocyanidins.

Proanthocyanidins (PAs) also known as condensed tannins, are oligomers of flavan-3-ols. Compared to monomeric flavan-3-ols, PAs have higher antioxidant capacity *in vitro* (Hagerman *et al.*, 1998), with 20 and 50 times more antioxidant capacity than vitamins C and E, respectively (Shi *et al.*, 2003). They likely possess a wide range of biological properties through against oxidative stress (Fine, 2000).

Dvoráková *et al.* (2008) studied the flavan-3-ols for ten barley varieties including eight malt and two hullless, and the results showed that proanthocyanidin oligomers, including two dimers (procyanidin B3 and prodelphinidin B3) and four trimers (procyanidin C2,

prodelphinidin C2, and two other prodelphinidins), are the main proanthocyanidins found in barley. The trimers are the most abundant species. Among these, prodelphinidin B3 is the most plentiful with the content varying from 90 to 197 mg/kg, while procyanidin C2 is a minor constituent with the content ranging from 5 to 19 mg/kg. Holtekjlen *et al.* (2006) also reported the proanthocyanidin content in different barley varieties, which included hulled, hullless, normal, waxy, and high-amylose starch varieties as well as two-rowed and six-rowed genotypes. The total flavanols ranged from 325 to 527 µg/g, with two dimers and four trimers as the major flavanols in those varieties. There was no association between the proanthocyanidin level and different barley genotypes. In another study, catechin was detected only in hulled PA-free barley (Quinde *et al.*, 2006). The total flavanols in hullless barley were higher than in hulled PA-containing barley. Hullless regular barley contained higher total flavanols than waxy varieties.

Anthocyanins are a major group of water-soluble pigments normally found in purple, blue, pink, and red colored flower, fruit, and vegetable as well as pigmented cereals. Some of the health benefits associated with anthocyanin consumption by humans, as reviewed by Lila (2004), include protection from DNA cleavage, alteration of development of hormone-dependent disease symptoms, enzyme inhibition, enhancement of the production of cytokines, lipid peroxidation, membrane strengthening, and reduction in capillary permeability and fragility. Jing *et al.* (2008) suggested that the structure of anthocyanin affected chemoprotection, which was measured as inhibition of colon cancer cell proliferation. Non-acylated anthocyanins had greater inhibitory effect on HT-29 cell proliferation than anthocyanins with pelargonidin, triglycoside, and/or acylation with cinnamic acid. Anthocyanin pigments and anthocyanin-rich foods have been reported to lower the risk of colon cancer through the inhibition of proliferation of human colon cancer *in vitro* (Zhao *et al.*, 2004). The role of anthocyanins in inhibiting colon cancer *in vivo* has also been reported (Jing *et al.*, 2008). In addition, the inhibition of cancer cell growth through induction of apoptosis by anthocyanins has been reported (Katsube *et al.*, 2003).

In barley, anthocyanins are found in the pericarp or in the aleurone layer causing the kernel color to appear blue. Some barley varieties (*Hordeum vulgare*) have black pigmentation due to a melanin-like pigment that may overlap purple or blue color as a result of anthocyanins (Siebenhandl *et al.*, 2007). As reported by Kim *et al.* (2007), the content of anthocyanins in barley varied from 13.0 to 1037.8 µg/g. Purple and blue barley groups contained higher average contents of anthocyanins than black barley. Unhulled genotypes had higher total anthocyanins than hulled genotypes. Purple barley bran had at least twice as high the level of anthocyanins than yellow barley bran (Bellido and Beta, 2009) confirming that pearling is effective in obtaining barley fractions high in antioxidant activity and phenolic contents (Rosa *et al.*, 2007). The most common anthocyanin in the purple barley groups was cyanidin 3-glucoside, whereas delphinidin 3-glucoside was the most abundant anthocyanin in the blue and black groups (Kim *et al.*, 2007).

### 2.3.3 Lignans in barley grain

Lignans are precursors to enterodiol and enterolactone in the mammalian system and this conversion is done by their gut microflora (Kuhnle *et al.*, 2009). The structure and function of lignans are similar to 17 β-estradiol. The latter is an estrogen that is important in reproductive and sexual functioning. It also influences other organs including the bones



in addition to acting as phytoestrogens (Kuhnle *et al.*, 2009). Epidemiological studies have concluded that lignans as phytoestrogens might play a role in the prevention of breast (Ingram *et al.*, 1997; Lime and Speirs, 2004), prostate, and colon (Aldercreutz, 2002) cancers through mechanisms likely based on alteration of hormone production, metabolism, and actions at the cellular level leading to reduction of cancer risks (Aldercreutz *et al.*, 2004; Orcheson *et al.*, 1998) and antioxidant properties that reduce cardiovascular diseases and mortality (Aldercreutz *et al.*, 2004). Lignans were found to improve menopausal symptoms. Phytoestrogens including lignans (matairesinol and secoisolariciresinol) were found to have beneficial roles in obesity and diabetes through mechanisms that modulate pancreatic insulin secretion or via antioxidant actions (Bhathena and Velasquez, 2002).

The major types of lignans found in barley include (–)-7-hydroxymatairesinol, (+)-syringaresinol, (+)-lariciresinol, (+)-pinoresinol, (–)-secoisolariciresinol (Kuhnle *et al.*, 2009; Meagher and Beecher, 2000), (–)-matairesinol, cyclolariciresinol, 7-oxomatairesinol, secoisolariciresinol-sesquiliglan, (+)-medioresinol, todolactol A,  $\alpha$ -conidendrin, nortrachelogenin, and lariciresinol-sesquiliglan, in order of decreasing concentration (Smeds *et al.*, 2007).

## **2.4 Potential of hulless barley in health promotion and disease prevention**

### **2.4.1 Hulled versus hulless barley**

The caryopsis of hulless barley is not covered with a husk unlike the hulled types. The dehulling is largely eliminated due to the absence of husk in the hulless varieties. Hulled varieties will need to be dehusked, a pre-processing step necessary to obtain hulless whole grain barley that is edible. The elimination of the dehulling step is attractive to food processors when it comes to hulless varieties. However, the brewing industry has preference for hulled varieties as the husk protects the grain during malting and serves as a filter during mashing.

### **2.4.2 The newly developed varieties of barley with enhanced macro-nutrients and phytochemicals for health promotion and disease prevention**

Varieties such as Prowashonupana, a waxy, hulless barley developed during the late 1970s through a conventional barley breeding program at Montana State University, are higher in fiber and protein, and lower in starch content, than many common cereal grains. They have four times more  $\beta$ -glucan than regular hulless barley.

## **2.5 Future studies**

Barley is one of the major cereal grains produced in various parts of the world for food and nonfood uses. As whole grain, it is a source of nutrients, dietary fiber, and other phytochemicals.  $\beta$ -glucans from different sources are associated with health benefits. Compared with  $\beta$ -glucans derived from bacteria, fungi, and oats, studies on  $\beta$ -glucan from

barley are limited. Literature reports on  $\beta$ -glucan from barley have focused on its effects on lipid cholesterol and blood glucose level and insulin responses. With respect to anti-cancer properties, more clinical trials on barley  $\beta$ -glucans are needed. Detailed relationships between barley  $\beta$ -glucan structure, molecular weight, viscosity, and biological activity need further studies.

Studies on bioavailability of barley phenolic compounds and lignans are still limited. More epidemiological evaluation and animal studies as well as clinical trials need to be done to establish the health promoting properties of barley grain.

## References

- Adom, K.K. and Liu, R.H. (2002) Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, **50**: 6182–6187.
- Akramiene, D., Grazeliee, G., Didziapetriene, J., and Kevelaitis, E. (2009) Treatment of lewis lung carcinoma by photodynamic therapy and glucan from barley. *Medicina (Kaunas)*, **45**: 480–485.
- Aldercreutz, H. (2002) Phytoestrogens and cancer. *Lancet Oncology*, **3**: 364–373.
- Aldercreutz, H., Heinonen, S., and Penalvo-Garcia, J. (2004) Phytoestrogens, cancer and coronary heart disease. *BioFactors*, **22**: 229–236.
- Ames, N.P., and Rhymer, C.R. (2008) Issues surrounding health claims for barley. *Journal of Nutrition*, **138**: 1237S–1243S.
- Anderson, J.W., Story, L., Sieling, B., Chen, W.J., and Petro, M.S. (1984) Hypercholesterolemic effect of oat-bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition*, **40**: 1146–1155.
- Anderson, J.W. and Gustafson, N.J. (1988) Hypocholesterolemic effects of oat and bean product. *American Journal of Clinical Nutrition*, **48**: 749–753.
- Andersson, A.A.M., Elfverson, C., Andersson, R., Regner, S., and Åman, P. (1999) Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture*, **79**: 979–986.
- Andersson, A.A.M., Lampi, A.M., Nystrom, L., Piironen, V., Li, L., Ward, J. L., Gebruers, K., Courtin, C.M., Delcour, J.A., Boros, D., Fras, A., Dyncowska, W., Rakszegi, M., Bedo, Z., Shewry, P.R., and Aman, P. (2008) Phytochemical and dietary fiber components in barley varieties in the health grain diversity screen. *Journal of Agricultural and Food Chemistry*, **56**: 9767–9776.
- Andreasen, M.F., Christensen, L.P., Meyer, A.S., and Hansen, A. (2000) Content of phenolic acids and ferulic acid dehydrotimers in 17 rye (*Secale cereale* L.) varieties. *Journal of Agricultural and Food Chemistry*, **48**: 2837–2842.
- Andreasen, M.F., Kroon, P.A., Williamson, G., and Garcia-Conesa, M. (2001) Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radicals in Biology and Medicine*, **31**: 304–314.
- Andreasen, M., Kroon, P., Williamson, G., and Garcia-Conesa, M.T. (2006) Esterase activity able to hydrolyze dietary antioxidants hydroxy-cinnamates is distributed along the intestine of mammals. *Journal of Agricultural and Food Chemistry*, **54**: 1256–1264.
- Angeli, J.P.F., Ribeir, L.R., Gonzaga, M.S.A., Soares, S.A., Ricardo, M.P.S.N., Tsuboy, M.S., Stidl, R., Knasmuller, S., Linhares, R.E., and Mantovani, M.S. (2006) Protective effects of  $\beta$ -glucan extracted from *Agaricus brasiliensis* against chemically induced DNA damage in human lymphocytes. *Cell Biology Toxicology*, **22**: 285–291.
- Behall, K.M., Scholfield, D.J., and Hallfrisch, J. (2004a) Lipid significantly reduced by diets containing barley in moderately hypercholesterolemic men. *Journal of the American College of Nutrition*, **23**: 55–62.
- Behall, K.M., Scholfield, D.J., and Hallfrisch, J. (2004b) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *American Journal Clinical Nutrition*, **80**: 1185–1193.
- Behall, K.M., Scholfield, D.J., and Hallfrisch, J. (2005) Comparison of hormone and glucose responses of overweight women to barley and oats. *Journal of the American College of Nutrition*, **24**: 182–188.
- Behall, K.M., Scholfield, D.J., and Hallfrisch, J. (2006) Barley  $\beta$ -glucan reduces plasma glucose and insulin responses compared with resistant starch in men. *Nutrition Research*, **26**: 644–650.
- Bellido, G., and Beta, T. (2009) Anthocyanins and ORAC values in pearled and milled purple, black and common barley. *Journal of Agricultural and Food Chemistry*, **57**: 1022–1028.

- Bhathena, S.J., and Velasquez, M.T. (2002) Beneficial role of dietary phytoestrogens in obesity and diabetes. *American Journal Clinical Nutrition*, **76**: 1191–1201.
- Cano, A., Arnao, M.B., Williamson, G., and Garcia-Conesa, M.T. (2002) Superoxide scavenging by polyphenols: effect of conjugation and dimerization. *Redox Report*, **7**: 379–383.
- Carpenter, M.W. (2007) Gestational diabetes, pregnancy hypertension, and late vascular disease. *Diabetes Care*, **30**: (suppl 2), S246–S250.
- Casiraghi, M.C., Garsetti, M, Testolin, G., and Brighenti, F. (2006) Post-prandial responses to cereal products enriched with barley  $\beta$ -glucan. *Journal of the American College of Nutrition* **25**: 313–320.
- Dvoráková, M., Moreira, M.M., Dostálek, P., Skulilová, Z., Guido, L.F., and Barros, A.A. (2008) Characterization of monomeric and oligomeric flavan-3-ols from barley and malt by liquid chromatography-ultraviolet detection-electrospray ionization mass spectrometry. *Journal of Chromatography A*, **1189**: 398–405.
- FDA (2005) Food labeling: health claims; soluble fiber from certain foods and coronary heart disease. *Interim final rule. Federal Register*, **70**: 76150–76162.
- Fine, A.M. (2000) Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. *Alternative Medicine Review*, **5**: 144–151.
- Fry, S.C., Willis, S.C., and Paterson, A.E. (2000) Intraprotoplasmic and wall-localized formation of arabinoxylan-bound diferules and larger ferulate coupling-products in maize cell-suspension cultures. *Planta*, **211**: 679–692.
- Grabber, J.H., Hatfield, R.D., and Ralph, J. (1998) Diferulate cross-links impede the enzymatic degradation of nonlignified maize walls. *Journal of the Science of Food and Agriculture*, **77**: 193–200.
- Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., & Riechel, T.L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry* **46**, 1887–1892.
- Hashimoto, T., Nonaka, Y., and Minato, K. (2002) Suppressive effect of polysaccharides from the edible and medicinal mushrooms, *Lentinus edodes* and *Agaricus blazei*, on the expression of cytochrome P450s in mice. *Bioscience Biotechnology, and Biochemistry*, **344**: 610–614.
- Hernanz, D., Nuea, V., Sancho, A.I., Faulds, C.B., Williamson, G., Bartolom, B., and Gmez-Cordove, C. (2001) Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. *Journal of Agricultural and Food Chemistry*, **49**: 4884–4888.
- Holtekjlen, A.K., Kinitz, C., and Knutsen, A.H. (2006) Flavanol and bound phenolic acid contents in different barley varieties. *Journal of Agricultural and Food Chemistry*, **54**: 2253–2260.
- Ingram, D., Sanders, K., Kolybaba, M., and Lopez, D. (1997) Case-control study of phytoestrogens and breast cancer. *The Lancet*, **35**: 990–994.
- Izydorczyk, M.S., and Dexter, J.E. (2008). Barley  $\beta$ -glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products- a review. *Food Research International*, **41**: 850–868.
- Jing, P., Bomser, J.A., Schwartz, S.J., He, J., Magnuson, B.A., and Giusti, M.M. (2008) Structure – function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. *Journal of Agricultural and Food Chemistry*, **56**: 9391–9398.
- Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., and Kobori, M. (2003) Induction of apoptosis in cancer cells by Bilberry (*Vaccinium myrtillus*) and the anthocyanins. *Journal of Agricultural and Food Chemistry*, **51**: 68–75.
- Kawabata, K., Yamamoto, T., Hara, A., Shimizu, M., Yamada, Y., Matsunaga, K., Tanaka, T., and Mori, H. (2000) Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Letters*, **157**: 15–21.
- Kim, M.J., Hyun, J.N., Kim, J.A., Park J.C., Kim, M.Y., Kim, J.G., Lee, S. C., Chun, S.C., and Chun, I.M. (2007) Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm. *Journal of Agricultural and Food Chemistry*, **55**: 4802–4809.
- Kim, M.J., Hong, S.Y., Kim, S.K., Cheong, C., Park, H.J., Chun, H.K., Jang, K.H., Yoon, B.D., Kim, C.H., and Kang, S.A. (2009)  $\beta$ -Glucan enhanced apoptosis in human colon cancer cells SNU-C4. *Nutrition Research and Practice*, **3**: 180–184.
- Kuhnle, G.G.C., Aquila, C.D., Aspinall, S.M., Runswick, S.A., Mulligan, A. A., and Bingham, S.A. (2009) Phytoestrogen content of cereals and cereal-based food consumed in the UK. *Nutrition and Cancer*, **61**: 302–309.
- Li, J., Kaneko, T., Qin, L.-Q., Wang, J., and Wang, J. (2003) Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. *Nutrition*, **19**: 926–929.

- Li, L., Shewry, P.R., and Ward, J.L. (2008) Phenolic acids in wheat varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, **56**: 9732–9739.
- Lila, M.A. (2004) Anthocyanins and human health: An in vitro investigative approach. *Journal of Biomedicine and Biotechnology*, **5**: 306–313.
- Lime, J.L., and Speirs, V. (2004) Phytoestrogens and breast cancer chemoprevention. *Breast Cancer Research*, **6**: 119–127.
- Madhujith, T., and Shahidi, F. (2007) Antioxidative and antiproliferative properties of selected barley (*Hordenum vulgare* L.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry*, **55**: 5018–5024.
- Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004) Polyphenols: Food sources and bioavailability. *American Journal Clinical Nutrition*, **79**: 727–747.
- Mattila, P., Pihlava, J.-M., and Hellstrom, J. (2005) Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, **53**: 8290–8295.
- McIntosh, G.H., Le, L.R.K., Royle, P.J., and Young, G.P. (1996) A comparative study of the influence of differing barley brans on DMH-induced intestinal tumours in male Sprague–Dawley rats. *Journal of Gastroenterology and Hepatology*, **11**: 113–119.
- McIntosh, G.H., Whyte, J., McArthur, R., and Nestel, P.J. (1991) Barley and wheat foods: influence on plasma cholesterol concentrations in hypercholesterolemic men. *American Journal of Clinical Nutrition*, **53**: 1205–1209.
- Meagher, L.P., and Beecher, G.R. (2000) Assessment of data on the lignan content of foods. *Journal of Food Composition and Analysis*, **13**: 935–947.
- Mori, H., Kawabata, K., Yoshimi, N., Tanaka, T., Murakami, T., Okada, T., and Murai, H. (1999) Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. *Anticancer Research*, **19**: 3775–3778.
- Morgan, G., and Riggs, T.J. (1981) Effects of drought on yield and on grain and malt characters in spring barley. *Journal of the Science of Food and Agriculture*, **32**: 339–346.
- Murakami, A., Nakamura, Y., Koshimizu, K., Takahashi, D., Matsumoto, K., Hagihara, K., Taniguchi, H., Nomura, E., Hosoda, A., Tsuno, T., Maruta, Y., Kim, H.W., Kawabata, K., and Ohigashi, H. (2002) FA15, a hydrophobic derivative of ferulic acid, suppresses inflammatory responses and skin tumor promotion: comparison with ferulic acid. *Cancer Letters*, **180**: 121–129.
- Okamoto, T., Kodoi, R., and Nonaka, Y. (2004) Lentinan from shiitake mushroom (*Lentinus edodes*) suppresses expression of cytochrome P450 1A subfamily in the mouse liver. *Biofactors*, **21**: 407–409.
- Oliveira, R.J., Ribeiro, L.R., Silva, A.F., Matuo, R., and Mantovani M.S. (2006) Evaluation of antimutagenic activity and mechanisms of action of  $\beta$ -glucan from barley, in CHO-k1 and HTC cell lines using the micronucleus test. *Toxicology In Vitro*, **20**: 1225–1233.
- Orcheson, L.J., Rickard, S.E., Seidl, M.M., and Thompson, L.U. (1998) Flaxseed and its mammalian lignan precursor cause a lengthening or cessation of estrous cycling in rats. *Cancer Letters*, **125**: 69–76.
- Pick, M.E., Hawrysh, Z.J., Gee, M.I., and Toth, E. (1998) Barley bread products improve glycemic control of type 2 subjects. *International Journal of Food Science and Nutrition*, **49**: 71–78.
- Pins, J.J., and Kaur, H. (2006) A review of the effects of barley  $\beta$ -glucan on cardiovascular and diabetic risk. *Cereal Foods World*, **51**: 8–11.
- Priefert, H., Rabenhorst, J., and Steinbuechel, A. (2001) Biotechnological production of vanillin. *Applied Microbiology and Biotechnology*, **56**: 296–314.
- Quinde, Z., Axtell, Q., and Baik, B.K. (2006) Phenolic compounds of barley grain and their implication in food product discoloration. *Journal of Agricultural and Food Chemistry*, **54**: 9978–9984.
- Rosa, C.F., Fulcher, G., Beta, T., and De Francisco, A. (2007) Efeito do perolamento na atividade antioxidante e composição fenólica de cevada (Effect of pearling in the antioxidant activity and the phenolic composition of barley). *Alimentos e Nutricao*, **18**: 69–75. (in Spanish)
- Rendell, M., Vanderhoof, J., Venn, M., Shehan, M.A., Arndt, E., Rao, C.S., Gill G., Newman R.K., and Newman C.W. (2005) Effect of a barley breakfast cereal on blood glucose and insulin response in normal and diabetic patients. *Plant Foods in Human Nutrition*, **60**: 63–67.
- Rother, K.I. (2007) Diabetes treatment – bridging the divide. *The New England Journal of Medicine*, **356**: 1499–1501.
- Sakai, S., Kawamata, H., Kogure, T., Mantani, N., Terasawa, K., Umatake, M., and Ochiai, H. (1999) Inhibitory effect of ferulic acid and isoferulic acid on the production of macrophage inflammatory

- protein-2 in response to respiratory syncytial virus infection in RAW264.7 cells. *Mediators of Inflammation*, **8**: 173–175.
- Sakamoto, K., Tabata, T., Shirasaki, K., Inagaki, T., and Nakayama, S. (1987) Effects of gamma-oryzanol and cycloartenol ferulic acid ester on cholesterol diet induced hyperlipidemia in rats. *Japanese Journal of Pharmacology*, **45**: 559–565.
- Schinner, S., Scherbaum, W.A., Bornstein, S.R., and Barthel, A. (2005) Molecular mechanisms of insulin resistance. *Diabetic Medicine*, **22**: 674–682.
- Shi, J., Yu, J., Pohorly, J.E., and Kakuda, Y. (2003) Polyphenolics in grape seeds-biochemistry and functionality. *Journal of Medicinal Food*, **6**: 291–299.
- Siebenhandl, S., Gausgruber, H., Pellegrini, N., Del Rio, D., Fogliano, V., Pernice, R., and Berghofer, E. (2007) Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. *Journal of Agricultural and Food Chemistry*, **55**: 8541–8547.
- Smeds, A.I., Eklundr, P.C., Sjöholm, R.E., Willför, S.M., Nishibe, S., Takeshi, D., and Holmbom, B.R. (2007) Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. *Journal of Agricultural and Food Chemistry* **55**: 1337–1346.
- Tohamy, A.A., EL-Gohr, A.A., El-Nahas, S.M., and Noshay, M.M. (2003)  $\beta$ -glucan inhibits the genotoxicity of cyclophosphamide, adriamycin and cisplatin. *Mutation Research*, **541**: 45–53.
- Wilson, T.A., Nicolosi, R.J., Delaney, B., Chadwell, K., Moolchandani, V., Kotyla, T., Ponduru, S., Zheng, G.H., and Hess, R. (2004) Reduced and high molecular weight barley  $\beta$ -glucans decrease plasma total and non-HDL-cholesterol in hypercholesterolemic Syrian golden hamsters. *Journal of Nutrition*, **134**: 2617–2622.
- Zhao, C., Giusti, M.M., Malik, M., Moyer, M.P., and Magnuson, B.A. (2004) Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. *Journal of Agricultural and Food Chemistry*, **52**: 6122–6128.

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# 3 Nutraceutical properties and health benefits of oats

Pu Jing and Xinzhong Hu

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## 3.1 Introduction

Oat belongs to the Poaceae family and the genus *Avena*, which comprises about 70 species, a few of which are cultivated (Suttie and Reynolds, 2004). *Avena sativa* (common oat) is the most important among cultivated oats (*A. sativa*, *A. byzantina*, *A. fatua*, *A. diffusa* and *A. orientalis*, among others) because of its multifunctional characteristics and nutrition (Butt *et al.*, 2008). *A. sativa* and *A. byzantina* K. Koch known as the white oat and red oat, respectively, are the primary oats grown commercially. Hulless oats (*A. nuda*), also called naked oats, are recorded as having been grown and used in food in China 2,100 years ago (Wang, 2004).

Oat ranks around the sixth in world cereal production statistics, following wheat, maize, rice, barley and sorghum. Oat grains have been grown mainly for livestock feeds and also for people food in forms of porridge or others, providing a good source of protein, fibre and minerals. Today, the eighth largest producers of oats are Russia, Canada, United States, Poland, Finland, Australia, Germany and China, producing around 70% of the world oat in the year 2005 (Food and Agricultural Organization of the United Nations). Oat has become more popular for human nutrition in western countries since its significant health benefits have been unfolded recently in the field of modern food and nutritional science, although oat has been recognised as one of the medical herbs in traditional Chinese medication for a long time. Currently, oats are consumed in many ways, such as breakfast cereal, oat bread, oat cakes, oat cookies, dairy products and juice, among others.

Of primary interest in oats are their dietary fibre that have been shown to significantly reduce the risk of several chronic diseases. Oat is a good source of different types of dietary fibre such as  $\beta$ -glucan, arabinoxylans and cellulose. The  $\beta$ -glucan is the primary component of oat soluble fibre responsible for its noted physiological effects. The physicochemical properties of  $\beta$ -glucan and processing effects are described in the first half of the chapter. With a focus on  $\beta$ -glucans, this chapter will discuss the well-documented contributions of oats to cardiovascular diseases, diabetes, prebiotic effects, as well as the relevant mechanisms.

### 3.2 Oat grain composition

Oat grains have soft kernels covered with 25~30% hull (husk) of total weight excluding naked oats (Butt *et al.*, 2008). Nutrient values for the edible portion for common oat (*Avena sativa* L.) are listed in Table 3.1. The oat kernel, also known as groat, is the part after removal of palea and lemma that contains high amounts of valuable nutrients, such as soluble fibre, proteins, unsaturated fatty acids, vitamins and minerals, among others. Groat contains 10~12.1% fibre, including 4.1~4.9% soluble fibre and 6.0~7.1% insoluble fibre (Manthey *et al.*, 1999). The hull is very high in lignified fibre, and is of less nutritional value.

Oat bran is the edible outermost layer of the oat kernel and is produced by grinding clean groats or rolled oats for separating the resulting flour by sieving, bolting and other suitable means into fractions such that the oat bran is not more than 50% of the starting material (Butt *et al.*, 2008). Oat bran contains approximately 9.7% water, 15.0~17.1% protein, 67.9% carbohydrate, 6.21~8.6% lipid, 15~22% dietary fibre, 10.4%  $\beta$ -glucan and some other minor components (Hahn *et al.*, 1990; Marlett, 1993).

**Table 3.1** Nutrient values and weights are for edible portion for common oat (*Avena sativa*)

Major Nutrients	Units	Value per 100 grams
Water	g	8.22
Protein	g	16.89
Total lipid (fat)	g	6.90
Carbohydrate, by difference	g	66.27
Fiber, total dietary	g	10.6
<b>Minerals</b>		
Calcium, Ca	mg	54
Iron, Fe	mg	4.72
Magnesium, Mg	mg	177
Potassium, K	mg	429
Zinc, Zn	mg	3.97
Manganese, Mn	mg	4.916
<b>Vitamins</b>		
Thiamin	mg	0.763
Riboflavin	mg	0.139
Niacin	mg	0.961
Pantothenic acid	mg	1.349
Vitamin B-6	mg	0.119
<b>Lipids</b>		
Saturated fatty acids	g	1.217
Monounsaturated fatty acids	g	2.178
Polyunsaturated fatty acids	g	2.535

Source: USDA Nutrient database.

### 3.3 The chemical and physical property of oat $\beta$ -glucan

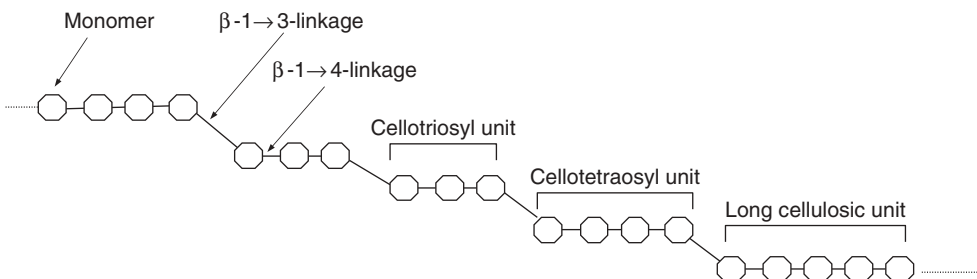
#### 3.3.1 Characterisation of oat $\beta$ -glucans

Oat  $\beta$ -glucan is a linear, unbranched polysaccharide mainly composed of (1  $\rightarrow$ 4)-O-linked (70%) and (1  $\rightarrow$ 3)-O-linked (30%)  $\beta$ -D-glucofuranosyl units (Figure 3.1). The oat  $\beta$ -glucan molecules are mainly composed of cellotriosyl and cellotetraosyl units connected singly by (1  $\rightarrow$ 3)-O-linkage, which account for 90% (Wood *et al.*, 1991) or 95% of the total (Johansson *et al.*, 2000). The long cellulosic units with degrees of polymerisation from 5 to 20 have been detected in oat  $\beta$ -glucan molecules as minor components (Wood *et al.*, 1994b; Izydorczyk *et al.*, 1998a; Johansson *et al.*, 2000). The mole ratios of cellotriosyl to cellotetraosyl units are low for oats (2.1–2.4) compared with barley (2.8–3.4), wheat (3.0–3.8), and rye (2.7–3.2) (Wood *et al.*, 1991; Wood *et al.*, 1994b). A wide range of molecular weight (MW) from 20 to 3000 kD has been reported for oat  $\beta$ -glucans (Varum *et al.*, 1992; Johansson *et al.*, 2000; Cui, 2001). The apparent discrepancies in molar mass of  $\beta$ -glucan are attributed to cultivars, isolation conditions and analysis methods (Jaskari *et al.*, 1995; Beer *et al.*, 1996; Beer *et al.*, 1997a; Cui and Wood, 2000; Izydorczyk and Biliaderis, 2000).

Depolymerisation of  $\beta$ -glucans may occur because of enzymatic hydrolysis in unheated raw materials, alkaline/acid hydrolyses during extraction or shearing damage in mechanical processing. The MW of  $\beta$ -glucan in oat bran, oat bran muffins and oat porridge, and the changes taking place during processing and storage, varied from 1400 to 1800 kD (Beer *et al.*, 1997b).

#### 3.3.2 Occurrence of $\beta$ -glucan in oat grain

$\beta$ -glucan accounts for at least 78% of the total soluble fibre in oats (Miller and Fulcher, 1994).  $\beta$ -glucan is distributed through the endosperm and is located primarily in the endosperm cell walls, and present at a less amount in the aleurone layer of the oat (Miller and Fulcher, 1994). The  $\beta$ -glucan concentration of oat groats varies from 4.5 to 5.5% (Cho and White, 1993) in typical cultivars and from 2.3 to 11.3% in wild cultivars (Welch and Brown, 2000), because of genetic variation of oat cultivars, environmental factors and analytic



**Figure 3.1** Scheme of oat  $\beta$ -glucan molecule structure.



**Table 3.2** Beta-glucan concentration in oat ingredients and commercial products

Product	Beta-glucan (g/100g dry weight)	Reference
<i>Oat ingredients</i>		
Oat groat	3.5~5.0	Mälkki and Virtanen, 2001 Cho <i>et al.</i> , 1993
	4.5~5.5	
Oat bran	5.5~9.0	Mälkki <i>et al.</i> , 2001
	8.7~8.8	Wood <i>et al.</i> , 1991
	8.3~20.0	Åman <i>et al.</i> , 2004
Oat gum	60~80	Hallfrisch <i>et al.</i> , 1995
Bran concentrate	11.5~17.0	Mälkki <i>et al.</i> , 2001
	16.0~25.0	
Instant oat	4.5	Wood <i>et al.</i> , 1991
Rolled oat	4.2	Wood <i>et al.</i> , 1991
	5.0	Åman <i>et al.</i> , 2004
<i>Commercial oat-based foods</i>		
Breakfast cereal	16.6	Åman <i>et al.</i> , 2004
Porridge	4.9	Åman <i>et al.</i> , 2004
Bread loaf	1.1	Åman <i>et al.</i> , 2004
Crisp bread	2.5	Åman <i>et al.</i> , 2004
Extruded oats	3.2	Åman <i>et al.</i> , 2004
Yogurt-like product	2.8	Åman <i>et al.</i> , 2004

methodologies.  $\beta$ -glucan is enriched in oat products such as oat-derived food ingredients and commercial oat products, which are summarised in Table 3.2. A recent research by Gajdošová *et al.* (2007) compared hullless oats (four cultivars) and hulled oats (29 cultivars) for the water soluble and insoluble  $\beta$ -glucan contents. The soluble  $\beta$ -glucan contents varied, 3.91~7.47% and 1.97~4.09% for hull-less oats and hulled oats, respectively, whereas the levels of insoluble glucans decreased in the order: hulled oats (13.79 ~ 33.7%)>naked oats (5.15 ~ 10.80%) (Gajdošová *et al.*, 2007).

### 3.3.3 Functional properties of oat $\beta$ -glucan in food

The aqueous solubility of  $\beta$ -glucan is defined as the maximum amount of  $\beta$ -glucan dissolving in water at selected temperature, and might also be referred to as extractability under specified conditions during sample preparation (Cui, 2001). The (1 $\rightarrow$ 3)-linkages break up the consistency of the (1 $\rightarrow$ 4)- $\beta$ -D-glucan molecule structure and make it water soluble and flexible, whereas the cellulose-like segments may exhibit a tendency for interchain aggregation via hydrogen bonds, contributing to the stiffness, crystalline and low-solubility in solution (Varum and Smidsrod, 1988). However, a high amount of consecutive cellotriosyl residues (more than three residues) may contribute to development of a stable motif (Tvaroska *et al.*, 1983) and thereby impose conformation regulation with a high organisation of those polymers in solution (i.e. low solubility) (Izydorczyk *et al.*, 1998b). Therefore, the high solubility of mixed-linked  $\beta$ -glucan is attributed to the presence of irregular (1 $\rightarrow$ 3)-linkages. The insolubility of freeze-dried cereal  $\beta$ -glucans follows the order of wheat>barley>oat, corresponding with the ratios of cellotriosyl to cellotetraosyl

units in  $\beta$ -glucans structure from wheat (3.0~3.8), barley (2.8~3.4) and oat (2.1~2.4), respectively (Lazaridou *et al.*, 2003). The molar mass has been found to be 500 kD for water-soluble  $\beta$ -glucans of oats and less than 200 kD for water-insoluble or alkali-soluble  $\beta$ -glucans, whereas the molar ratio of cellotriosyl to cellotetraosyl residues is 1.8 for soluble  $\beta$ -glucan and 2.3 for water-insoluble  $\beta$ -glucan (Johansson *et al.*, 2004). A lower water-solubility of  $\beta$ -glucan with a higher ratio of cellotriosyl to cellotetraosyl units has also been observed in other studies (Skendi *et al.*, 2003; Johansson *et al.*, 2007). Factors including temperature, moisture content and processing operations might affect the solubility or extractability of  $\beta$ -D-glucan (Beer *et al.*, 1997b; Cui, 2001; Johansson *et al.*, 2007). For instance, drying may reduce the extractability or decrease the solubility by creating new barriers for water penetration or for diffusion of the dissolved material.

Viscosity is an important physical property not only for  $\beta$ -glucan functionality in food matrix and processing operations but also for health benefits in humans.  $\beta$ -glucan solution is highly viscous even at low concentration (>0.3%) (Doublier and Wood, 1995). The viscosity, shear thinning and viscoelastic properties of  $\beta$ -glucan solution have been enhanced with increasing concentration and/or molecular weight of  $\beta$ -glucan (Lazaridou and Biliaderis, 2007).

$\beta$ -glucans have been reported to form gels (Lazaridou *et al.*, 2003). It has been suggested that a longer gelation time with a lower gelation rate was needed for the high molecular weight of  $\beta$ -glucan samples than for the low molecular weight of counterparts since the storage modulus ( $G'$ ) of  $\beta$ -glucan cryogels increased with reduced  $\beta$ -glucan MW (Lazaridou *et al.*, 2003; Lazaridou *et al.*, 2004). The ratio of  $\beta$ -(1 $\rightarrow$ 3)-linkages to  $\beta$ -(1 $\rightarrow$ 4)-linkages in the  $\beta$ -glucan molecular structure also has an effect on the gelation characteristics and elasticity of  $\beta$ -glucan gel samples (Tosh *et al.*, 2004). The storage modulus and the apparent melting enthalpy values increased not only with decreasing molecular size but also with increasing cellotriose/cellotetraose ratios (Lazaridou *et al.*, 2004).

### 3.4 Effects of processing on oat $\beta$ -glucan

$\beta$ -glucans that impart high viscosity to solutions are known to be important for serum cholesterol lowering and insulin and glucose lowering effects. Factors that influence the viscosity of  $\beta$ -glucan solution are its molecular weight, molecular structure (ratio of cellotriosyl/cellotetraosyl units) and concentration. Food processing conditions including milling, extrusion, malting, cooking and baking may affect the molecular chemical structure, degree of polymerisation, molecular interactions and functional properties (e.g. water solubility, water binding capacity and viscosity). The physicochemical changes may be ultimately reflected in health benefits of  $\beta$ -glucans. For instance, fruit juice with the addition of oat  $\beta$ -glucan appeared to be more effective in producing a cholesterol-lowering response than were the bread and cookies enriched with the same oat  $\beta$ -glucan (Kerkhoffs *et al.*, 2003).

Baking of muffins has been shown to increase the extractability (30~85% of total  $\beta$ -glucans) but decreased the MW of  $\beta$ -glucan when compared to original bran (12~33% of total  $\beta$ -glucans) in an *in vitro* digestion system. The extractability of muffins was influenced by recipes. Baking of cookies and bread has also been found to decrease MW of  $\beta$ -glucan (Kerkhoffs *et al.*, 2003). However, frozen storage decreased the extractability of  $\beta$ -glucan by more than 50% in all muffins (Beer *et al.*, 1997b), but did not change the MW of  $\beta$ -glucan in muffins (Beer *et al.*, 1997b) or bread (Kerkhoffs *et al.*, 2003). Extremely high temperature

or enzymatic hydrolysis during dough development may cause the depolymerisation of  $\beta$ -glucan, to a degree depending on other food ingredients. On the contrary, low temperature may promote interchain aggregation via hydrogen bonds and contribute to the stiffness, crystalline and low extractability. Fermentation with lactic acid bacteria decreased the amount of soluble  $\beta$ -glucan and insoluble  $\beta$ -glucan in oat concentrates. The maximum viscosities of the soluble fibre in oat fibre concentrates were also reduced after fermentation. However, no change in molecular weight was detected (Lambo *et al.*, 2005).

The effects of cooking, baking and drying on the extractability of oat  $\beta$ -glucan were comprehensively evaluated by Johansson *et al.* (2007). Cooking was found to release more soluble  $\beta$ -glucan, while baking decreased the amount of soluble  $\beta$ -glucan, probably due to enzyme activity in the flour towards  $\beta$ -glucan. Drying (overnight at 60 °C) decreased the amount of soluble  $\beta$ -glucan both in bread and fermentate but not in porridge. All processing conditions did not influence the ratio of cellotriosyl to cellotetraosyl residues in soluble  $\beta$ -glucan molecular structure (Johansson *et al.*, 2007). Major viscosity losses in oat gum have been observed during centrifuging, which produced high shear damage to  $\beta$ -glucan molecules (Wood *et al.*, 1989). Extrusion processing at high temperature might decrease the molecular weight of  $\beta$ -glucan (Suortti *et al.*, 2000).

The food matrix could alter the molecular properties of oat  $\beta$ -glucan. Presence of food acids or other food components might affect the  $\beta$ -glucan MW (Åman and Graham, 1987). The variation in average  $\beta$ -glucan MWs of oat-derived ingredients including oats, rolled oats and oat bran (four sources) was 2060–2300 kD. The average  $\beta$ -glucan MWs (1880–1920 kD) after different processing were obviously reduced in extruded flakes, macaroni and muffin. However, the average  $\beta$ -glucan MWs in apple juice, fresh pasta and teacakes were in range of 450–580 kD, even dramatically lower than those in extruded flakes, macaroni and muffin. Acid hydrolysis of glycosidic bonds was proposed to cause the depolymerisation in apple juice, whereas enzymatic hydrolysis of  $\beta$ -glucan may occur in fresh pasta and teacakes (Åman and Graham, 1987).

### 3.5 Oat and health

Consumers' interest in oat products has increased principally because of the health beneficial effects of oats in research papers, general discussions and the marketing efforts of industries. Most health benefits of oat are attributed primarily to water-soluble fibre, of which  $\beta$ -glucan is the major component. Oat dietary fibre has been shown to have numerous physiological effects in addition to the well-known cholesterol-lowering and postprandial hyperglycaemia ones. Health benefits associated significantly with oat dietary fibre will be reviewed in this chapter. Other minor phytochemicals, such as phenolic compounds and fatty acids in oats, are discussed in another chapter of this book.

#### 3.5.1 Lowering cholesterol levels

##### 3.5.1.1 Clinical studies

Cardiovascular disease is the top cause of death in the United States and most western countries. Saturated fat and *trans* fat intake, serum cholesterol and obesity are regarded as major risk factors. By the early 1960s, de Groot *et al.* had observed the cholesterol-lowering effects

of rolled oats in humans. Since then, many human studies have confirmed this health benefit (Ripsin *et al.*, 1992; Brown *et al.*, 1999). From a meta-analysis of 20 trials, incorporating oat products into the diet was found to cause a modest reduction in blood cholesterol level and larger reductions were observed in hypercholesterolemic subjects particularly when a dose of 3 g or more of soluble oat fibre was employed. In another meta-analysis of 67 controlled trials by Brown *et al.* (1999), it was concluded that various types of soluble fibre reduced total and LDL (low-density lipoprotein) cholesterol by similar amounts. Hence, soluble fibre from oats in a practical dose range (2~10 g/day) was associated with small but significant decreases in total cholesterol [ $-0.040 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{g soluble fibre}^{-1}$  (95% CI:  $-0.054, -0.026$ )] and LDL cholesterol [ $-0.037 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{g soluble fibre}^{-1}$  (95% CI:  $-0.040, -0.034$ )] based on 25 trials of oat products.

The cholesterol-lowering effect of oat products has been attributed specifically to oat  $\beta$ -glucans. In a subsequent study, oat gum containing 80% of  $\beta$ -glucans significantly reduced the total and LDL cholesterol levels of hypercholesterolemic human subjects without effects on plasma HDL (high-density lipoprotein) cholesterol, suggesting that  $\beta$ -glucan could be the major component of oats responsible for the overall cholesterol-lowering effect (Braaten *et al.*, 1994b).

Based on numerous convincing studies, the US FDA authorised 'a health claim that soluble fibre sources from whole oats, as a part of a diet low in saturated fat, cholesterol and total fat, may reduce the risk of heart diseases'. The daily dietary intake level of soluble fibre from oat sources associated with reduced risk of coronary heart disease is 3 g or more per day of soluble  $\beta$ -glucan fibre from either whole oats or barley, or a combination of the two. Oat sources include oat bran, rolled oats (oatmeal), whole oat flour and oatrim. In order to have the health claim on the label, the minimum contents of  $\beta$ -glucans required by US FDA are, on dry weight basis, 5.5% for oat bran, 4% for rolled oats, 4% for whole oat flour and 10% for oatrim.

### 3.5.1.2 Mechanisms

$\beta$ -glucan has been demonstrated to be the principal component for serum cholesterol-lowering effects of oats (Braaten *et al.*, 1994b). Even though not fully understood, two major mechanisms have been proposed to explain the serum cholesterol-lowering effect observed with oat soluble fibre.

One mechanism is that  $\beta$ -glucans lower serum cholesterol concentrations by interfering with the absorption of bile acids and fats, which increases bile acid synthesis from serum cholesterol. These effects could possibly be explained by entrapment of whole micelles in the gut owing to higher viscosity to prevent dietary cholesterol from reaching the intestinal epithelium. Oat soluble fibre may decrease absorption of dietary cholesterol by altering the composition of the bile acid pool. Marlett *et al.* (1994) found that oat bran increased the portion of the total bile acid pool that was deoxycholic acid, which has been noted to decrease the absorption of exogenous cholesterol in humans (Hillman *et al.*, 1986).

Consumption of a diet rich in soluble fibre including oat bran or of a diet supplemented with oat bran has been shown to increase fecal excretion of bile acids in many studies (Judd and Truswell, 1981; Kirby *et al.*, 1981a; Anderson *et al.*, 1984; Jenkins *et al.*, 1993; Marlett *et al.*, 1994). Increased small bowel bile acid excretion has also been reported after consumption of oat fibre by use of subjects with ileostomies (Zhang *et al.*, 1992; Lia *et al.*, 1995). The model for *in vivo* determination of dietary fibre and its effect on the absorption

of nutrients in the small intestine was established by Sandberg (Sandberg *et al.*, 1981). The ileostomy effluents collected quantitatively are less degraded by intestinal bacteria than are the feces of healthy subjects. By alternative detection of 7 $\alpha$ -hydroxy-4-cholesten-3-one ( $\alpha$ -HC) that is a metabolite in the synthesis of bile acids (Cohen, 1999), consumption of oat bran breakfast containing 11 g of  $\beta$ -glucan nearly doubled the serum  $\alpha$ -HC concentration within 8 h whereas wheat bran breakfast containing 1 g of  $\beta$ -glucan did not influence serum  $\alpha$ -HC concentrations (Andersson *et al.*, 2002).

The excretion and synthesis of bile acids are the major approaches to eliminating cholesterol. Thus, oat  $\beta$ -glucan may act in a manner similar to that of cholestyramine, which increased bile acid excretion and thereby stimulated cholesterol uptake from the circulation followed by a decreased serum cholesterol concentration (Grundy *et al.*, 1971; Miettinen, 1979). This binding action may stimulate bile acid synthesis from cholesterol that is either made endogenously or captured from the circulation (Kirby *et al.*, 1981b; Glore *et al.*, 1994).

Inhibition of endogenous cholesterol synthesis could possibly be another major mechanism. Short-chain fatty acids (SCFAs) as fermentation products in the colon from dietary fibre including oat  $\beta$ -glucan may enter the portal vein and mediate the inhibitory effect. Bridges *et al.* (1992) found that oat bran decreased serum cholesterol with a significantly elevated level of serum acetate in hypercholesterolemic men (Bridges *et al.*, 1992). Based on a model intestinal fermentation, concentrated oat  $\beta$ -glucan produced total SCFAs and acetate concentrations similar to inulin and guar gum, propionate much higher than inulin, and butyrate, higher than both inulin and guar gum (Queenan *et al.*, 2007). It was suggested that propionate may inhibit cholesterol synthesis by inhibition of hydroxymethylglutaryl (HMG) CoA reductase that is the enzyme catalysing the rate-limiting reaction for cholesterol biosynthesis or by increasing catabolism of LDL cholesterol (Chen *et al.*, 1984; Cummings and Macfarlane, 1997). Therefore, colonic propionate is a gluconeogenic substrate in humans and may inhibit the utilisation of acetate for cholesterol synthesis (Wolever *et al.*, 1989, 1991). Soluble fibre may delay gastric emptying, thereby reducing post-prandial serum insulin concentrations that can reduce hepatic cholesterol production through mediation of HMG-CoA reductase (Bell *et al.*, 1999).

### 3.5.2 Postprandial effects

#### 3.5.2.1 Clinical studies

Dietary consumption of a high content of whole grains has been inversely associated with low incidence of type 2 diabetes in several population studies (Fung *et al.*, 2002; McKeown *et al.*, 2002; Montonen *et al.*, 2003; Jensen *et al.*, 2006). For example, wholegrain consumption was associated with a reduced risk of type 2 diabetes in a cohort study of 2286 men and 2030 women initially free of diabetes based on food consumption data collected from 1966 through 1972 (Montonen *et al.*, 2003). Those health benefits of wholegrain intake may be attributable to the synergistic effects of dietary fibre and micronutrients found in wholegrain foods (Jacobs and Steffen, 2003).

On the aspects of dietary fibre, soluble fibre has been studied as active components since they could provide water solution with a high viscosity, which is reported to delay gastric emptying and slow glucose absorption in the intestine (Würsch and Pi-Sunyer, 1997). As early as 1978, Jenkins and co-workers documented that several types of soluble reduced

glucose and insulin responses of healthy adults when undergoing 50 g glucose tolerance tests and were potential in modifying postprandial hyperglycaemia.

Therefore, interest in oats has been increased due to their high concentration of soluble fibre (Manthey *et al.*, 1999). Braaten *et al.* (1991) compared effects of oat gum with other gums on glucose and insulin response, and found that oat gum was comparable with or greater than guar gum in lowering postprandial plasma glucose and insulin concentration in human subjects (Braaten *et al.*, 1991). Later in 1994, Braaten *et al.* found that both the native cell wall fibre of oat bran and isolated oat gum reduced postprandial blood glucose and insulin in subjects with and without type 2 diabetes (Braaten *et al.*, 1994a). Hallfrisch *et al.* (1995) reported that diets containing soluble oat extracts (rich in  $\beta$ -glucans) improved glucose and insulin response in both men and women who had moderately high cholesterol concentrations (Hallfrisch *et al.*, 1995). In 1996, Pick *et al.* evaluated the 24-week effects of bread products containing oat bran concentrate in the free-living subjects with non-insulin-dependent diabetes via dietary, clinical and biochemical methods and found that oat bran concentrate bread products improved glycemic, insulinemic and lipidemic responses (Pick *et al.*, 1996). Jenkins *et al.* (2002) found that oat products with enrichment of  $\beta$ -glucan reduced the glycemic index (GI, an indicator of carbohydrate's ability to raise blood glucose levels) in human subjects. GI has been associated with the risk of developing type 2 diabetes in another study (Salmerón *et al.*, 1997). Tapola *et al.* (2005) reported that oat bran products high in  $\beta$ -glucans had resulted in a lower glycemic response and decreased postprandial glycemic response of an oral glucose load in type 2 diabetic patients.

### 3.5.2.2 Mechanisms

Although mechanisms for health benefits of oats relating to diabetes are still not clear,  $\beta$ -glucan is believed to be the primary functional component responsible for the physiological effect. Interests in  $\beta$ -glucan have been increased since oat  $\beta$ -glucans have been found to be an inverse association with postprandial blood glucose and insulin responses in healthy and diabetic human subjects (Braaten *et al.*, 1994a; Hallfrisch *et al.*, 1995; Pick *et al.*, 1996; Tapola *et al.*, 2005).

The dose response of  $\beta$ -glucan has been investigated in several studies. Tappy *et al.* (1996) observed that consumption of breakfast cereals with addition of oat  $\beta$ -glucan could reduce the postprandial glycaemic response by up to 50% in subjects with non-insulin-dependent diabetes mellitus. The maximum reductions in plasma glucose were 33%, 58% and 62% after breakfast cereal meals that had different levels of  $\beta$ -glucan (4, 6 and 8.4 g, respectively) at the same carbohydrate load (35 g). A linear inverse relationship between dose of  $\beta$ -glucan and plasma glucose response was clearly observed. The 50% decrease in glycemic response should occur after the consumption of 35 g of carbohydrate with approximately 5 g of  $\beta$ -glucan (Tappy *et al.*, 1996). Similarly, Jenkins *et al.* (2002) reported that GIs of the prototype  $\beta$ -glucan cereal ( $\beta$ -glucan=7.3 g, GI=52 $\pm$ 5) and  $\beta$ -glucan bar ( $\beta$ -glucan=6.2 g, GI=43 $\pm$ 4.1) were significantly lower than the commercial oat bran breakfast cereal ( $\beta$ -glucan=3.7 g, GI=86 $\pm$ 6) and white bread (GI=100) at the same carbohydrate intake (50 g), suggesting the effect of  $\beta$ -glucan may be dose-dependent. Muesli with 4 g of  $\beta$ -glucans has been found to significantly reduce the glucose and insulin responses whereas muesli with 3 g of  $\beta$ -glucans gave no significant effects compared to a reference meal without muesli and  $\beta$ -glucans at the same carbohydrate load (50 g), suggesting a total of 4 g of  $\beta$ -glucans from oats might be a critical level for a significant decrease in glucose and insulin responses in healthy people (Granfeldt *et al.*, 2008).

It has been demonstrated that the viscosity of  $\beta$ -glucan is an important factor for the low glycemic response. In 1978, Jenkins found that the effect of guar gum on the postprandial glycemic response was abolished when non-viscous guar gum hydrolysed by acid was used, suggesting the importance of viscosity for the health property of the dietary fibre. The relationship of the viscosity of oat gum rich in  $\beta$ -glucan with plasma glucose and insulin levels has been quantitatively studied in healthy humans consuming 50 g glucose in a drink model (Wood *et al.*, 1994a). A significant linear relationship between log (viscosity) of the mixtures consumed and the glucose and insulin responses has shown that the viscosity could account for 79~96% of the changes in plasma glucose and insulin. The increase of intestinal viscosity due to high molecular weight  $\beta$ -glucan is crucial for achieving the positive effect of  $\beta$ -glucan on the peak blood glucose.

Viscosity of hydrocolloids was usually determined by concentration in solution and molecular weight. The glycemic response was significantly correlated to a combination of logarithm of the concentration and logarithm of molecular weight of  $\beta$ -glucans (Wood *et al.*, 2000). Panahi *et al.* (2007) evaluated the effect of viscosity of two oat  $\beta$ -glucan concentrates prepared by different processing methods on postprandial glycemia in healthy individuals. The enzymatic method rather than the aqueous method preserved the viscosity and improved postprandial glycemic control activity (Panahi *et al.*, 2007).

### 3.5.3 Prebiotic benefits

Prebiotics have been defined as non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system that are beneficial to the health of the host (Gibson and Roberfroid, 1995). A prebiotic substrate, such as dietary fibre, is selectively utilised by beneficial bacteria in the colon including *Bifidobacteria* and *Lactobacilli*, but does not promote intestinal bacteria such as *Clostridia*, *Bacteroides* and *Escherichia coli* (Manning and Gibson, 2004).

Feeding rats with oat bran diets has been shown to increase the content of *Lactobacilli* and *Bifidobacteria* in fecal flora as compared with fibre-free diets (Ryhänen *et al.*, 1996). Oat  $\beta$ -glucan has been shown to stimulate the growth of *Bifidobacterium* and *Lactobacillus* in a dose-dependent manner and also inhibit *Enterobacillus* growth in the colon in mice (Shen *et al.*, 2006). The contents of short-chain fatty acids in the colon were significantly higher in mice fed with oat  $\beta$ -glucan compared to those fed with the control diet. Shen *et al.* (2006) indicated that the prebiotic effects of oat  $\beta$ -glucan were associated with their molecular weight. However, in the study by Jaskari *et al.* (1993) oat  $\beta$ -glucan enhanced the growth of two *Bifidobacterium* strains but inhibited the growth of two *Lactobacillus* strains *in vitro* (Jaskari *et al.*, 1993). Kedia *et al.* (2008) compared fermentation ability of oat fractions (pearlings, whole flour and bran) by human *Lactobacillus* strains *in vitro*. Oat fractions with high concentration of soluble fibre have resulted in greater growth of *Lactobacillus reuteri*, *Lactobacillus plantarum* and *Lactobacillus acidophilus*. The 1~3% of pearling oat sample containing the highest amount of soluble fibre and  $\beta$ -glucan had the greatest fermentation ability and the non-digestible components of this fraction treated with an *in vitro* digestion model also showed the greatest growth of the three *Lactobacillus* strains (Kedia *et al.*, 2008).

Polysaccharides are generally not prebiotic in their metabolism, since breakdown of polysaccharides in the colon is a complex process involving various enzymes from many species and cross-feeding by micro-flora (Wang and Gibson, 1993). *Bacteroides*, for

instance, the predominant genus in the colon, were capable of synthesising a wide range of inducible cell-associated polysaccharidases and glucosidases (Salyers and Leedle, 1983).

Therefore, there is an increasing interest in non-digestible oligomers that would selectively promote the growth of health-promoting probiotic strains.  $\beta$ -glucoooligomers ( $\beta$ -glucotriose and  $\beta$ -glucotetraose) and xyloooligomers (xylose, xylobiose and xylotriose), enzymatically hydrolysed from oat  $\beta$ -glucan and oat xylan respectively, stimulated the probiotic growth compared with the growth of *Clostridia*, *Bacteroides* and *Escherichia coli* (Jaskari *et al.*, 1998). In this *in vitro* study,  $\beta$ -glucoooligomers and xyloooligomers have shown selective stimulation of the growth of two *Lactobacillus* and three *Bifidobacteria*.  $\beta$ -glucoooligomers were utilised preferentially by *L. rhamnosus* GG whilst three *Bifidobacterium* strains utilised xyloooligomers. In a study by Kontula *et al.* (1998), lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactococcus lactis*) have been shown to utilise oat  $\beta$ -glucoooligomers ( $\beta$ -glucotriose and  $\beta$ -glucotetraose) while only *L. plantarum* utilised oat xyloooligomers (xylose, xylobiose and xylotriose) to form the end products, including lactic acid, acetic acid, formic acid and ethanol. Those two types of oat oligosaccharides affected both qualitatively and quantitatively fermentation end products of lactic acid bacteria (Kontula *et al.*, 1998).

### 3.6 Conclusions

Interests in oats have been increasing over the last decades since oat  $\beta$ -glucan has been found to offer many health benefits and rheological advantages. Thus, the physicochemical and physiological properties of  $\beta$ -glucan are of commercial and nutritional importance. In general, oat products may lower serum cholesterol levels and reduce postprandial glycemic and insulinemic responses. The effectiveness is related to the viscosity of oat  $\beta$ -glucan, particularly for postprandial effects, although the evidence for a role of viscosity has not been directly demonstrated for the serum cholesterol-lowering effect. With regards to prebiotic effect,  $\beta$ -glucoooligomers produced from oat  $\beta$ -glucan appeared preferable to the growth of probiotic bacterial strains. In aspects of food functionalities of oat  $\beta$ -glucans, the molecular structure and size are important determinants of physical properties of the  $\beta$ -glucan system, such as water solubility, water binding capacity, viscosity and gelation, among others. The molecular structure and size of oat  $\beta$ -glucans should be tailored for both different health benefits and rheological properties of oat-based foods. Furthermore, oat  $\beta$ -glucan molecules are determined primarily by genetic variation of cultivars and also affected by extraction or processing approaches. The relationships of health benefits, functionalities in food and pressing operations of  $\beta$ -glucans need to be further bridged in the food system.

### References

- Åman, P. and Graham, H. (1987) Analysis of total and insoluble mixed-linked (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucans in barley and oats. *Journal of Agriculture and Food Chemistry*, **35**: 704–709.
- Anderson, J.W., Story, L., Sieling, B., Chen, W.J., Petro, M.S. and Story, J. (1984) Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition*, **40**: 1146–1155.



- Andersson, M., Ellegard, L. and Andersson, H. (2002) Oat bran stimulates bile acid synthesis within 8 h as measured by 7 $\alpha$ -hydroxy-4-cholesten-3-one. *American Journal of Clinical Nutrition*, **76**: 1111–1116.
- Beer, M.U., Arrigoni, E. and Amado, R. (1996) Extraction of oat gum from oat bran: effects of process on yield, molecular weight distribution, viscosity and (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucan content of the gum. *Cereal Chemistry*, **73**: 58–62.
- Beer, M.U., Wood, P.J. and Weisz, J. (1997a) Molecular weight distribution and (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chemistry*, **74**: 476–480.
- Beer, M.U., Wood, P.J., Weisz, J. and Fillion, N. (1997b) Effect of cooking and storage on the amount and molecular weight of (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan extracted from oat products by an *in vitro* digestion system. *Cereal Chemistry*, **74**: 705–709.
- Bell, S., Goldman, V.M., Bistran, B.R., Arnold, A.H., Ostroff, G. and Forse, R.A. (1999) Effect of  $\beta$ -glucan from oats and yeast on serum lipids. *Critical Reviews in Food Science and Nutrition*, **39**: 189–202.
- Braaten, J.T., Scott, F.W., Wood, P.J., Riedel, K.D., Wolynetz, M.S., Brulé, D. and Collins, M.W. (1994a) High  $\beta$ -glucan oat bran and oat gum reduce postprandial blood glucose and insulin in subjects with and without type 2 diabetes. *Diabetic Medicine*, **11**: 312–318.
- Braaten, J.T., Wood, P.J., Scott, F.W., Riedel, K.D., Poste, L.M. and Collins, M.W. (1991) Oat gum lowers glucose and insulin after an oral glucose load. *American Journal of Clinical Nutrition*, **53**: 1425–1430.
- Braaten, J.T., Wood, P.J., Scott, F.W., Wolynetz, M.S., Lowe, M.K., Bradley-White, P. and Collins, M.W. (1994b) Oat  $\beta$ -glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition*, **48**: 465–474.
- Bridges, S.R., Anderson, J.W., Deakins, D.A., Dillon, D.W. and Wood, C.L. (1992) Oat bran increases serum acetate of hypercholesterolemic men. *American Journal of Clinical Nutrition*, **56**: 455–459.
- Brown, L., Rosner, B., Willett, W.W. and Sacks, F.M. (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition*, **69**: 30–42.
- Butt, M.S., Tahir-Nadeem, M., Khan, M.K.I., Shabir, R. and Butt, M.S. (2008) Oat: unique among the cereals. *European Journal of Nutrition*, **47**: 68–79.
- Chen, W.J., Anderson, J.W. and Jennings, D. (1984) Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *Proceedings of the Society for Experimental Biology and Medicine*, **175**: 215–218.
- Cho, K.C. and White, P.J. (1993) Enzymatic analysis of  $\beta$ -glucan content in different oat genotypes. *Cereal Chemistry*, **70**: 539–542.
- Cohen, J.C. (1999) Contribution of cholesterol 7  $\alpha$ -hydroxylase to the regulation of lipoprotein metabolism. *Current Opinion in Lipidology*, **10**: 303–307.
- Cui, S.W. (2001) *Polysaccharides gums from agricultural products: processing, structure and functionality*. Lancaster: Technomic Publishing Company Inc.
- Cui, W. and Wood, P.J. (2000) Relationships between structural features, molecular weight and rheological properties of cereal  $\beta$ -D-glucan. In K. Nishinari (ed.), *Hydrocolloids-Part 1* (pp. 159–168). Amsterdam: Elsevier.
- Cummings, J.H. and Macfarlane, G.T. (1997) Colonic microflora: nutrition and health. *Nutrition*, **13**: 476–478.
- Doublier, J.L. and Wood, P.J. (1995) Rheological properties of aqueous solutions of (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan from oats (*Avena sativa* L.). *Cereal Chemistry*, **72**: 335–340.
- Food and Agricultural Organization of the United Nations. (2005). Major food and agricultural commodities and producers. Economic and Social Department: The Statistics Division.
- Fung, T.T., Hu, F.B., Pereira, M.A., Liu, S., Stampfer, M.J., Colditz, G.A. and Willett, W.C. (2002) Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *American Journal of Clinical Nutrition*, **76**: 535–540.
- Gajdošová, A., Petrušáková, Z., Havrlentová, M., Červená, V., Hozová, B., Šturdík, E. and Kogan, G. (2007) The content of water-soluble and water-insoluble  $\beta$ -D-glucans in selected oats and barley varieties. *Carbohydrate Polymers*, **70**: 46–52.
- Gibson, G.R. and Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, **125**: 1401–1412.
- Glore, S.R., Van, T.D., Knehans, A.W. and Guild, M. (1994) Soluble fiber and serum lipids: a literature review. *Journal of the American Dietetic Association*, **94**: 425–436.
- Granfeldt, Y., Nyberg, L. and Björck, I. (2008) Muesli with 4 g oat  $\beta$ -glucans lowers glucose and insulin responses after a bread meal in healthy subjects. *European Journal of Clinical Nutrition*, **62**: 600–607.

- Grundy, S.M., Ahrens, E.H., Jr and Salen, G. (1971) Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *Journal of Laboratory and Clinical Medicine*, **78**: 94–121.
- Hahn, J.D., Chung, T.K. and Baker, D.H. (1990) Nutritive value of oat flour and oat bran. *Journal of Animal Science* **68**: 4253–4260.
- Hallfrisch, J., Scholfield, D.J. and Behall, K.M. (1995) Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. *American Journal of Clinical Nutrition*, **61**: 379–384.
- Hillman, L.C., Peters, S.G., Fisher, C.A. and Pomare, E.W. (1986) Effects of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. *Gut*, **27**: 29–36.
- Izydorczyk, M.S. and Biliaderis, C.G. (2000) Structural and functional aspects of cereal arabinoxylans and  $\beta$ -glucans. In G. Doxastakis and V. Kiosseoglou (eds.), *Novel macromolecules in food systems* (pp. 361–384). Amsterdam: Elsevier.
- Izydorczyk, M.S., Biliaderis, C.G., Macri, L.J. and MacGregor, A.W. (1998a) Fractionation of oat (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)- $\beta$ -D-glucans and characterisation of the fractions. *Journal Of Cereal Science*, **27**: 321.
- Izydorczyk, M.S., Macri, L.J. and MacGregor, A.W. (1998b) Structure and physicochemical properties of barley non-starch polysaccharides – I. Water-extractable  $\beta$ -glucans and arabinoxylans. *Carbohydrate Polymers*, **35**: 249–258.
- Jacobs, D.R., Jr and Steffen, L.M. (2003) Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. *American Journal of Clinical Nutrition*, **78**(suppl): 508S–513S.
- Jaskari, J., Henriksson, K., Nieminen, A., Suortti, T., Salovaara, H. and Poutanen, K. (1995) Effects of hydrothermal and enzymic treatments on the viscous behavior of dry- and wet-milled oat brans. *Cereal Chemistry*, **72**: 625–631.
- Jaskari, J., Kontula, P., Siitonen, A., Jousimies-Somer, H., Mattila-Sandholm, T. and Poutanen, K. (1998) Oat  $\beta$ -glucan and xylan hydrolysates as selective substrates for *Bifidobacterium* and *Lactobacillus* strains. *Applied Microbiology and Biotechnology*, **49**: 175–181.
- Jaskari, J., Salovaara, H., Mattila-Sandholm, T. and Poutanen, K. (1993) The effect of oat  $\beta$ -glucan on the growth of selected *Lactobacillus spp.* and *Bifidobacterium spp.* In T. Aalto-Kaarlehto and H. Salovaara (eds.), *Proceedings of the 25th Nordic Cereal Congress* (pp. 242–244). Univ. Helsinki, Helsinki.
- Jenkins, D.J., Wolever, T.M., Rao, A.V., Hegele, R.A., Mitchell, S.J., Ransom, T., Boctor, D.L., Spadafora, P.J., Jenkins, A.L., Mehling, C., Relle, L.K., Connelly, P.W., Story, J.A., Furumoto, E.J., Corey, P. and Wursch, P. (1993) Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. *New England Journal of Medicine*, **329**: 21–26.
- Jenkins, A.L., Jenkins, D.J. Zdravkovic, U., Wursch, P., and Vuksan, V. (2002) Depression of the glycemic index by high levels of  $\beta$ -glucan fiber in two functional foods tested in type 2 diabetes. *Eur J Clin Nutr*, **56**, 622–628.
- Jensen, M.K., Koh-Banerjee, P., Franz, M., Sampson, L., Gronbaek, M. and Rimm, E.B. (2006) Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids, and inflammation. *American Journal of Clinical Nutrition*, **83**: 275–283.
- Johansson, L., Tuomainen, P., Anttila, H., Rita, H. and Virkki, L. (2007) Effect of processing on the extractability of oat  $\beta$ -glucan. *Food Chemistry*, **105**: 1439–1445.
- Johansson, L., Tuomainen, P., Ylilinen, M., Ekholm, P. and L. Virkki. (2004) Structural analysis of water-soluble and -insoluble  $\beta$ -glucans of whole-grain oats and barley. *Carbohydrate Polymers*, **58**: 267–274.
- Johansson, L., Virkki, L., Maunu, S., Lehto, M., Ekholm, P. and Varo, P. (2000) Structural characterization of water soluble  $\beta$ -glucan of oat bran. *Carbohydrate Polymers*, **42**: 143–148.
- Judd, P.A. and Truswell, A.S. (1981) The effect of rolled oats on blood lipids and fecal steroid excretion in man. *American Journal of Clinical Nutrition*, **34**: 2061–2067.
- Kedia, G., Vázquez, J.A. and Pandiella, S.S. (2008) Enzymatic digestion and *in vitro* fermentation of oat fractions by human *lactobacillus* strains *Enzyme and Microbial Technology*, **43**: 355–361.
- Kerkhoffs, D., Hornstra, G. and Mensink, R.P. (2003) Cholesterol-lowering effect of  $\beta$ -glucan from oat bran in mildly hypercholesterolemic subjects may decrease when  $\beta$ -glucan is incorporated into bread and cookies. *American Journal of Clinical Nutrition*, **78**: 221–227.
- Kirby, R.W., Anderson, J.W., Sieling, B., Rees, E.D., Chen, W.J., Miller, R.E. and Kay, R.M. (1981a) Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. *American Journal of Clinical Nutrition*, **34**: 824–829.

- Kirby, R.W., Anderson, J.W., Sieling, B., Rees, E.D., Chen, W.J.L., Miller, R.E. and Kay, R.M. (1981b) Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentration of hypercholesterolemic men. *American Journal of Clinical Nutrition*, **34**: 824–829.
- Kontula, P., Wright, A.V. and Mattila-Sandholm, T. (1998) Oat bran  $\beta$ -gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. *International Journal of Food Microbiology*, **45**: 163–169.
- Lambo, A.M., Öste, R. and Nyman, M.E.G.-L. (2005) Dietary fibre in fermented oat and barley  $\beta$ -glucan rich concentrates. *Food Chemistry*, **89**: 283–293.
- Lazaridou, A. and Biliaderis, C.G. (2007) Molecular aspects of cereal beta-glucan functionality: physical properties, technological applications and physiological effects. *Journal Of Cereal Science*, **46**: 101–118.
- Lazaridou, A., Biliaderis, C.G. and Izydorczyk, M.S. (2003) Molecular size effects on rheological properties of oat  $\beta$ -D-glucans in solution and gels. *Food Hydrocolloids*, **17**: 693–712.
- Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M. and Steele, B.R. (2004) A comparative study on structure-function relations of mixed-linkage (1 $\rightarrow$ 3), (1 $\rightarrow$ 4) linear  $\beta$ -D-glucans. *Food Hydrocolloids*, **18**: 837–855.
- Lia, Å., Hallmans, G., Sandberg, A. S., Sundberg, B., Åman, P. and Andersson, H. (1995) Oat  $\beta$ -glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. *American Journal of Clinical Nutrition* **62**: 1245–1251.
- Manning, T.S. and Gibson, G.R. (2004) Microbial-gut interactions in health and disease. Prebiotics. *Best Practice and Research: Clinical Gastroenterology*, **18**: 287–298.
- Manthey, F.A., Hareland, G.A. and Huseby, D.J. (1999) Soluble and insoluble dietary fiber content and composition in oat. *Cereal Chemistry*, **76**: 417–420.
- Marlett, J.A. (1993) Comparison of dietary fiber and selected nutrient compositions of oat and other grain fractions. In P.J. Wood (ed.), *Oat Bran* (pp. 49–82). St. Paul, MN: American Association of Cereal Chemists.
- Marlett, J.A., Hosig, K.B., Vollendorf, N.W., Shinnick, F.L., Haack, V.S. and Story, J.A. (1994) Mechanism of serum cholesterol reduction by oat bran. *Hepatology*, **20**: 1450–1457.
- McKeown, N.M., Meigs, J.B., Liu, S., Wilson, P.W. and Jacques, P.F. (2002) Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *American Journal of Clinical Nutrition*, **76**: 390–398.
- Miettinen, T.A. (1979) Effects of neomycin alone and in combination with cholestyramine on serum cholesterol and fecal steroids in hypercholesterolemic subjects. *Journal of Clinical Investigation*, **64**: 1485–1493.
- Miller, S.S. and Fulcher, R.G. (1994) Distribution of (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucan in kernels of oats and barley using microspectrofluorometry. *Cereal Chemistry*, **71**: 64–68 (26 ref.).
- Montonen, J., Knekt, P., Jarvinen, R., Aromaa, A. and Reunanen, A. (2003) Whole-grain and fiber intake and the incidence of type 2 diabetes. *American Journal of Clinical Nutrition*, **77**: 622–629.
- Panahi, S., Ezatagha, A., Temelli, F., Vasanthan, T. and Vuksan, V. (2007)  $\beta$ -glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. *Journal of the American College of Nutrition*, **26**: 639–644.
- Pick, M.E., Hawrysh, Z.J., Gee, M.I., Toth, E., Garg, M.L. and Hardin, R.T. (1996) Oat bran concentrate bread products improve long-term control of diabetes: a pilot study. *Journal of the American Dietetic Association*, **96**: 1254–1261.
- Queenan, K.M., Stewart, M.L., Smith, K.N., Thomas, W., Fulcher, R.G. and Slavin, J.L. (2007) Concentrated oat  $\beta$ -glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutrition Journal*, **6**: 6–14.
- Ripsin, C.M., Keenan, J.M., Jacobs, D.R., Jr, Elmer, P.J., Welch, R.R., Van Horn, L., Liu, K., Turnbull, W.H., Thyne, F.W., Kestin, M., Hegsted, M., Davidson, D.M., Davidson, M.H., Dugan, L.D., Demark-Wahnefried, W. and Beling, S. (1992) Oat products and lipid lowering. A meta-analysis. *Journal of the American Medical Association*, **267**: 3317–3325.
- Ryhänen, E.L., Mantere-Alhonen, S. and Salovaara, H. (1996) Effects of oat bran and rye bran diet on intestinal *Lactobacillus* and *Bifidobacterium* flora of Wistar rats. In Y. Malkki and J. H. Cumming (eds.), *Cost Action 92. Dietary Fibre and Fermentation in the Colon* (pp. 55–57). Luxembourg: Office for Official Publications of the European Communities.
- Salmerón, J., Ascherio, A., Rimm, E.B., Colditz, G.A., Spiegelman, D., Jenkins, D.J., Stampfer, M.J., Wing, A.L. and Willett, W.C. (1997) Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care*, **20**: 545–550.

- Salyers, A.A. and Leedle, J.A.Z. (1983) Carbohydrate metabolism in the human colon. In D. J. Hentges (ed.), *Human intestinal microflora in health and disease* (pp. 129–146). New York: Academic Press.
- Sandberg, A.S., Andersson, H., Hallgren, B., Hasselblad, K., Isaksson, B. and Hulten, L. (1981) Experimental model for in vivo determination of dietary fibre and its effect on the absorption of nutrients in the small intestine. *British Journal of Nutrition*, **45**: 283–294.
- Shen, R.L., Wang, Z.C., Dong, J.L. and Yao, H.Y. (2006) Effect of oat  $\beta$ -glucan on colon flora and its function in mice. *Acta Nutrimenta Sinica*, **28**: 430–433.
- Skendi, A., Biliaderis, C.G., Lazaridou, A. and Izydorczyk, M.S. (2003) Structure and rheological properties of water soluble  $\beta$ -glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal Of Cereal Science*, **38**: 15–31.
- Suortti, T., Johansson, L. and Autio, K. (2000) Effect of heating and freezing on molecular weight of oat  $\beta$ -glucan. *American Association of Clinical Chemistry (AACC) Annual Meeting*: Abstract **332**.
- Suttie, J.M. and Reynolds, S.G. (2004) Chapter I - Background to fodder oats worldwide. In J.M. Suttie and S.G. Reynolds (eds.), *Fodder oats: a world overview* (pp. 1–10). Viale delle Terme di Caracalla, 00100 Rome: FAO.
- Tapola, N., Karvonen, H., Niskanen, L., Mikola, M. and Sarkkinen, E. (2005) Glycemic responses of oat bran products in type 2 diabetic patients. *Nutrition, Metabolism and Cardiovascular Diseases*, **15**: 255–261.
- Tappy, L., Gügölz, E. and Würsch, P. (1996) Effects of breakfast cereals containing various amounts of beta glucans fibres on plasma glucose and insulin responses in NIDDM subjects. *Diabetes Care*, **19**: 831–834.
- Tosh, S.M., Wood, P.J., Wang, Q. and Weisz, J. (2004) Structural characteristics and rheological properties of partially hydrolyzed oat  $\beta$ -glucan: the effects of molecular weight and hydrolysis method. *Carbohydrate Polymers*, **55**: 425–436.
- Tvaroska, I., Ogawa, K., Deslandes, Y. and Marchessault, R.H. (1983) Crystalline conformation and structure of lichenan and barley,  $\beta$ -glucan. *Canadian Journal of Chemistry*, **61**: 1608–1616.
- Varum, K.M. and Smidsrod, O. (1988) Partial chemical and physical characterisation of (1  $\rightarrow$  3), (1  $\rightarrow$  4)- $\beta$ -D-glucans from oat (*Avena sativa* L.) aleurone. *Carbohydrate Polymers*, **9**: 103–117.
- Varum, K.M., Smidsrod, O. and Brant, D.A. (1992) Light scattering reveals micelle-like aggregation in the (1  $\rightarrow$  3), (1  $\rightarrow$  4)- $\beta$ -D-glucans from oat aleurone. *Food Hydrocolloids*, **5**: 497–511.
- Würsch, P. and Pi-Sunyer, F. (1997) The role of viscous soluble fiber in the metabolic control of diabetes. *A review with special emphasis on cereals rich in  $\beta$ -glucan*. *Diabetes Care*, **20**: 1774–1780.
- Wang, S. (2004) Chapter VIII - Fodder oat in China. In J.M. Suttie and S.G. Reynolds (eds.), *Fodder Oats: A World Overview* (pp. 123–144). Viale delle Terme di Caracalla, 00100 Rome: FAO.
- Wang, X. and Gibson, G.R. (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Microbiology*, **75**: 373–380.
- Welch, R.W. and Brown, J.C.W. (2000) Interspecific and intraspecific variation in grain and great characteristics of wild oat (*Avena*) species: Very high great (1  $\rightarrow$  3), (1  $\rightarrow$  4)- $\beta$ -D-glucan in an *Avena atlantica* genotype. *Journal Of Cereal Science*, **31**: 273–279.
- Wolever, T.M., Brighenti, F., Royall, D., Jenkins, A.L. and Jenkins, D.J. (1989) Effect of rectal infusion of short chain fatty acids in human subjects. *American Journal of Gastroenterology*, **84**: 1027–1033.
- Wolever, T.M., Spadafora, P. and Eshuis, H. (1991) Interaction between colonic acetate and propionate in humans. *American Journal of Clinical Nutrition*, **53**: 681–687.
- Wood, P., Weisz, J., Fedec, P. and Burrows, V.D. (1989) Large-scale preparation and properties of oat fractions enriched in (1  $\rightarrow$  3)(1  $\rightarrow$  4)- $\beta$ -D-glucan. *Cereal Chemistry*, **66**: 97–103.
- Wood, P.J., Beer, M.U. and Butler, G. (2000) Evaluation of role of concentration and molecular weight of oat  $\beta$ -glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*, **84**: 19–23.
- Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S. and Collins, M.W. (1994a) Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*, **72**: 731–743.
- Wood, P.J., Weisz, J. and Blackwell, B.A. (1991) Molecular characterization of cereal  $\beta$ -D-glucans. Structural analysis of oat  $\beta$ -D-glucan and rapid structural evaluation of  $\beta$ -D-glucans from different sources by High-Performance Liquid Chromatography of Oligosaccharides released by Lichenase. *Cereal Chemistry*, **68**: 31–39.

Wood, P.J., Weisz, J. and Blackwell, B.A. (1994b) Structural studies of (1→3),(1→4)-β-D-glucans by <sup>13</sup>C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chemistry*, **71**: 301–307.

Zhang, J.X., Hallmans, G., Andersson, H., Bosaeus, I., Aman, P., Tidehag, P., Stenling, R., Lundin, E. and Dahlgren, S. (1992) Effect of oat bran on plasma cholesterol and bile acid excretion in nine subjects with ileostomies. *American Journal of Clinical Nutrition*, **56**: 99–105.

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# 4 Nutraceutical properties and health benefits of rice

Jinsong Bao

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## 4.1 Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and is the staple food for about half of the world's population. According to the FAO/STAT database, the top ten production countries are China, India, Indonesia, Bangladesh, Viet Nam, Thailand, Myanmar, Philippines, Brazil, and Japan, all in Asia. Their production accounts for over 90% of the world total rice productions ( $\sim 623 \times 10^6$  t).

Rice has two major subspecies, *Oryza sativa* ssp. *indica* and *O. sativa* ssp. *japonica*, which were domesticated in India and China, respectively (Londo *et al.*, 2006). They account for most of the rice cultivars and landraces currently grown. Domestication of wild rice probably started about 11 000 years ago. Recent archaeological research argues that the process of rice domestication culminated  $\sim 6500$  years ago in Tianluoshan, the Lower Yangtze region of Zhejiang, China, and that consumption of domesticated rice increased at that time (Fuller *et al.*, 2008).

In addition to being a staple food, rice has also a medicinal value, which was clearly recognized thousands of years ago. The philosophy that rice is both a food crop and a natural medicine has been well recorded in the ancient Chinese literature. For example, the most important book of Chinese medicine, *Huangdi neijing* (*Yellow Emperor's Classic of Medicine*), indicated that "The five cereals are for nutrition, the five vegetables aid recuperation ...", which is the first description of the health benefit of rice (one of five cereals) for humans. Since then, rice as food appears frequently in other literature, such as *Bencaogangmu* (*Catalogue of Chinese Herbs*, edited by Li Shizhen in the Ming dynasty) that has recorded the nutraceutical properties and health benefits of black rice, *japonica* rice and *indica* rice, respectively. In India, ancient Ayurvedic treatises laud the Raktashali red rice as a nutritive food and medicine and the medicinal value of other types of rice such as Sashtika, Sali, and parched rice have been documented in the Charaka Samhita (c. 700 BC) and the Susruta Samhita (c. 400 BC), in the treatment of various ailments such as diarrhea, vomiting, fever, hemorrhage, chest pain, wounds, and burns (Ahuja *et al.*, 2008).

Rice provides the necessary nutrition for people in underdeveloped countries. Due to the role of rice in the diet, its composition and nutritional characteristics are important

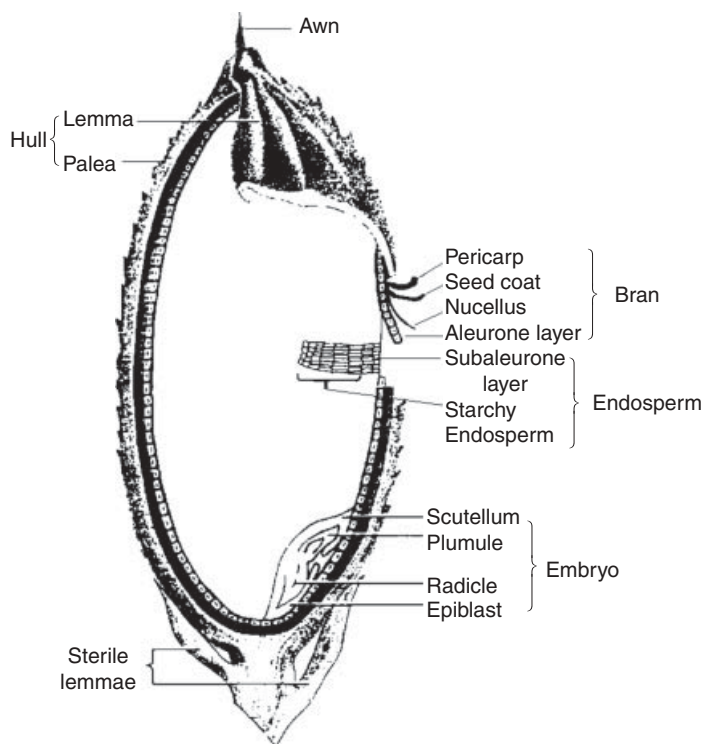
to human health. This chapter reviews the nutritional and nutraceutical composition of rice grain and current understandings of its contribution to human health.

## 4.2 Rice grain structure and nutritional composition distribution

The mature rice grain harvested is called rough rice or paddy rice, which is enveloped by the hull, composed of two modified leaves, lemmae and palea. After removing the hull, the caryopsis (or brown rice) is a single-seeded fruit, wherein the pericarp is fused to the seed, which is composed of seed coat, nucellus, endosperm, and embryo (see Figure 4.1).

When the outer layers of brown rice are removed during milling, the milled or polished or white rice is produced. The byproducts during milling are rice bran and polish, with the former containing more of the pericarp, seed coat, nucellus, aleurone layer, and germ than the latter, which contains relatively more starchy endosperm. Roughly estimated, there is around 6–11% bran and 2.5–3.8% of polish in brown rice (Juliano, 2003).

Some nutrients of the whole rice grain are mainly deposited in rice bran, including tocopherols,  $\gamma$ -oryzanol, other vitamins, minerals (such as iron), and phenolics etc. These rice components are higher in rice bran than brown or milled rice (Table 4.1). In the rice bran, the level of chemical components in outer bran fraction still differed from those in the inner bran fraction (Jang and Xu, 2009).



**Figure 4.1** Longitudinal section of the rice grain.

Modified from Juliano and Bechtel (1985) and [www.fao.org/docrep/t0567e/T0567E07.htm](http://www.fao.org/docrep/t0567e/T0567E07.htm). Used with permission of the Food and Agricultural Organization of the United Nations.

**Table 4.1** Comparison of nutrient composition of brown rice, milled rice, and rice bran

<b>Nutrient<sup>1</sup></b>	<b>Units</b>	<b>Brown rice (long-grain)<sup>2</sup></b>	<b>White rice (long-grain)<sup>2</sup></b>	<b>Rice bran<sup>2</sup></b>
<b>Proximates</b>				
Water	g	10.37	11.62	6.13
Energy	kcal	370	365	316
Energy	kJ	1548	1527	1322
Protein	g	7.94	7.13	13.35
Total lipid (fat)	g	2.92	0.66	20.85
Ash	g	1.53	0.64	9.98
Carbohydrate	g	77.24	79.95	49.69
Total dietary fiber	g	3.5	1.3	21
Total sugars	g	0.85	0.12	0.9
Sucrose	g	0.85	–	0.5
Glucose	g	–	–	0.2
Fructose	g	–	–	0.2
<b>Minerals</b>				
Calcium, Ca	mg	23	28	57
Iron, Fe	mg	1.47	0.8	18.54
Magnesium, Mg	mg	143	25	781
Phosphorus, P	mg	333	115	1677
Potassium, K	mg	223	115	1485
Sodium, Na	mg	7	5	5
Zinc, Zn	mg	2.02	1.09	6.04
Copper, Cu	mg	0.277	0.22	0.728
Manganese, Mn	mg	3.743	1.088	14.21
Selenium, Se	µg	23.4	15.1	15.6
<b>Vitamins</b>				
Thiamin	mg	0.401	0.07	2.753
Riboflavin	mg	0.093	0.049	0.284
Niacin	mg	5.091	1.6	33.995
Pantothenic acid	mg	1.493	1.014	7.39
Vitamin B-6	mg	0.509	0.164	4.07
Total folate	µg	20	8	63
Total choline	mg	30.7	5.8	32.2
Lutein + zeaxanthin	µg	0	0	220
Vitamin E (alpha-tocopherol)	mg	1.2	0.11	4.92
Vitamin K (phyloquinone)	µg	1.9	0.1	1.9
<b>Lipids</b>				
Fatty acids (total saturated)	g	0.584	0.18	4.171
12:00	g	0.003	0	0.019
14:00	g	0.011	0.004	0.078
16:00	g	0.498	0.161	3.557
18:00	g	0.052	0.012	0.373
Fatty acids (total monounsaturated)	g	1.056	0.206	7.549
16:1 undifferentiated	g	0.01	0.002	0.075
18:1 undifferentiated	g	1.046	0.203	7.475
Fatty acids (total polyunsaturated)	g	1.044	0.177	7.459
18:2 undifferentiated	g	1	0.146	7.143
18:3 undifferentiated	g	0.044	0.031	0.316

(continued)



**Table 4.1** (cont'd)

Nutrient <sup>1</sup>	Units	Brown rice (long-grain) <sup>2</sup>	White rice (long-grain) <sup>2</sup>	Rice bran <sup>2</sup>
<b>Amino acids</b>				
Tryptophan	g	0.101	0.083	0.108
Threonine	g	0.291	0.255	0.555
Isoleucine	g	0.336	0.308	0.568
Leucine	g	0.657	0.589	1.022
Lysine	g	0.303	0.258	0.65
Methionine	g	0.179	0.168	0.306
Cystine	g	0.096	0.146	0.317
Phenylalanine	g	0.41	0.381	0.635
Tyrosine	g	0.298	0.238	0.411
Valine	g	0.466	0.435	0.881
Arginine	g	0.602	0.594	1.058
Histidine	g	0.202	0.168	0.355
Alanine	g	0.463	0.413	0.97
Aspartic acid	g	0.743	0.67	1.308
Glutamic acid	g	1.618	1.389	1.854
Glycine	g	0.391	0.325	0.875
Proline	g	0.372	0.335	0.668
Serine	g	0.411	0.375	0.662

Source:

<sup>1</sup>Data adapted from USDA National Nutrient Database for Standard Reference, Release 22 (2009).

<sup>2</sup>Value per 100g.

### 4.3 Nutrient compositions and their health benefits

Humans need to consume relatively large amounts of carbohydrate, protein, and lipid, as well as small amounts of macroelements and trace amounts of microelements (Fe, Zn, Cu, I, and Se) and vitamins, to grow normally and remain healthy during their life (Welch and Graham, 2004). Rice grain contains most of these beneficial components for humans, some are present in large amounts, while others only in small quantities (Table 4.1). Table 4.2 summarizes the health benefits of individual phytochemicals, rice bran, and whole grain in model animals and in human volunteers.

#### 4.3.1 Carbohydrate

Carbohydrates are the major component of the rice grain. Total starch content of brown rice ranged between 72 and 82%, on a dry matter basis (Frei *et al.*, 2003), and around 90% in the milled rice. There are two structural components of starch, amylose and amylopectin. Amylose molecules are essentially linear chains (although they contain occasional branch points) containing  $10^4$ – $10^5$  glucose residues. Amylopectin molecules are much larger (about ten times as many glucose residues) and their structure is more complex than that of amylose. The apparent amylose content, or amylose/amylopectin ratio, serves as one of the most

**Table 4.2** Health benefits of individual phytochemicals, rice bran, and whole grain in model animals and human volunteers

Health benefit		
Nutrients	Animal feeding study (rat/mouse/rabbit/others)	Human study
Dietary fiber (including resistant starch)	Hypoglycemia (dietary fiber, Seki, 2005); Prevent large bowel carcinogenesis (hemicellulose, Aoe <i>et al.</i> , 1993)	Hypoglycemia and decrease insulinaemic responses (resistant starch, Li <i>et al.</i> , 2009; fiber, Silva <i>et al.</i> , 2005); Activate humoral immunity (hemicellulose, Yamagishi <i>et al.</i> , 2008); Attenuate irritable bowel syndrome (hemicellulose, Kanauchi <i>et al.</i> , 2010)
Protein	Hypocholesterolemia (Yang and Kadowaki, 2009); Anti-atherogenic effect (Ni <i>et al.</i> , 2003)	
$\gamma$ -Oryzanol	Anti-inflammatory (Islam <i>et al.</i> , 2008); Antiatherogenic properties (Wilson <i>et al.</i> , 2007)	
Vitamins (Tocotrienol)	Hypercholesterolemia (chicken, Gureshi <i>et al.</i> , 2000; swine, Gureshi <i>et al.</i> , 2001b); Hypolipidemic and antioxidant effect (Minhajuddin <i>et al.</i> , 2005); Anticancer (Iqbal <i>et al.</i> , 2004)	
Phytic acid	Reduced risk of colon cancer (Norazalina <i>et al.</i> , 2010); Hypoglycemia (Eun <i>et al.</i> , 2007; Ohnishi <i>et al.</i> , 2004)	
Anthocyanins	Ameliorate high-fructose-induced insulin resistance (Guo <i>et al.</i> , 2008); Reduce the adverse effect of alcohol (Hou <i>et al.</i> , 2010); Prevent cancer metastasis (Chen <i>et al.</i> , 2006); Inhibit scratching behaviors (Han <i>et al.</i> , 2009). Reduced risks of cardiovascular disease (Xia <i>et al.</i> , 2006)	Cardioprotective effect (anthocyanins, Wang <i>et al.</i> , 2007)
Rice bran	Hypotension, hypolipidemia, and hypoglycemia (Ardiansyah <i>et al.</i> , 2006); Prevent colorectal carcinogenesis (Verschoyle <i>et al.</i> , 2007); Antioxidant and hypocholesterolemia (Revilla <i>et al.</i> , 2009); Stress relief (Jabeen <i>et al.</i> , 2007); Reduced risks of cardiovascular disease (Ling <i>et al.</i> , 2002; Xia <i>et al.</i> , 2003)	Improve the function and redox state of immune cells (Álvarez <i>et al.</i> , 2006); Ameliorate lipid and glycemic anomalies in type 2 diabetic subjects (Cheng <i>et al.</i> , 2010)
Whole grain	Hypoglycemia (Seki <i>et al.</i> , 2005); Reduced risks of hypertension (Ebizuka <i>et al.</i> , 2010) and cardiovascular disease (rabbit, Ling <i>et al.</i> , 2001; rat, Lee <i>et al.</i> , 2007); Hypocholesterolaemia (Roohinejad <i>et al.</i> , 2010)	Hypoglycemia (Ito <i>et al.</i> , 2005; Panlasigui and Thompson, 2006; Hsu <i>et al.</i> , 2008); Reduced risk of hypertension and cardiovascular disease (Hallfrisch <i>et al.</i> , 2003); Reduce risks of obesity (Lee <i>et al.</i> , 2006; Kim <i>et al.</i> , 2008)

important indices for evaluation of the quality of rice products. Waxy rice has <2% amylose, while non-waxy rice has an amylose content ranging from 8 to 39% (Bao *et al.*, 2006).

Most of the starch in the human diet is ingested in cooked foods and is digested rapidly in the small intestine. Glycemic index (GI), which is commonly used to estimate the blood glucose response of a test food to that of a reference food, usually fresh white bread, has been used as a guide for the diets for non-insulin-dependent diabetes mellitus patients. Rice can be classified into high and low GI types, depending on its amylose content and processing methods. In a study by Frei *et al.* (2003), the estimated GI of freshly cooked rice samples ranged between 109 for the waxy rice and 69 for the high amylose rice, whereas those of retrograded starch ranged from 64 to 87. Diets with low GI may improve metabolic profiles in diabetes and are associated with a reduced risk of developing type 2 diabetes and cardiovascular disease (Amano *et al.*, 2004; Murakami *et al.*, 2006; Villegas *et al.*, 2007).

Resistant starch is the sum of starch and products of starch hydrolysis, which is not absorbed in the small intestine of healthy individuals. It reaches the large intestine and serves as a substrate for fermentation by the gut microflora, producing short chain fatty acids, which might promote the optimal function of the viscera. Consumption of foods high in resistant starch is associated with improved gut health through raising short chain fatty acids, while low supply of these acids is associated with increased risk of non-infectious diseases (Rahman *et al.*, 2007; Topping, 2007). The resistant starch content was shown to range from 0 to 1.6% for uncooked raw rice (Eggum *et al.*, 1993a; Frei *et al.*, 2003), and from 0.4 to 2.8% for cooked rice (Eggum *et al.*, 1993a). The high amylose rice, such as *amylose extender* mutant with the apparent amylose content of 39% (Bao *et al.*, 2006), had 1.8 and 1.5% of the resistant starch for uncooked and cooked rice, respectively (Eggum *et al.*, 1993b). Parboiled rice had higher resistant starch content than either the raw or the cooked rice (Eggum *et al.*, 1993ab). In general, amylose content significantly affects the rate and degree of rice starch digestion in the gastrointestinal tract, and some other biologically relevant parameters. For instance, the rats fed with high amylose diets had lower apparent starch digestibility, higher wet and dry fecal weights and faecal nitrogen excretion, reduced fecal pH, lower postprandial blood glucose response and serum triacylglycerol levels and liver weights, and higher serum total cholesterol levels (Denardin *et al.*, 2007). Consumption of a rice meal high in resistant starch decreased the postprandial glycemic and insulinaemic responses and promoted fermentation-related production of H<sub>2</sub> in the large bowel of young and healthy adults (Li *et al.*, 2009).

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 (AACC, 2001). Dietary fiber includes cellulose, hemicellulose, galactooligosaccharides, pectins, resistant starch, lignin, and substances associated with nonstarch polysaccharides and lignin complexes – waxes, phytate, cutin, saponins, suberin, and tannins (AACC, 2001). Dietary fiber constitutes 2.9–4.0% of brown rice, 0.7–2.3% of milled rice, and 17–29% of rice bran, mostly insoluble (Juliano and Bechtel, 1985; Abdul-Hamid and Luan, 2000). The Indian medicinal rice Njavara had the total dietary fiber content of 8.08%, higher than that of other two non-medicinal rice samples (Deepa *et al.*, 2008). The neutral detergent fiber content is around 4.0% in brown rice and 1.1% in milled rice (Miyoshi *et al.*, 1986). The effects of the total dietary fiber, insoluble and soluble fibers separated from rice grain on the fermentative activity of human fecal microflora, were studied. It was found that the acetate was the most abundant short chain fatty acid formed by feeding all rice fibers. Total dietary fiber of different rice varieties

were reported to produce a greater level of short chain fatty acids than either the soluble fiber or insoluble fiber fraction alone (Fernando *et al.*, 2008).

Greater intake of dietary fiber may lead to beneficial physiological effects including laxation, attenuation of blood cholesterol and blood glucose, and prevention or alleviation of maladies such as cardiovascular disease, diabetes, diverticulosis, and colon cancer (AACC, 2001). After the diabetic patients (both type 1 and type 2) received a low-fiber diet enriched with 40 g of fiber (30.6% insoluble and 11.7% soluble components) from rice bran for one week, their mean fasting and postprandial serum glucose levels were reduced, but values of the high fiber diet group were significantly lower than that of the lower fiber diet group (Silva *et al.*, 2005). Seki *et al.* (2005) reported that the post-prandial blood glucose concentration lowering effect of the pre-germinated brown rice was attributable to the major constituent of insoluble fiber. However, Vitaglione *et al.* (2008) argued that the beneficial effects attributed to the cereal dietary fiber are due not only to the polysaccharide moiety, but also to the associated phenolic compounds.

The water-soluble rice bran hemicellulose might play a preventive role in 1,2-dimethylhydrazine-induced large bowel carcinogenesis in rats (Aoe *et al.*, 1993), and its ability to bind bile acids may play a role in lowering cholesterol and phospholipid contents in blood serum (Normand *et al.*, 1987). Yamagishi *et al.* (2008) reported the effects of non-starch polysaccharides on immunological reactions in humans. Human immunological reaction is classified as humoral and cellular immunity. Anticomplementary activity belongs to humoral immunity, which is responsible for over 99% of the human immunological defense system. Rice polysaccharides were identified as a stimulator of both the classical and alternative pathways of complement activation of humoral immunity (Yamagishi *et al.*, 2008). The polysaccharides extracted with hot-water from rice bran were composed of Glu, Man, Gal, Rib, and Ara, and showed good capability of scavenging superoxide and hydroxyl free radicals, and suppressing lipid peroxidation, suggesting their role in protecting human body from free radicals and retarding the progress of many chronic diseases (Zha *et al.*, 2009). The MGN-3/Biobran, a modified form of arabinoxylan from rice bran, is a potent biological response modifier to sensitize human leukemia cells to death receptor [CD95]-induced apoptosis (Ghoneum and Gollapudi, 2003). It is also an effective chemo-sensitizer and may represent a potential novel adjuvant for the treatment of breast cancer (Gollapudi and Ghoneum, 2008).

Irritable bowel syndrome is a common health issue that is characterized by abdominal pain, abnormal bowel movements, altered visceral perception, and abnormal metabolism of 5-hydroxytryptamine secretion. Diets supplemented with insoluble dietary fiber from rice bran (~70% hemicellulose) could significantly ameliorate irritable bowel syndrome, reflecting attenuating urgent fecal excretion, colonic mucosal 5-hydroxytryptamine secretion, and hyperalgesia (Kanauchi *et al.*, 2010).

### 4.3.2 Protein

The brown rice protein content of 17587 accessions in the world ranged from 4.3 to 18.2%, with a mean of 9.5% (Champagne *et al.*, 2004), whereas other studies with fewer samples reported the protein content to be within that range, such as 6.99–10.17% in some Malaysian brown rice (Roohinejad *et al.*, 2009a). The protein content of milled rice ranged from 4.5 to 10.5% (Table 4.1) or from 5.1 to 11.3% (Champagne *et al.*, 1999) depending on different genotype and environmental effects (Champagne *et al.*, 2004). Mean protein content of

milled rice is 7.3% from food composition tables, but lower values are now apparent due to reduced soil fertility (Juliano, 2003). The diets consumed in developed countries usually contain various sources of dietary protein (cereals, legume seeds, meat, etc.), so protein content in rice grain is less important in relation to nutritional requirements. However, this is not the case in some developing countries where a single cereal may account for a major part of the total protein intake. Nutritional quality (i.e. content of essential amino acids) of rice protein, as well as the amount, may be important (Shewry, 2007). Rice protein contains all ten essential amino acids with lysine as the limiting amino acid (Shewry, 2007). The rice bran is rich in glutamic acid, aspartic acid, valine, methionine, serine, lysine, arginine, tyrosine, glutamine, and threonine (Table 4.1).

Based on the protein solubility fraction of Osborne (1907), rice grain has about 10% albumin, 5% globulin, 20% prolamin, and 65% glutelin. Yang and Kadowaki (2009) studied the effects of rice proteins with different contents of glutelin and prolamin from two cultivars on hepatic cholesterol secretion by isolated perfused livers of rats fed with cholesterol-enriched diets. The results indicated that both rice proteins had similar hypocholesterolemic effects. Chen *et al.* (2010) identified a prolamin that could augment anti-leukaemia immune response. The human peripheral blood mononuclear cells-conditioned medium prepared from prolamin treatment showed an increment in production of tumor necrosis factor- $\alpha$ . Human leukaemia U937 cells cultured in the presence of prolamin-prepared medium were inhibited in growth capacity and triggered differentiation toward monocytes (Chen *et al.*, 2010). For patients with chronic renal failure, limiting the ingestion of protein is essential. It is possible for rice mutants low in  $\alpha$ -globulin or glutelin, or both, to reduce the easy-to-digest protein content by approximately 50% (Morita *et al.*, 2009). Ni *et al.* (2003) demonstrated that rice protein and soy protein isolates have anti-atherogenic potentials in rat. Zhang *et al.* (2009) found that Neutrased hydrolysate from rice endosperm protein produced the antioxidative peptides with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity similar to that of  $\alpha$ -tocopherol. The active constituent contained eight amino acids in its sequence (Lys-His-Asn-Arg-Gly-Asp-Glu-Phe), with the molecular mass of 1 kDa. After sequence interpretation and database searching, the MS/MS spectrum was matched to a glutelin protein. Kannan *et al.* (2008) showed that gastrointestinal resistant peptide hydrolysates from direct hydrolysis of heat-stabilized defatted rice bran was found to inhibit growth of human colon (Caco-2) and liver (HepG2) cancer cells.

### 4.3.3 $\gamma$ -aminobutyric acid

As a well known non-protein amino acid,  $\gamma$ -aminobutyric acid is a flavor compound in rice.  $\gamma$ -aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the sympathetic nervous system, and is also known to potentiate the insulin secretion in the pancreas. GABA contents in brown rice seeds ranged between 0.01 and 0.1 mg/g (Roohinejad *et al.*, 2009a). In a study of the influence of brown rice varieties containing different GABA levels on blood cholesterol in rats, no correlation was found between GABA content and serum total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol, or glucose level (Roohinejad *et al.*, 2009b). Pro-germinated brown rice is produced by soaking brown rice in water for slight germination. Amounts of GABA are remarkably increased in the pro-germinated brown rice (Hsu *et al.*, 2008).

### 4.3.4 Lipid

The lipid content of brown rice ranged from 2.76 to 3.84% on a dry weight basis depending on different varieties and was affected by the growing environments (Kitta *et al.*, 2005). The major fatty acids were oleic (18:1) and linoleic (18:2) acids, followed by palmitic acid (16:0). These three fatty acids accounted for more than 90% of the total fatty acid content (Kitta *et al.*, 2005). Black/purple varieties were found to be higher in crude lipid content than the red/brown and colorless varieties (Frei and Becker, 2005). Major portions of the lipids in the rice grain are in the lipid bodies in the embryo and the aleurone layer, and also in the subaleurone layer of the starchy endosperm. The lipid content in rice bran varied relatively strongly, ranging from 17.3 to 27.4% (w/w). The major fat acids in bran oil were oleic, linoleic, and palmitic acids, which were 35.9–49.2, 27.3–41.0, and 13.9–22.1%, respectively (Goffman *et al.*, 2003). Rice bran oil (20–25 wt% in rice bran) is unique among edible oil due to its rich source of commercially and nutritionally important phytochemicals such as oryzanol, lecithin, tocopherols, and tocotrienols. However, most of these phytochemicals are removed from the rice bran oil as waste byproducts during the refining process (Patel and Naik, 2004). Consumption of rice bran oil may protect the liver from oxidative damage caused by lipid peroxidation. Black rice bran oil was shown to be more effective than brown rice bran oil in the improvement of overall cholesterol metabolism and antioxidant status (Yean *et al.*, 2008). Other health benefits of rice bran oil are summarized in Chapter 5. The nutraceutical properties and health benefits of  $\gamma$ -oryzanol and vitamins are described in sections 4.3.5 and 4.3.6, respectively.

### 4.3.5 $\gamma$ -oryzanol

Rice  $\gamma$ -oryzanol is mainly composed of esters of *trans*-ferulic acid (trans-hydroxycinnamic acid) with phytosterols (sterols and triterpenic alcohols) extracted from rice bran oil or brown rice. In brown rice, the  $\gamma$ -oryzanol content ranged from 26 to 63 mg/100 g (Miller and Engel, 2006). The content was significantly higher in *japonica* (24.63 mg/100 g) than in *indica* rice (19.01 mg/100 g) (Heinemann *et al.*, 2008). In rice bran,  $\gamma$ -oryzanol content ranged from 251 to 686.5 mg/100 g across different genotypes and environments (Bergman and Xu, 2003). The level of  $\gamma$ -oryzanol in rice germ was five times lower than in rice bran (Yu *et al.*, 2007). The major phytosterol components of rice  $\gamma$ -oryzanol are cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and  $\beta$ -sitosteryl ferulate (Miller and Engel, 2006). The proportion of individual steryl ferulates exhibited enormous variability (Miller and Engel, 2006; Britz *et al.*, 2007). Steryl ferulates have long been studied for their health-promoting properties, partially owing to their capacity to inhibit oxidation (Nyström *et al.*, 2006). Kong *et al.* (2009) showed that rice cycloartenyl ferulate was most prominent *in vitro* growth inhibition on human colorectal adenocarcinoma SW480, but had low toxicity on normal colon CCD-18-Co cells, suggesting its role in reducing the risk of colon cancer.

The function of rice  $\gamma$ -oryzanol was reviewed by Lerma-Garcia *et al.* (2009), but new functions are still under discovery. Rice  $\gamma$ -oryzanol is a natural antioxidant (Xu and Godber, 2001). A mouse lymphatic endothelial cell (SVEC4–10) *in vitro* model was developed and found to be effective in the study of antioxidant activity of  $\gamma$ -oryzanol in rice bran. Huang *et al.* (2009) showed that, in some situations,  $\gamma$ -oryzanol was a more effective antioxidant

than  $\alpha$ -tocopherol, and the three major components of rice  $\gamma$ -oryzanol (cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, and campesterol ferulate) had higher antioxidant activity than  $\gamma$ -oryzanol, with 24-methylene cycloartanyl ferulate relatively more effective.

A test rice diet enriched with functional components ( $\gamma$ -oryzanol, inositol, and  $\gamma$ -aminobutyric acid (GABA)) from brown extract can possibly adjust the serum cholesterol level, but only in people with an already high total cholesterol level (Murata *et al.*, 2007). Berger *et al.* (2005) revealed that the low density lipoprotein-cholesterol decreased by 12% in two weeks after the change from a peanut oil-based diet containing no  $\gamma$ -oryzanol to a rice bran oil-based diet containing varied  $\gamma$ -oryzanol content, and the ratio of low to high density lipoprotein-cholesterol ratio decreased by 19% in four weeks following the diet change (Berger *et al.*, 2005). It is noted that other bioactive compounds such as unsaturated fatty acids, tocotrienols, and ferulic acid may have contributed to the cholesterol-lowering effects of rice bran oil consumption besides  $\gamma$ -oryzanol (Berger *et al.*, 2005).

Both  $\gamma$ -oryzanol and *trans*-ferulic acid may exert similar antiatherogenic properties, but through different mechanisms (Wilson *et al.*, 2007). At equal dietary levels, oryzanol has a greater effect on lowering plasma non-high-density lipoprotein cholesterol levels and raising plasma high-density lipoprotein cholesterol than ferulic acid, possibly through a greater extent to increase fecal excretion of cholesterol and its metabolites. However, ferulic acid may have a greater antioxidant capacity via its ability to maintain serum vitamin E levels compared to oryzanol (Wilson *et al.*, 2007).

A colitis mice *in vivo* model was used to investigate the anti-inflammatory effect of  $\gamma$ -oryzanol and Islam *et al.* (2008) found that phytosteryl ferulates could be new potential therapeutic and/or preventive agents for gastrointestinal inflammatory diseases. Their anti-inflammatory effect could be mediated by inhibition of NF- $\kappa$ B activity (Islam *et al.*, 2008), which was at least partly due to the antioxidant effect of the ferulic acid moiety in the structure of phytosteryl ferulates.

### 4.3.6 Vitamins

Rice has thiamin (B1), riboflavin (B2), niacin, pantothenic acid, folate, and vitamin E, but usually does not have vitamins A, C, and D. Vitamins are great in number in brown rice than milled rice because of their higher levels in the bran (Table 4.1).

The thiamin content of 79 varieties of rice was in the range of 0.117–1.74 mg/100 g, with an average of 0.457 mg/100 g; riboflavin ranged from 0.011 to 0.403 mg/100 g and averaged 0.087 mg/100 g; and niacin had a range of 1.972–9.218 mg/100 g, an average of 5.322 mg/100 g (Kennedy and Burlingame, 2003). An Indian medicinal rice, Njavara, had higher amounts of thiamin (27–32%), riboflavin (4–25%), and niacin (2–36%) than the other two cultivated varieties (Deepa *et al.*, 2008).

Rice is a poor source of folates (vitamin B9). Overexpressing two *Arabidopsis thaliana* genes of the pterin and para-aminobenzoate branches of the folate biosynthetic pathway, Storozhenko *et al.* (2007) obtained transgenic rice with a maximal folate content (38.3 nmol/g) enhancement as high as 100 times above wild type (0.42 nmol/g), with 100 g of polished raw grains containing up to four times the adult daily folate requirement.

None of the polished cultivars was identified as containing  $\beta$ -carotene, when unpolished, a majority of the rice accessions showed both lutein and  $\beta$ -carotene peaks, but some had only one peak (Tan *et al.*, 2005). Black upland rice grain samples from the Philippines had

$\beta$ -carotene values up to 0.13 mg/kg, which was significantly higher than in red/brown or colorless varieties (Frei and Becker, 2004). Among a set of 54 rice landrace grain samples, the  $\beta$ -Carotene values were significantly higher in the black/purple category as well, with values ranging from 0 to 0.22 mg/kg. In red/brown and colorless varieties, the  $\beta$ -carotene level was generally low, ranging from 0 to 0.01 mg/kg and from 0 to 0.02 mg/kg, respectively (Frei and Becker, 2005). Carotenoids is a precursor of vitamin A. Vitamin A deficiency is common in developing countries, including highly populated areas of Asia, Africa, and Latin America, causing severe visual impairment, and this correlates with fatal infections and death from diarrhoea, respiratory diseases, and measles. Ye *et al.* (2000) introduced the entire  $\beta$ -carotene biosynthetic pathway in rice endosperm. The transgenic rice, Golden Rice 1, can accumulate a maximal level of 1.6  $\mu$ g/g total carotene in the endosperm. The second generation of golden rice, Golden Rice 2, contains as much as 37  $\mu$ g total carotenoids per gram of dry weight of grain, of which 31  $\mu$ g/g is  $\beta$ -carotene (Paine *et al.*, 2005). Both vitamin A and  $\beta$ -carotene can improve nonheme iron absorption from rice by humans (García-Casal *et al.*, 1998).

Vitamin E is the generic term used to describe a family of eight lipid-soluble antioxidants with two types of structures, the tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol) and tocotrienols ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienol). Brown rice seeds contained about 100 nmol/g of dry matter of total tocols, except for rice *Italica Livorna* (216 nmol/g) that had about double this amount. The major individual tocol was  $\gamma$ -tocotrienols, except again for rice *Italica Livorna*, which had very high levels of  $\alpha$ -tocopherol (Britz *et al.*, 2007). The amount of vitamin E compounds in rice bran ranged from 179 to 389 mg/kg, the amounts of  $\alpha$ -tocotrienols,  $\gamma$ -tocotrienols,  $\alpha$ -tocopherol and  $\gamma$ -tocopherol were 165, 130, 94, 59 mg/kg, respectively, and the tocopherols and tocotrienols levels were 27.5 and 72.5% of the total vitamin E content (Bergman and Xu, 2003). Tocotrienol is the predominant vitamin E in rice with an average content of 830 mg/kg among 109 kinds of rice bran samples (Sookwong *et al.*, 2007). The level of vitamin E in rice germ was five times greater than in rice bran (Yu *et al.*, 2007).

Two new tocotrienols, i.e. desmethyl tocotrienol and didesmethyl tocotrienol, have been isolated from stabilized and heated rice bran (Qureshi *et al.*, 2000). They had much greater *in vitro* antioxidant activities and greater suppression of B16 melanoma cell proliferation than  $\alpha$ -tocopherol and known tocotrienols. These tocotrienols significantly lowered serum total and low density lipoprotein cholesterol levels and inhibited 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in chickens (Qureshi *et al.*, 2000) and in swine expressing hereditary hypercholesterolemia (Qureshi *et al.*, 2001a). They also resulted in a significant reduction in the atherosclerotic lesion size the C57BL/6 apolipoprotein (apo)E-deficient mice, suggesting that dietary tocotrienol supplements may provide a unique approach to promoting cardiovascular health (Qureshi *et al.*, 2001b).

The most important functions of vitamin E in the body are antioxidant activity and maintenance of membrane integrity. Vitamin E has also been shown to play a role in immune function, in DNA repair and other metabolic processes (Traber, 1999). It has also been demonstrated that vitamin E has a central role in maintaining neurological structure and function (Sen *et al.*, 2006). Tocotrienols may possess powerful antioxidant, anticancer, and cholesterol-lowering properties. Sen *et al.* (2006) have observed that  $\alpha$ -tocotrienol is multi-fold more potent than  $\alpha$ -tocopherol in protecting HT4 and primary neuronal cells against the toxicity induced by glutamate, as well as by a number of other toxins. At nanomolar concentration, tocotrienol, but not tocopherol, completely protected neurons by an antioxidant-independent mechanism. The current findings point towards tocotrienol as a potent neuroprotective form of natural vitamin E (Sen *et al.*, 2006).



Tocotrienol rich fraction isolated from rice bran oil displayed a dose-dependent hypolipidemic and antioxidant effect in an induced hyperlipidemic rats model (Minhajuddin *et al.*, 2005). Feeding with an atherogenic diet (5% hydrogenated fat, 0.5% cholic acid, and 1% cholesterol) for three weeks resulted in a significant increase in plasma triacylglycerol (3.3-fold) and total cholesterol (2.4-fold) levels. After the induction of hyperlipidemia for three weeks, rats were supplemented with different doses of a Tocotrienol rich diet for one week, which decreased the lipid parameters in a dose-dependent manner. These results suggest that rice diet supplementation rich in tocotrienol may have significant health benefits through the modulation of physiological functions that include various atherogenic lipid profiles and antioxidants in hypercholesterolemia.

A crude lipophilic rice bran extract, Ricetrienol®, which contains  $\alpha$ -tocopherol, tocotrienol and phytosterol, exerted a protective effect against oxidative damage in diabetes mellitus in obese diabetic KKAY mice (Kanaya *et al.*, 2004). The anticancer efficacy of tocotrienol-rich fraction isolated from rice bran oil was evaluated during diethylnitrosamine (DEN)/2-acetylaminofluorene (AAF)-induced hepatocarcinogenesis in rats (Iqbal *et al.*, 2004). The administration of a tocotrienol-rich diet to DEN/AAF-treated rats substantially decreased hepatic activity of glutathione S-transferase, lipid peroxidation, and low-density lipoprotein oxidation and, thus, limited the action of DEN/AAF. It might be expected that long-term intake of a tocotrienol-rich diet could reduce cancer risk by preventing hepatic lipid peroxidation and protein oxidation damage due to its antioxidant actions.

$\gamma$ -tocotrienol could be a potential, new chemopreventive agent for human gastric cancer. Sun *et al.* (2009) revealed that  $\gamma$ -tocotrienol inhibited human gastric cancer SGC-7901 cell growth with inhibitory effects in relation to the DNA damage and arresting cell cycle at G<sub>0</sub>/G<sub>1</sub> phase.  $\gamma$ -tocotrienol also induced activation of caspase-3 and increased the cleavage of the downstream substrate poly(ADP-ribose) polymerase, which might induce the apoptosis of SGC-7901 cells.

### 4.3.7 Minerals

Rice grain contains a number of minerals with the contents varying with different analyses (Heinemann *et al.*, 2005; also see Table 4.1). In the milled rice, the mean contents of K, Ca, Na, and Mg were 804.83, 119.50, 20.78, and 194.79  $\mu\text{g/g}$ , respectively, which were macronutrients for the human body. The elements of Fe, Zn, Cu, and Mn were micronutrients for the human body with mean values of 5.40, 25.97, 9.96, and 10.73  $\mu\text{g/g}$  in milled rice, respectively (Jiang *et al.*, 2009). In the milled rice, the average Se content in different samples in China was  $0.025 \pm 0.011 \mu\text{g}$  of  $\text{Se g}^{-1}$  (Chen *et al.*, 2002), or was  $0.020 \text{ mg kg}^{-1}$  with a range of  $0.003\text{--}0.049 \text{ mg kg}^{-1}$  (Fang *et al.*, 2008). Thus, rice is regarded as seriously low-selenium food. The mineral contents in rice are too low to meet the micronutrient demands for humans feeding on the rice as staple.

### 4.3.8 Phytate

Rice contains phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate), the most important anti-nutritional factor impeding availability of divalent minerals. It forms complexes with mineral ions, such as Fe, Zn, and Ca, which cannot be digested by humans. Phytate may affect mineral bioavailability and, thus, food nutritional value, which may lead to public

health problems in human populations. Rice contributed 50% to the total estimated daily zinc intake and also contributed 68% to the daily intake of phytate in the nonpregnant women of reproductive age in developing countries (Chandyo *et al.*, 2009). The brown rice had the mean phytic acid content of 9.6 g/kg, ranging from 7.2 to 11 g/kg (Liang *et al.*, 2007). Several low phytic acid rice mutant lines with the aim of increasing the bioavailability of the minerals of rice (Liu *et al.*, 2007) have been isolated. One brown rice derived low phytic acid mutant had a content of phytate P about 58.4% that of the parent line, while total P levels were not significantly different between the mutant and its parent (Ren *et al.*, 2007). Expression of a thermotolerant phytase gene from *Aspergillus fumigatus* in rice endosperm is expected to decrease the phytic acid and increase iron bioavailability (Lucca *et al.*, 2001).

Apart from negative effects, consumption of phytate provides protection against a variety of cancers mediated through antioxidative properties. It has therapeutic use against diabetes mellitus, atherosclerosis, and coronary heart disease and reduces kidney stone formation, HIV-1, and heavy metal toxicity (Kumar *et al.*, 2010; Schlemmer *et al.*, 2009).

Phytate has the potential to treat pancreatic cancer, which is one of the malignancies most resistant to therapy. Pancreatic adenocarcinoma is characterized by its poor prognosis. A resistance to apoptosis contributes to its insensitivity to conventional therapies. Somasundar *et al.* (2005) reported that rice or corn-derived phytate significantly reduced the proliferation of human pancreatic adenocarcinoma cells, ranging from 37.1 to 91.5%. Phytic acid administration also reduced the risk of colon cancer in rats (Norazalina *et al.*, 2010). Rats with colon carcinogenesis induced by azoxymethane were administered with phytic acid extract from rice bran and the formation of aberrant crypt foci were greatly reduced. In addition, phytic acid significantly suppressed the number of aberrant crypt foci in the distal, middle, and proximal colon compared to those treated with azoxymethane alone (Norazalina *et al.*, 2010).

### 4.3.9 Phenolics

Phenolics are compounds containing a benzene ring with one or more hydroxyl groups, and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Liu, 2007). Brown rice had a total phenolic content of 5.56  $\mu\text{mol}$  of gallic acid equiv/g of the grains tested, lower than that of other cereals such as corn, wheat, and oats (Adom and Liu, 2002). The major portion of phenolics in rice grain existed in the bound form, accounting for 62% of the total. Other studies also showed that brown rice had a large variation in their total free phenolic content, ranging from 0.69 to 2.74 mg gallic acid equivalents (GAE)/g grain (Goffman and Bergman, 2004), and from 108.1 to 1244.9 mg GAE/100 g (Shen *et al.*, 2009) depending on the color of grain; black rice usually has higher phenolic content than the red and brown rice (Goffman and Bergman, 2004; Shen *et al.*, 2009). The rice bran had the total free phenolic content ranging from 3.1 to 45.4 mg GAE/g bran, much higher than that in the brown rice (Goffman and Bergman, 2004).

The most common phenolic compounds found in whole rice grains are phenolic acids and flavonoids, which are mainly present in the bound form, linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds. The major phenolic acids in rice grain were identified as *p*-coumaric acid, ferulic acid, and sinapinic acid (Zhou *et al.*, 2004; Yawadio *et al.*, 2007; Zhu *et al.*, 2010). The *p*-coumaric, ferulic, and sinapinic acids accounted for 22.5, 67.6, and 9.9% of the total phenolic acids in red rice, 29.4, 60.4,

and 10.2% in brown rice, and 10.7, 79.7, and 9.6% in black rice, respectively (Zhu *et al.*, 2010). Ferulic acid was primary phenolic acid in the bran, whereas vanillic and *p*-coumaric acids were mostly found in the husk (Butsat and Siriamornpun, 2010). Chung and Shin (2007) identified four phenolic acids, ethyl-3,4-dihydroxybenzoic, 4-hydroxy-3-methoxyphenylacetic, 3,4-dihydroxybenzoic, and 4-hydroxy-3-methoxycinnamic acids, which showed a strong antioxidant activity and can serve as a good source of dietary bio-functional phytochemicals.

Rice bran is a good source of ferulic acids, which are esterified to hemicellulose of the cell walls. The total ferulic acid content in brown rice was 153.39  $\mu\text{mol}$  of ferulic acid/100 g of grain, with the free, soluble-conjugated, and bound ferulic acids in the ratio 0.1:1:100 (Adom and Liu, 2002). It is abundant in the aleurone, pericarp, and embryo cell walls of various grains, but occurs only in trace amounts in the starchy endosperm. It exhibits a wide range of therapeutic effects against various diseases such as cancer, diabetes, cardiovascular, and neurodegenerative. A wide spectrum of beneficial activity for human health has been advocated for this phenolic compound, at least in part, because of its strong antioxidant activity (Srinivasan *et al.*, 2007). Eun *et al.* (2007) evaluated the hypoglycemic effects of the ethyl acetate-soluble phenolic acid fraction of rice bran and of ferulic acid in the type 2 diabetic mice model. The results suggested that phenolic acid fraction and ferulic acid might be beneficial for treatment of type 2 diabetes because they regulated blood glucose levels by elevating glucokinase activity and production of glycogen in the liver. The study by Ohnishi *et al.* (2004) also showed that the dietary ferulic acid may be useful in alleviating oxidative stress and attenuating the hyperglycemic response associated with diabetes in both the insulin-dependent and non-insulin dependent diabetes mellitus rat models.

Brown rice had 0.33  $\mu\text{mol/g}$  free and 0.60  $\mu\text{mol/g}$  bound flavonoids in the grain (Adom and Liu, 2002). Shen *et al.* (2009) reported that the free flavonoid of brown rice averaged 131.6 mg rutin equivalent/100 g, lower than in red rice (147.2 mg rutin equivalent/100 g) and black rice (240 mg rutin equivalent/100 g). It should be noted that some red rice accessions might be lower in the flavonoid contents than brown rice. Flavonoids are lacking in the endosperm of rice. Expression of maize C1 and R-S regulatory genes driven by an endosperm specific promoter of a rice prolamin gene in rice grain resulted in dark brown pericarp of the C1/R-S homozygous lines, and the major flavonoids, dihydroquercetin (taxifolin), dihydroisorhamnetin (3'-O-methyltaxifolin), and 3'-O-methyl quercetin were identified in the rice grain (Shin *et al.*, 2006). These rice lines have the potential to be developed further as a novel variety that can produce various flavonoids in its endosperm.

The kernel of black rice is characterized by the presence of anthocyanins. Anthocyanins are natural colorants belonging to the flavonoid family. The major anthocyanins components isolated from black rice are peonidin 3-glucoside and cyanidin 3-glucoside (Hu *et al.*, 2003; Abdel-Aal *et al.*, 2006; Zhu *et al.*, 2010). Black rice had the highest total anthocyanin content (3276  $\mu\text{g/g}$ ) among all colored grains studied, which was about 35 times higher than that of the red rice (94  $\mu\text{g/g}$ ). The kernel of red rice is characterized by the presence of proanthocyanidins, which are also called condensed tannins consisting of polymerized flavanol units. The red-grained genotypes contained no detectable anthocyanins and one black rice contained no detectable proanthocyanidins. However, another black-grained rice (Artemide) had large amounts of both proanthocyanidins and anthocyanins, which was also characterized by the highest total anthocyanin content and polyphenol content: its total anthocyanins content was about twice that of the other pigmented rices, and it had a polyphenol content two to three times that found in other pigmented rices (Finocchiaro *et al.*, 2010).

All the phenolic compounds of rice grain have antioxidant activities, and may contribute to protection against degenerative diseases (i.e. heart disease and cancer) in which reactive oxygen species are involved. Brown rice had the total antioxidant activity of 55.77  $\mu\text{mol}$  of vitamin C equiv/g of grain, lower than that of corn, wheat, and oats. Bound phytochemicals contributed 71% to the total antioxidant activity in brown rice (Adom and Liu, 2002). Shen *et al.* (2009) reported that the total antioxidant capacity, measured using the ABTS assay, varied to a great extent, averaging 0.413 mM trolox equivalents/100g, ranging from 0.012 to 5.533 mM trolox equivalents/100g among the total rice accessions. Goffman and Bergman (2004) reported that rice bran had antiradical activity ranging from 10.0 to 345.3  $\mu\text{M}$  trolox equivalents/g.

Black rice is a popular specialty food that has been regarded as being highly beneficial to human health due to its high phenolics and other phytochemicals together with its potent antioxidant capacities. Hu *et al.* (2003) showed that anthocyanins extracted from black rice exhibited strong antioxidant activities and free radical scavenging capacities in a battery of *in vitro* model systems, and significant prevention of supercoiled DNA strand scission induced by reactive oxygen species, suppression of the oxidative modification of human low-density lipoprotein, and also showed anti-inflammatory properties for potential use in nutraceuticals or functional food. Han *et al.* (2004) showed that black colored rice extracts had a dose-dependent suppressive activity on thrombin time inhibition, which might be attributed in part to their unique phytochemical composition, such as high level of anthocyanin pigments. The cyanidin-3-glucoside from black rice and its metabolites exhibited an anti-allergic effect, showing inhibitory effects against histamine- or compound 48/80-induced scratching behaviors in mice (Han *et al.*, 2009). Black rice and its pigment fraction showed anti-atherogenic activities in mice and rabbit models (Xia *et al.*, 2003; Ling *et al.*, 2002). The exact component contributing to the beneficial effect might be the anthocyanins. Chronic diet intake of anthocyanin-rich extract from black rice may enhance atherogenic plaque stabilization in old apoE-deficient mice. The underlying mechanism is related mainly to inhibiting proinflammatory factors and improving the serum lipid profile (Xia *et al.*, 2006). Supplementation of black rice pigment fraction to patients with coronary heart disease could exert cardioprotective effects on patients by improving plasma antioxidant status and inhibiting inflammatory factors (Wang *et al.*, 2007).

The anthocyanins, including cyanidin-3-glucoside, peonidin-3-glucoside, and phtochemicals (quercetin, ferulic acid, and tocopherol), showed significant inhibitory activity against aldose reductase, suggesting that the pigmented rice varieties might contribute significantly to combating diabetic complications as health-promoting food ingredients (Yawadio *et al.*, 2007). Yao *et al.* (2010) showed that black rice had the highest total anthocyanin contents, highest antioxidant activity, and  $\alpha$ -glucosidase inhibitory activity. The inhibition of intestinal  $\alpha$ -glucosidase could delay the digestion and absorption of carbohydrates and, thus, suppress the postprandial hyperglycemia. Black rice pigment extract rich in anthocyanin prevented and ameliorated high-fructose-induced insulin resistance in rats. Cyanidin 3-glucoside showed a protective role against  $\text{H}_2\text{O}_2$ - or tumor necrosis factor  $\alpha$ -induced insulin resistance in 3T3-L1 adipocytes by inhibiting the c-Jun  $\text{NH}_2$ -terminal kinase signal pathway (Guo *et al.*, 2008).

The anthocyanin-rich extract from black rice also showed a benefit in reducing the adverse effect of alcohol (Hou *et al.*, 2010). The protective effect on chronic ethanol-induced biochemical changes in male Wistar rats was evaluated in rats administrated with

ethanol to induce liver damage. The administration of the anthocyanin-rich extract decreased the activities of liver enzymes (aspartate transaminase, alanine transaminase, gamma glutamyl transferase) in serum, the hepatic malondialdehyde levels and the concentrations of serum, and hepatic triglyceride and total cholesterol. Rats treated with anthocyanin-rich extract showed a better profile of the antioxidant system.

Tumor metastasis is the most important cause of cancer death and various treatment strategies have targeted prevention of the occurrence of metastasis. Chen *et al.* (2006) reported that the anti-metastatic effects of black rice anthocyanins (i.e. peonidin 3-glucoside and cyanidin 3-glucoside) through an inhibition on the invasion and motility of SKHep-1 cells from human hepatocellular carcinoma. Rice anthocyanins also exerted an inhibitory effect on the DNA binding activity and the nuclear translocation of AP-1, and also exerted an inhibitory effect of cell invasion on various human cancer cells (SCC-4, Huh-7, and HeLa). Male immunodeficient nude mice, a nude mice xenograft model, were used to test the anti-tumor effect of anthocyanins. It was found that small solid tumors were observed after ten days following SKHep-1 cell inoculation, and anthocyanins feeding resulted in a 1.9-fold reduction of tumor volume on day 35 and a 1.7-fold reduction in tumor weight on day 40 (Chen *et al.*, 2006).

Recently, the effect of consumption of the red color strain of Thai brown rice, a high source of phenolic compounds, on the oxidative stress prevention was studied in the rat model. Results indicated that the rats consuming Thai brown rice possessed low level of oxidative stress and serum malondialdehyde through both radical and non radical defenses, suggesting its role in the prevention of oxidative stress (Suwannalert *et al.*, 2010).

Serotonin derivatives such as p-coumaroylserotonin and feruloylserotonin, a family of plant polyphenol compounds, have been implicated in an array of biological activities including antioxidative activity, but neither their production nor identification has been reported in crop plants. Transgenic rice expressing the pepper hydroxycinnamoyl-CoA:serotonin N-(hydroxycinnamoyl) transferase gene produced on average 274 ng/g, which was nine-fold higher than the wild-type (30 ng/g grain weight) (Kang *et al.*, 2005). Chemical treatments such as trans-cinnamic acid and tyramine increased the serotonin derivatives contents by two- to three-fold in both wild-type and transgenic rice. The transgenic rice had higher radical scavenging activities than that of wild-type, suggesting that nutraceutical serotonin derivative could be enriched by transgenic engineering (Kang *et al.*, 2005).

Lignans are biphenolic compounds found ubiquitously in foods of plant origin including the bran layer of whole rice grain; they are converted by friendly flora in our intestines into mammalian lignans, including one called enterolactone that has a role in protection against breast and other hormone-dependent cancers, as well as heart disease. Serum enterolactone concentrations can be raised by eating a diet rich in whole grains including brown rice. Overweight, hyperinsulinaemic, non-diabetic men and women who ate wholegrain foods had higher serum enterolactone concentrations than those who ate the refined-grain diet (Jacobs *et al.*, 2002).

Coenzyme Q (CoQ), also called ubiquinone, is an electron transfer molecule in the respiratory chain. CoQ is a lipid-soluble antioxidant. Most cereal crops produce mainly CoQ9, which has nine isoprene units, whereas humans produce mainly CoQ10, with 10 isoprene units. CoQ10 is a very popular food supplement. Takahashi *et al.* (2009) produced CoQ10-enriched rice plants by introduction of the gene for decaprenyl diphosphate synthase. In CoQ10-enriched rice plants, seed CoQ10 content in brown rice was 7.7–11 µg/g, which was

up to ten times greater than that of wild-type rice (0.4–1.5 µg/g), although this level is still insufficient for practical use. Combination of the transgene with giant embryo mutant lines produced giant embryo CoQ10-enriched rice with seed CoQ10 content per weight increased to up to 1.4–1.8 times. It was found that CoQ was preferentially accumulated in bran and germ of rice seed.

#### 4.3.10 Alkaloid

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Chung and Shin (2007) isolated and identified 4-carboethoxy-6-hydroxy-2-quinolone from the aleurone layer of one black rice that showed potent antioxidant activity for the first time. Kang *et al.* (2010) also isolated and characterized a new alkaloid, oryzadine, from the same tissue of black rice. Oryzadine showed radical scavenging effects and protected H<sub>2</sub>O<sub>2</sub>-induced cell damage. Oryzadine might be considered a significant natural source of antioxidant.

### 4.4 Biofortification of nutrients in rice grain to improve its health benefits

Agronomic management may increase the nutrients dense in the grain, which is especially successful for increasing the mineral contents. For example, foliar application of zinc, selenium, and iron fertilizers could increase their content by 36.7%, 194.1%, and 37.1%, respectively, under the optimal application condition (Fang *et al.*, 2008). Breeding for improved nutritional quality would be beneficial if it could be accomplished without any yield loss. Wide genotypic diversity for most nutrient compositions in rice grain exists in rice germplasm, which could be exploited by breeders to enhance the nutritional value of the new breeding lines. For example, pigmented rice varieties with high antioxidative activities provide a source of antioxidants and a genetic resource for developing new health-promoting rice cultivars (Nam *et al.*, 2006). The possibility to select the genotypes accumulating both anthocyanins and proanthocyanidins provides a way to substantially increase the polyphenol content and total anthocyanin content of the rice caryopsis (Finocchiaro *et al.*, 2010). Mutation breeding has been commonly used to select the mutants high in nutritional properties among the variants generated with the treatment of a mutagen. Mutants high in nutrients such as lysine and tryptophan (Kim *et al.*, 2004) or low in antinutrients such as phytic acid (Liu *et al.*, 2007) have been isolated. Rapid prediction method, such as near-infrared spectroscopy (Zhang *et al.*, 2008), could replace the wet chemistry methods for testing nutritional quality, in order to save time and cost. Molecular breeding, selection on DNA markers (so-called marker assisted selection), and transgenic engineering have comparative advantage over the conventional breeding, and have been widely used in plant breeding programs provided that prior knowledge on the molecular markers tagged for the nutritional quality such as phenolic content (Jin *et al.*, 2009; Shao *et al.*, 2011) and gene resources for metabolic pathway (Storozhenko *et al.*, 2007; Ye *et al.*, 2000) for target traits were known. Biofortification of resistant starch (Li *et al.*, 2009), Va (Ye *et al.*, 2000), folate (Storozhenko *et al.*, 2007), flavonoids (Shin *et al.*, 2006), serotonin (Kang *et al.*, 2005), and CoQ (Takahashi *et al.*, 2009) in rice grains have been accomplished by transgenic engineering.

## 4.5 Health benefits of rice bran

The beneficial properties of rice bran, which make it a health-promoting functional food product, have widely been recognized (Kahlon and Smith, 2004; Cicero and Derosa, 2005; see also Table 4.2). The total dietary fiber content of rice bran ranges from 20 to 27%, with less than 2% as soluble dietary fiber (Kahlon and Smith, 2004). Consumption of these rice bran containing foods could slow down glucose uptake and has displayed significant health benefits (Patel, 2007). Rice bran protein is a complete protein, hypoallergenic and easily digestible, and it has a high nutritional value with a protein efficiency ratio of 1.6. It is energy dense, containing 22–29% crude fat, including 1.8% gum and 0.4% wax (Kahlon and Smith, 2004). However, rice bran needs stabilization to prevent rancidity and protect its nutritional value (Patel, 2007).

Since most of the phytochemicals are deposited in the bran layer, compared to brown rice powder, rice bran contained most of the antioxidants and had correspondingly higher values of antioxidant capacity. Nutraceutical properties and health benefits of rice bran reflect a mixed role of individual phytochemicals, and may also be derived from the synergetic effects.

Type 2 diabetes human patients with a dietary supplement of stabilized rice bran had lower postprandial glucose, glycated hemoglobin, serum total cholesterol, and low density lipoprotein cholesterol concentrations than the control group, and the adiponectin concentration was significantly higher in the rice bran group than the control group (Cheng *et al.*, 2010).

The stroke-prone spontaneously hypertensive rats were fed with the animal diet containing a rice bran fraction obtained by treating the bran with Driselase (a commercial plant cell wall-degrading enzyme mixture containing cellulase, xylanase, and laminarinase) or an ethanol extract rice bran fraction (containing, respectively, 64.74 and 139.60 Gallic Acid Equivalents mg/g) (Ardiansyah *et al.*, 2006). After eight weeks, the blood pressure decreased in the bran feeding groups in comparison with the control group, and the plasma angiotensin-1-converting enzyme inhibitory activity, blood urea nitrogen, albumin, triglyceride, glucose, plasma nitric oxide, and urinary 8-hydroxy-2'-deoxyguanosine levels were lower in the bran treatment groups than in the control. The authors concluded that rice bran fractions appeared to have a beneficial dietary component that ameliorates hypertension, hyperlipidemia, and hyperglycemia, and that the improvement was due to the fatty acid and to the total phenolic compound present in both fractions (Ardiansyah *et al.*, 2006).

Verschoyle *et al.* (2007) tested the cancer chemopreventive efficacy of rice bran in genetic mouse models of breast, prostate, and intestinal carcinogenesis. The results indicated that while rice bran possesses cancer chemopreventive efficacy in the mouse model of colorectal carcinogenesis, it lacked anticarcinogenic activity in the other mouse models of mammary and prostate cancers. Ethanol-water extracts from rice bran samples removed from seeds of two black/purple pigmented brown rice cultivars showed high antioxidative, anti-tumor promoting, and anticarcinogenic activities assayed in mammalian cells (human leukemia HL-60, marmoset B lymphoblastoid B95–8, and Chinese hamster V79 lung cells) (Nam *et al.*, 2005). Water-soluble rice bran enzymatic extract with main components of protein, fat, and carbohydrate showed anti-proliferative effect on the leukemia tumor cell growth *in vitro*, suggesting that it could potentially be used as a functional food for the treatment and prevention of chronic pathological states associated with abnormal proliferation of cells, as is the case with cancer (Parrado *et al.*, 2006).

Revilla *et al.* (2009) showed that where water-soluble enzymatic extract from rice bran had antioxidant and hypocholesterolemic activities evaluated in a rat model, a reduction in total cholesterol levels and an increase in high density lipoprotein-cholesterol were found. Most *et al.* (2005) compared the effects of defatted rice bran and rice bran oil in an average American diet on blood lipids in moderately hypercholesterolemic persons, and found that the defatted rice bran did not lower lipid concentrations, but the total cholesterol was significantly lower with consumption of the diet containing rice bran oil than with consumption of the control diet. Moreover, with consumption of the rice bran oil diet, low density lipoprotein-cholesterol decreased, whereas the high density lipoprotein-cholesterol was unchanged. Thus, the author argued that it is rice bran oil, not fiber, that lowers cholesterol in healthy, moderately hypercholesterolemic adults.

Rice bran dietary supplementation showed that an improvement of the function and redox state of immune cells in unhealthy or aged subjects comes from their properties as powerful antioxidant compounds (Álvarez *et al.*, 2006).

Jabeen *et al.* (2007) showed potential use of a stabilized-rice bran rich diet in stress relief in rats, which might be due to the antioxidant and serotonergic effect of the bran. It was found that the body weights decreased and exploratory activity in an open field increased in rice bran rich diet treated rats. Learning and spatial memory monitored in the Morris water test was enhanced. An episode of 2h restraint stress decreased food intake of rice bran, which was also the case for normal diet treated animals. Deficits were smaller in rice bran diet than normal diet treated animals. Exposure to 2h restraint stress increased brain serotonin (5-hydroxytryptamine) metabolism. The increases were smaller in SRB rich than normal diet animal groups.

## 4.6 Health benefits of whole rice grain consumption

Many reports have shown that the health benefits including reduced risk of chronic diseases such as cardiovascular disease (Liu *et al.*, 1999; Anderson *et al.*, 2000; Jensen *et al.*, 2004; Pereira *et al.*, 2002), type 2 diabetes (Pereira *et al.*, 2002), some cancers (Jacobs *et al.*, 1998), and all-cause mortality are associated with the consumption of whole grain, including rice (Liu 2007; see also Table 4.2). With only the inedible hull removed, brown rice is a whole grain. Brown rice whole grains have more fiber, antioxidants such as vitamin E, and trace minerals compared to milled rice, which are responsible for its health benefits.

Substituting brown rice for milled rice in diets for healthy humans resulted in an increase of fecal weight and a decrease of digestibility of energy, protein, and fat, which is assumed to be effective in preventing colonic disease (Miyoshi *et al.*, 1986). Brown rice is reported to be a more health beneficial food for diabetics and hyperglycemic individuals than milled rice (Panlasigui and Thompson, 2006). The intake of pre-germinated brown rice instead of white rice also ameliorates the hyperglycemia (Seki *et al.*, 2005; Ito *et al.*, 2005; Hsu *et al.*, 2008), suggesting consumption of pro-germinated brown rice is a better choice for patients with type 2 diabetes. The blood glucose-lowering and hypocholesterolemic effects of a pre-germinated brown rice diet may be caused by its higher content of dietary fiber,  $\gamma$ -oryzanol, and  $\gamma$ -aminobutyric acid in the pro-germinated brown rice compared to white rice (Seki *et al.*, 2005; Ito *et al.*, 2005; Hsu *et al.*, 2008).



Non-hypertensive men with elevated plasma cholesterol levels had reduced systolic, diastolic, and mean arterial pressures after consuming diets with brown rice/whole wheat, barley, or a combination for five weeks, regardless whether the fiber was predominantly soluble (barley) or insoluble (brown rice and whole wheat) (Hallfrisch *et al.*, 2003). The result suggested that increasing whole grain foods in a healthy diet may reduce cardiovascular risk. Ebizuka *et al.* (2010) indicated that pre-germinated brown rice had an antihypertensive effect and decreased serum cholesterol in spontaneously hypertensive rats. Roothinejad *et al.* (2010) also indicated that intake of pre-germinated brown rice and brown rice had the cardio-protective effect in hypercholesterolaemic rats. The protective effects of whole grain cereals against heart diseases and certain cancers may be due, at least partly, to the antioxidant effects of phenolics concentrated in the bran.

Consumption of fiber-rich rice was found to significantly lower body weight in both non-obese and obese subjects, which was associated with lower serum triacylglycerol, total cholesterol, low-density lipoprotein cholesterol, etc. in the obese subjects, suggesting that fiber-rich rice might have beneficial effects and may be therapeutically useful for obese subjects (Lee *et al.*, 2006). Meal replacement with black and brown rice was superior to replacement with white rice in weight control for overweight women (Kim *et al.*, 2008). Consumption of white rice or mixed rice (black rice and brown rice) showed a significant reduction in weight, body mass index, and body fat during the experimental period, with the mix rice group exhibiting levels of all three parameters significantly lower than those of the white rice group. The levels of total cholesterol and triacylglycerols decreased gradually. High-density lipoprotein cholesterol was significantly elevated in the mixture of brown rice and black rice but not in the white rice group.

Consumption of whole grains is associated with a lowered risk of coronary heart disease (Jacobs and Gallaher, 2004). The effects of white, red, and black rice consumption on atherosclerotic plaque formation induced by hypercholesterolemia were investigated in rabbits (Ling *et al.*, 2001). The area of atherosclerotic plaque was 50% lower in rabbits fed with the red or black rice diets than in those fed with the white rice diet. Red or black rice consumption reduced or retarded the progression of atherosclerotic plaque development induced by dietary cholesterol. The enhanced serum high-density lipoprotein cholesterol and apolipoprotein A-I concentrations, and the increased antioxidant and decreased oxidative status may be mechanisms of the antiatherogenic effect of red or black rice. Similarly, Lee *et al.* (2007) investigated the effect of diets made of germinated giant embryonic rice, giant embryonic rice, or conventional brown rice on the lipid metabolism and antioxidant system in rats fed with a high-cholesterol diet. The results also showed that consumption of germinated giant embryonic rice is effective in lowering atherosclerosis cardiovascular disease risk.

The nutraceutical functions of brown rice are derived from the sum of the entire grain, which are greater than any of its individual components. The mechanisms behind are thought to be brought about by a synergistic effect of several nutrients and non nutrients. For example, the antihypertensive effect and decreases serum cholesterol of the pre-germinated brown rice may be due to complex actions of various components abundantly found in the rice, including  $\gamma$ -aminobutyric acid, dietary fiber, phytic acid, and ferulic acid (Ebizuka *et al.*, 2010). Pigmented rice (black or red) has higher antioxidant activities than brown rice due to having more phenolics and flavonoids in the colored bran layers, it is no surprise that consumption of whole pigmented grain has higher nutraceutical benefit than of the non-pigmented brown whole rice grain.

## 4.7 Future trends

Rice grain contains a range of bioactive substances and there is growing interest in the potential health benefits that these substances may provide. Further research is required to identify other health substances within rice and the factors altering their bioavailability. The exact mechanisms by which cereals convey beneficial effects on health are not fully understood. Synergistic and stimulus effects among the phytochemicals contributing to health are important for the overall benefits and potential toxic effects and need further study. The biological interactions of these dietary components within the human body are still unclear. In addition, means to promote whole rice consumption are needed.

To obtain high nutritional components whole rice grain, mutation breeding and transgenic breeding are two of the most efficient approaches. Others could also be considered to further enrich the components. Smaller whole grain should be further developed by integration with other high-nutrient traits for improving the nutraceutical properties and health benefits. In addition, organic rice production techniques need to be developed to produce green rice without arsenic contamination so as to secure the safety of the food for the public.

## References

- AACC (American Association for Clinical Chemistry). (2001) The definition of dietary fiber. *Cereal Foods World*, **46**: 112.
- Abdel-Aal E.S.M., Young, J.C., and Rabalski, I. (2006) Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, **54**: 4696–4704.
- Abdul-Hamid, A. and Luan, Y.S. (2000) Functional properties of dietary fibre prepared from defatted rice bran. *Food Chemistry*, **68**: 15–19.
- Adom, K.K. and Liu, R.H. (2002) Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, **50**: 6182–6187
- Ahuja, U., Ahuja, S.C., Thakrar, R., and Singh, R.K. (2008) Rice – A nutraceutical. *Asian Agri-History*, **12**: 93–108.
- Álvarez, P., Alvarado, C., Mathieu, F., Jiménez, L., and De La Fuente, M. (2006) Diet supplementation for 5 weeks with polyphenol-rich cereals improves several functions and the redox state of mouse leucocytes. *European Journal of Nutrition*, **45**: 428–438.
- Amano, Y., Kawakubo, K., Lee, J.S., Tang, A.C., Suqiyama, M., and Mori, K. (2004) Correlation between dietary glycemic index and cardiovascular disease risk factors among Japanese women. *European Journal of Clinical Nutrition*, **58**: 1472–1478.
- Anderson, J.W., Hanna, T.J., Peng, X., and Kryscio, R.J. (2000) Whole grain foods and heart disease risk. *Journal of the American College of Nutrition*, **19**: 291S–2999S.
- Aoe, S., Oda, T., Tojima, T., Tanaka, M., Tatsumi, K., and Mizutani, T. (1993) Effects of rice bran hemicellulose on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in Fischer 344 rats. *Nutrition and Cancer*, **20**: 41–49.
- Ardiansyah, Shirakawa, H., Koseki, T., Ohinata, K., Hashizume, K., and Komai, M. (2006) Rice bran fractions improve blood pressure, lipid profile, and glucose metabolism in stroke-prone spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry*, **54**: 1914–1920.
- Bao, J.S., Shen, S.Q., Sun, M., and Corke, H. (2006) Analysis of genotypic diversity in the starch physicochemical properties of nonwaxy rice, apparent amylose content, pasting viscosity and gel texture. *Starch/Stärke*, **58**: 259–267.
- Berger, A., Rein, D., Schafer, A., Monnard, I., Gremaud, G., Lambelet, P., and Bertoli, C. (2005) Similar cholesterol-lowering properties of rice bran oil, with varied  $\gamma$ -oryzanol, in mildly hypercholesterolemic men. *European Journal of Nutrition*, **44**: 163–173.

- Bergman, C.J. and Xu, Z. (2003) Genotype and environment effects on tocopherol, tocotrienol, and  $\gamma$ -Oryzanol contents of southern U.S. rice. *Cereal Chemistry*, **80**: 446–449.
- Britz, S.J., Prasad, P.V.V., Moreau, R.A., Allen Jr. L.H., Kremer, D.F., and Boote, K.J. (2007) Influence of growth temperature on the amounts of tocopherols, tocotrienols, and  $\gamma$ -oryzanol in brown rice. *Journal of Agricultural and Food Chemistry*, **55**: 7559–7565.
- Butsat, S. and Siriamornpun, S. (2010) Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chemistry*, **119**: 606–613.
- Champagne, E.T., Bett-Garber, K.L., Vinyard, B.T., McClung, A.M., Barton, II. F.E., Moldenhauer, K., Linscombe, S., and McKenzie, K. (1999) Correlation between cooked rice texture and rapid visco analyser measurements. *Cereal Chemistry*, **76**: 764–771.
- Champagne, E.T., Bett-Garber, K.L., McClung, A.M., and Bergman, C. (2004) Sensory characteristics of diverse rice cultivars as influenced by genetic and environmental factors. *Cereal Chemistry*, **81**: 237–243.
- Chandyo, R.K., Strand, T.A., Mathisen, M., Ulak, M., Adhikari, R.K., Bolann, B.J., and Sommerfelt, H. (2009) Zinc deficiency is common among healthy women of reproductive age in Bhaktapur, Nepal. *Journal of Nutrition*, **139**: 594–597.
- Chen, Y.J., Chen, Y.Y., Wu, C.T., Yu, C.C., and Liao, H.F. (2010) Prolamin, a rice protein, augments anti-leukaemia immune response. *Journal of Cereal Science*, **51**: 189–197.
- Chen, L., Yang, F., Xu, J., Hu, Y., Hu, Q., Zhang, Y., and Pan, G. (2002) Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry*, **50**: 5128–5130.
- Chen, P.N., Kuo, W.H., Chiang, C.L., Chiou, H.L., Hsieh, Y.S., and Chu, S.C. (2006) Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. *Chemico-Biological Interactions*, **163**: 218–229.
- Cheng, H.H., Huang, H.Y., Chen, Y.Y., Huang, C.L., Chang, C.J., Chen, H.L., and Lai, M.H. (2010) Ameliorative effects of stabilized rice bran on type 2 diabetes patients. *Annals of Nutrition and Metabolism*, **56**: 45–51.
- Chung, H.S., and Shin, J.C. (2007) Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. Heugjinjubyeo) *Food Chemistry*, **104**: 1670–1677.
- Cicero, A.F.G., and Derosa, G. (2005) Rice bran and its main components, Potential role in the management of coronary risk factors. *Current Topics in Nutraceutical Research*, **3**: 29–46.
- Deepa, G., Singh, V., and Naidu, K.A. (2008) Nutrient composition and physicochemical properties of Indian medicinal rice – Njavara. *Food Chemistry*, **106**: 165–171.
- Denardin, C.C., Walter, M., da Silva, L.P., Souto, G.D., and Fagundes, C.A.A. (2007) Effect of amylose content of rice varieties on glycemic metabolism and biological responses in rats. *Food Chemistry*, **105**: 1474–1479.
- Ebizuka, H., Ihara, M., and Arita, M. (2010) Antihypertensive effect of pre-germinated brown rice in spontaneously hypertensive rats. *Food Science and Technology Research*, **15**: 625–630.
- Eggum, B.O., Juliano, B.O., Perez, C.M., and Acedo, E.F. (1993a) The resistant starch, undigestible energy and undigestible protein contents of raw and cooked milled rice. *Journal of Cereal Science*, **18**: 159–170.
- Eggum, B.O., Juliano, B.O., Perez, C.M., and Acedo, E.F. (1993b) Starch, energy and protein utilization by rats in milled rice of IR36-based amylose extender mutant. *Cereal Chemistry*, **70**: 275–279.
- Eun, H.J., Sung, R.K., In, K.H., and Tae, Y.H. (2007) Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. *Journal of Agricultural and Food Chemistry*, **55**: 9800–9804.
- Fang, Y., Wang, L., Xin, Z., Zhao, L., An, X., and Hu, Q. (2008) Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. *Journal of Agricultural and Food Chemistry*, **56**: 2079–2084.
- Fernando, W., Ranaweera, K., Bamunuarachchi, A., and Brennan, C. (2008) The influence of rice fibre fractions on the in vitro fermentation production of short chain fatty acids using human faecal micro flora. *International Journal of Food Science & Technology*, **43**: 2237–2244.
- Finocchiaro, F., Ferrari, B., and Gianinetti, A. (2010) A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype. *Journal of Cereal Science*, **51**: 28–34.
- Frei, M. and Becker, K. (2004) Agro-biodiversity in subsistence-oriented farming systems in a Philippine upland region, nutritional considerations. *Biodiversity Conservation*, **13**: 1591–1610.

- Frei, M., and Becker, K. (2005) Fatty acids and all-trans- $\beta$ -carotene are correlated in differently colored rice landraces. *Journal of the Science of Food and Agriculture*, **85**: 2380–2384.
- Frei, M., Siddhuraju, P., and Becker, K. (2003) Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chemistry*, **83**: 395–402.
- Fuller, D.Q., Qin, L., Zheng, Y.F., Zhao, Z.J., Chen, X.G., Hosoya, L. A., and Sun, G.P. (2008) The domestication process and domestication rate in rice, spikelet bases from the lower Yangtze. *Science*, **323**: 1607–1610.
- García-Casal, M.N., Layrisse, M., Solano, L., Barón, M.A., Arguello, F., Llovera, D., Ramírez, J., Leets, I., and Tropper, E. (1998) Vitamin A and  $\beta$ -carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *Journal of Nutrition*, **128**: 646–650.
- Ghoneum, M. and Gollapudi, S. (2003) MGN-3 sensitizes human T cell leukemia cells to death receptor (CD95)-induced apoptosis. *Cancer Letter*, **201**: 41–49.
- Goffman, F.D. and Bergman, C.J. (2004) Rice kernel phenolic content and its relationship with antiradical efficiency. *Journal of the Science of Food and Agriculture*, **84**: 1235–1240.
- Goffman, F.D., Pinson, S., and Bergman, C. (2003) Genetic diversity for lipid content and fatty acid profile in rice bran. *Journal of the American Oil Chemists' Society*, **80**: 485–490.
- Gollapudi, S. and Ghoneum, M. (2008) MGN-3/biobran, modified arabinoxylan from rice bran, sensitizes human breast cancer cells to chemotherapeutic agent, daunorubicin. *Cancer Detection and Prevention*, **32**: 1–6.
- Guo, H., Ling, W., Wang, Q., Liu, C., Hu, Y., and Xia, M. (2008) Cyanidin 3-glucoside protects 3T3-L1 adipocytes against H<sub>2</sub>O<sub>2</sub>- or TNF- $\alpha$ -induced insulin resistance by inhibiting c-Jun NH<sub>2</sub>-terminal kinase activation. *Biochemical Pharmacology*, **75**: 1393–1401.
- Hallfrisch, J., Scholfield, D.J., and Behall, K.M. (2003) Blood pressure reduced by whole grain diet containing barley or whole wheat and brown rice in moderately hypercholesterolemic men. *Nutrition Research*, **23**: 1631–1642.
- Han, S.J., Ryu, S.N., Trinh, H.T., Joh, E.H., Jang, S.Y., Han, M.J., and Kim, D.H. (2009) Metabolism of cyanidin-3-o- $\beta$ -d-glucoside isolated from black colored rice and its antiscratching behavioral effect in mice. *Journal of Food Science*, **74**: H253–H258.
- Han, S.J., Kim, J.S., Chae, S., Kang, S.S., Ryu, S.N., Hyun, J.W., Son, K.H., Sohn, H.Y., and Chang, H.W. (2004) Biological screening of extracts from the colored rice cultivars. *Korean Journal of Pharmacognosy*, **35**: 346–349.
- Heinemann, R.J.B., Fagundes, P.L., Pinto, E.A., Penteado, M.V.C., and Lanfer-Marquez, U.M. (2005) Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. *Journal of Food Composition and Analysis*, **18**: 287–296.
- Heinemann, R.J.B., Xu, Z., Godber, J.S., and Lanfer-Marquez, U.M. (2008) Tocopherols, tocotrienols, and  $\gamma$ -oryzanol contents in Japonica and Indica subspecies of rice (*Oryza sativa* L.) cultivated in Brazil. *Cereal Chemistry*, **85**: 243–247.
- Hou, Z.H., Qin, P.Y., and Ren, G.X. (2010) Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. Japonica) on chronically alcohol-induced liver damage in Rats. *Journal of Agricultural and Food Chemistry*, **58**: 3191–3196.
- Hsu, T.F., Kise, M., Wang, M.F., Ito, Y., Yang, M.D., Aoto, H., Yoshihara, R., Yokoyama, J., Kunii, D., and Yamamoto, S. (2008) Effects of Pre-Germinated Brown Rice on Blood Glucose and Lipid Levels in Free-Living Patients with Impaired Fasting Glucose or Type 2 Diabetes. *Journal of Nutritional Science and Vitaminology*, **54**: 163–168.
- Hu, C., Zawistowski, J., Ling, W., and Kitts, D.D. (2003) Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *Journal of Agricultural and Food Chemistry*, **51**: 5271–5277.
- Huang, C.C.J., Xu, Z., and Godber, J.S. (2009) Potential antioxidant activity of  $\gamma$ -oryzanol in rice bran as determined using an in vitro mouse lymph axillary endothelial cell model. *Cereal Chemistry*, **86**: 679–684.
- Iqbal, J., Minhajuddin, M., and Beg, Z.H. (2004) Suppression of diethylnitrosamine and 2-acetylaminofluorene-induced hepatocarcinogenesis in rats by tocotrienol-rich fraction isolated from rice bran oil. *European Journal of Cancer Prevention*, **13**: 515–520.
- Islam, M.S., Murata, T., Fujisawa, M., Nagasaka, R., Ushio, H., Bari, A. M., Hori, M., and Ozaki, H. (2008) Anti-inflammatory effects of phytosteryl ferulates in colitis induced by dextran sulphate sodium in mice. *British Journal of Pharmacology*, **154**: 812–824.

- Ito, Y., Mizukuchi, A., Kise, M., Aoto, H., Yamamoto, S., Yoshihara, R., and Yokoyama, J. (2005) Postprandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. *Journal of Medical Investigation*, **52**: 159–164.
- Jabeen, B., Badaruddin, M., Ali, R., and Haleem, D. (2007) Attenuation of restraint-induced behavioral deficits and serotonergic responses by stabilized rice bran in rats. *Nutritional Neuroscience*, **10**: 11–16.
- Jacobs, Jr. D.R. and Gallaher, D.D. (2004) Whole grain intake and cardiovascular disease, A review. *Current Atherosclerosis Reports*, **6**: 415–423.
- Jacobs, Jr. D.R., Marquart, L., Slavin, J., and Kushi, L.H. (1998) Whole-grain intake and cancer, An expanded review and meta-analysis. *Nutrition and Cancer*, **30**: 85–96.
- Jacobs, Jr. D.R., Pereira, M.A., Stumpf, K., Pins, J.J., and Adlercreutz, H. (2002) Whole grain food intake elevates serum enterolactone. *The British Journal of Nutrition*, **88**: 111–116.
- Jang, S. and Xu, Z. (2009) Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. *Journal of Agricultural and Food Chemistry*, **57**: 858–862.
- Jensen, M.K., Koh-Banerjee, P., Hu, F.B., Franz, M., Sampson, L., Gronbaek, M., and Rimm, E.B. (2004) Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *The American Journal of Clinical Nutrition*, **80**: 1492–1499.
- Jiang, S., Shi, C., and Wu, J. (2009) Studies on mineral nutrition and safety of wild rice (*Oryza L.*). *International Journal of Food Sciences and Nutrition*, **60** (Suppl. 1): 139–147.
- Jin, L., Xiao, P., Lu, Y., Shao, Y. F., Shen, Y., and Bao, J.S. (2009) Quantitative trait loci for brown rice color, total phenolics and, flavonoid contents and antioxidant capacity in rice grain. *Cereal Chemistry*, **86**: 609–615.
- Juliano, B.O. (2003) *Rice Chemistry and Quality*. Island Publishing House, Inc. pp. 25–54.
- Juliano, B.O. and Bechtel, D.B. (1985) The rice grain and its gross composition. In B.O. Juliano (ed.) *Rice, Chemistry and Technology*, 2nd ed (pp. 17–57), AACCC, St Paul, MN, USA.
- Kahlon, T.S. and Smith, G.E. (2004) Rice bran, A health-promoting ingredient. *Cereal Foods World*, **49**: 188–194.
- Kanauchi, O., Mitsuyama, K., Komiyama, Y., Yagi, M., Andoh, A., and Sata, M. (2010) Preventive effects of enzyme-treated rice fiber in a restraint stress-induced irritable bowel syndrome model. *International Journal of Molecular Medicine*, **25**: 547–555.
- Kanaya, Y., Doi, T., Sasaki, H., Fujita, A., Matsuno, S., Okamoto, K., Nakano, Y., Tsujiwaki, S., Furuta, H., Nishi, M., Tsuno, T., Taniguchi, H., and Nanjo, K. (2004) Rice bran extract prevents the elevation of plasma peroxylipid in KKAY diabetic mice. *Diabetes Research and Clinical Practice*, **66** (Suppl.): S157–S160.
- Kang, K., Jang, S.-M., Kang, S., and Back, K. (2005) Enhanced neutraceutical serotonin derivatives of rice seed by hydroxycinnamoyl-CoA,serotonin N-(hydroxycinnamoyl) transferase. *Plant Science*, **168**: 783–788.
- Kang, K.A., Lee, J.H., Zhang, R., Piao, M.J., Chung, H.S., and Hyun, J.W. (2010) Oryzadine, a new alkaloid of *Oryza sativa* cv. Heugjinjubyeo, attenuates oxidative stress-induced cell damage via a radical scavenging effect. *Food Chemistry*, **119**: 1135–1142.
- Kannan, A., Hettiarachchy, N., Johnson, M.G., and Nannapaneni, R. (2008) Human colon and liver cancer cell proliferation inhibition by peptide hydrolysates derived from heat-stabilized defatted rice bran. *Journal of Agricultural and Food Chemistry*, **56**: 11643–11647.
- Kennedy, G. and Burlingame, B. (2003) Analysis of food composition data on rice from a plant genetic resources perspective. *Food Chemistry*, **80**: 589–596.
- Kim, D.S., Lee, I.S., Jang, C.S., Lee, S.J., Song, H.S., Lee, Y.I., and Seo, Y.W. (2004) AEC resistant rice mutants induced by gamma-ray irradiation may include both elevated lysine production and increased activity of stress related enzymes. *Plant Science*, **167**: 305–316.
- Kim, J.Y., Kim, J.H., Lee, D.H., Kim, S.H., and Lee, S.S. (2008) Meal replacement with mixed rice is more effective than white rice in weight control, while improving antioxidant enzyme activity in obese women. *Nutrition Research*, **28**: 66–71.
- Kitta, K., Ebihara, M., Iizuka, T., Yoshikawa, R., Isshiki, K., and Kawamoto, S. (2005) Variations in lipid content and fatty acid composition of major non-glutinous rice cultivars in Japan. *Journal of Food Composition and Analysis*, **18**: 269–278.
- Kong, C.K.L., Lam, W.S., Chiu, L.C.M., Ooi, V.E.C., Sun, S.S.M., and Wong, Y.S. (2009) A rice bran polyphenol, cycloartenyl ferulate, elicits apoptosis in human colorectal adenocarcinoma SW480 and sensitizes metastatic SW620 cells to TRAIL-induced apoptosis. *Biochemical Pharmacology*, **77**: 1487–1496.

- Kumar, V., Sinha, A.K., Makkar, H.P.S., and Becker, K. (2010) Dietary roles of phytate and phytase in human nutrition, A review. *Food Chemistry*, **120**: 945–959.
- Lee, K.W., Song, K.E., Lee, H.S., Kim, Y.K., Lee, S.W., Kim, D.J., Hwang, W.S., Choe, S.J., Kim, Y.S., and Kim, T.Y. (2006) The effects of goami No. 2 rice, a natural fiber-rich rice, on body weight and lipid metabolism. *Obesity*, **14**: 423–430.
- Lee, Y.R., Kim, C.E., Kang, M.Y., and Nam, S.H. (2007) Cholesterol-lowering and antioxidant status-improving efficacy of germinated giant embryonic rice (*Oryza sativa* L.) in high cholesterol-fed rats. *Annals of Nutrition and Metabolism*, **51**: 519–526.
- Lerma-Garcia, M.J., Herrero-Martinez, J.M., Simo-Alfonso, E.F., Mendonca, C.R.B., and Ramis-Ramos, G. (2009) Composition, industrial processing and applications of rice bran  $\gamma$ -oryzanol. *Food Chemistry*, **115**: 389–404.
- Li, M., Piao, J.H., Tian, Y., Li, W.D., Li, K.J., and Yang, X.G. (2009) Postprandial glycaemic and insulinaemic responses to GM-resistant starch-enriched rice and the production of fermentation-related H<sub>2</sub> in healthy Chinese adults. *British Journal of Nutrition*, **103**: 1–6.
- Liang, J., Hang, B.Z., Hang, L., Nout, M.J.R., and Hamer, R.J. (2007) Iron, zinc and phytic acid content of selected rice varieties from China. *Journal of the Science of Food and Agriculture*, **87**: 504–510.
- Ling, W.H., Cheng, Q.X., Ma, J., and Wang, T. (2001) Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *Journal of Nutrition*, **131**: 1421–1426.
- Ling, W.H., Wang, L.L., and Ma, J. (2002) Supplementation of the black rice outer layer fraction to rabbits decreases atherosclerotic plaque formation and increases antioxidant status. *Journal of Nutrition*, **132**: 20–26.
- Liu, R.H. (2007) Whole grain phytochemicals and health. *Journal of Cereal Science*, **46**: 207–219.
- Liu, S., Stampfer, M.J., Hu, F.B., Giovannucci, E., Rimm, E., Manson, J.E., Hennekens, C.H., and Willett, W.C. (1999) Whole grain consumption and risk of coronary heart disease, results from the Nurse's Health Study. *The American Journal of Clinical Nutrition*, **70**: 412–419.
- Liu, Q.L., Xu, X.H., Ren, X.L., Fu, H.W., Wu, D.X., and Shu, Q.Y. (2007) Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **114**: 803–814.
- Londo, J.P., Chiang, Y.C., Huang, K.H., Chiang, T.Y., and Schaal, B.A. (2006) Phylogeography of Asian wild rice, *Oryza rufipogon* reveals multiple independent domestications of cultivated rice, *Oryza sativa*. *Proceedings of the National Academy of Sciences, USA*, **103**: 9578–9583.
- Lucca, P., Hurrell, R., and Potrykus, I. (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theoretical and Applied Genetics*, **102**: 392–397.
- Miller, A., and Engel, K.H. (2006) Content of  $\gamma$ -oryzanol and composition of steryl ferulates in brown rice (*Oryza sativa* L.) of European origin. *Journal of Agricultural and Food Chemistry*, **54**: 8127–8133.
- Minhajuddin, M., Beg, Z.H., and Iqbal, J. (2005) Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food and Chemical Toxicology*, **43**: 747–753.
- Miyoshi, H., Okuda, T., Oi, Y., and Koishi, H. (1986) Effects of rice fiber on fecal weight, apparent digestibility of energy, nitrogen and fat, and degradation of neutral detergent fiber in young men. *Journal of Nutritional Science and Vitaminology*, **32**: 581–589.
- Morita, R., Kusaba, M., Iida, S., Nishio, T., and Nishimura, M. (2009) Development of PCR markers to detect the *glb1* and *Lgc1* mutations for the production of low easy-to-digest protein rice varieties. *Theoretical and Applied Genetics*, **119**: 125–130.
- Most, M.M., Tulley, R., Morales, S. and Lefevre, M. (2005) Rice bran oil, not fiber, lowers cholesterol in humans. *American Journal of Clinical Nutrition*, **81**: 64–68.
- Murakami, K., Sasaki, S., Takahashi, Y., Okubo, H., Hosoi, Y., Horiuchi, H., Oquma, E., and Kayama, F. (2006) Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. *American Journal of Clinical Nutrition*, **83**: 1161–1169.
- Murata, K., Kamei, T., Toriumi, Y., Kobayashi, Y., Iwata, K., Fukumoto, I., and Yoshioka, M. (2007) Effect of processed rice with brown rice extracts on serum cholesterol level. *Clinical and Experimental Pharmacology and Physiology*, **34**: S87–S89.
- Nam, S.H., Choi, S.P., Kang, M.Y., Koh, H.J., Kozukue, N., and Friedman, M. (2006) Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. *Food Chemistry*, **94**: 613–620.
- Nam, S.H., Choi, S.P., Kang, M.Y., Kozukue, N., and Friedman, M. (2005) Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. *Journal of Agricultural and Food Chemistry*, **53**: 816–822.

- Ni, W., Tsuda, Y., Takashima, S., Sato, H., Sato, M., and Imaizumi, K. (2003) Anti-atherogenic effect of soya and rice-protein isolate, compared with casein, in apolipoprotein E-deficient mice. *British Journal of Nutrition*, **90**: 13–20.
- Norazalina, S., Norhaizan, M.E., Hairuszah, I., and Norashareena, M.S. (2010) Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. *Experimental and Toxicologic Pathology*, **62**: 259–268.
- Normand, F.L., Ory, R.L., and Mod, R.R. (1987) Binding of bile acids and trace minerals by soluble hemicellulose of rice. *Food Technology*, **41**: 86–99.
- Nyström, L., Achrenius, T., Lampi, A.-M., Moreau, R.A., and Piironen, V. (2006) A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures. *Food Chemistry*, **101**: 947–954.
- Ohnishi, M., Matuo, T., Tsuno, T., Hosoda, A., Nomura, E., Taniguchi, H., Sasaki, H., and Morishita, H. (2004) Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. *BioFactors*, **21**: 315–319.
- Osborne, T.B. (1907) The protein of the wheat kernel. Carnegie Inst, Washington, DC, USA Publ 84.
- Paine, J.A., Shipton, C.A., Chaggar, S., Howells, R.M., Kennedy, M.J., Vernon, G., Wright, S., Hincliffe, E., Adams, J.L., Silverstone, A.L., and Drake, R. (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology*, **23**: 482–487.
- Panlasigui, L.N. and Thompson, L.U. (2006) Blood glucose lowering effects of brown rice in normal and diabetic subjects. *International Journal of Food Sciences and Nutrition*, **57**: 151–158.
- Parrado, J., Miramontes, E., Jover, M., Gutierrez, J.F., Collantes de Terán, L., and Bautista, J. (2006) Preparation of a rice bran enzymatic extract with potential use as functional food. *Food Chemistry*, **98**: 742–748.
- Patel, M. and Naik, S.N. (2004) Gamma-oryzanol from rice bran oil - A review. *Journal of Scientific and Industrial Research*, **63**: 569–578.
- Patel, R.M. (2007) Stabilized Rice Bran – The functional food for the 21st century! *Agro Food Industry Hi-Tech*, **18**: 39–41.
- Pereira, M.A., Jacobs, Jr. D.R., Pins, J.J., Raatz, S.K., Gross, M.D., Slavin, J.L., and Seaquist, E.R. (2002) Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *American Journal of Clinical Nutrition*, **75**: 848–855.
- Qureshi, A.A., Mo, H., Packer, L., and Peterson, D.M. (2000) Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. *Journal of Agricultural and Food Chemistry*, **48**: 3130–3140.
- Qureshi, A.A., Peterson, D.M., Hasler-Rapacz, J.O., and Rapacz, J. (2001a) Novel tocotrienols of rice bran suppress cholesterologenesis in hereditary hypercholesterolemic swine. *Journal of Nutrition*, **131**: 223–230.
- Qureshi, A.A., Salser, W.A., Parmar, R., and Emeson, E.E. (2001b) Novel tocotrienols of rice bran inhibit atherosclerotic lesions in C57BL/6 apoE-deficient mice. *Journal of Nutrition*, **131**: 2606–2618.
- Rahman, S., Bird, A., Regina, A., Li, Z., Ral, R.P., McMaugh, S., Topping, D., and Morell, M. (2007) Resistant starch in cereals: Exploiting genetic engineering and genetic variation. *Journal of Cereal Science*, **46**: 251–260.
- Ren, X.L., Liu, Q.L., Fu, H.W., Wu, D.X., and Shu, Q.Y. (2007) Density alteration of nutrient elements in rice grains of a low phytate mutant. *Food Chemistry*, **102**: 1400–1406.
- Revilla, E., Maria, C.S., Miramontes, E., Bautista, J., García-Martínez, A., Cremades, O., Cert, R., and Parrado, J. (2009) Nutraceutical composition, antioxidant activity and hypocholesterolemic effect of a water-soluble enzymatic extract from rice bran. *Food Research International*, **42**: 387–393.
- Roohinejad, S., Mirhosseini, H., Saari, N., Mustafa, S., Alias, I., Meor Hussin, A.S., Hamid, A., and Manap, M.Y. (2009a) Evaluation of GABA, crude protein and amino acid composition from different varieties of Malaysian's brown rice. *Australian Journal of Crop Science*, **3**: 184–190.
- Roohinejad, S., Omidzadeh, A., Mirhosseini, H., Rasti, B., Saari, N., Mustafa, S., Yusof, R.M., Hussin, A.S.M., Hamid, A., and Manap, M.Y.A. (2009b) Effect of hypocholesterolemic properties of brown rice varieties containing different gamma aminobutyric acid (GABA) levels on Sprague–Dawley male rats. *Journal of Food, Agriculture and Environment*, **7**: 197–203.
- Roohinejad, S., Omidzadeh, A., Mirhosseini, H., Saari, N., Mustafa, S., Yusof, R.M., Hussin, A.S.M., Hamid, A., and Manap, M.Y.A. (2010) Effect of pre-germination time of brown rice on serum cholesterol levels of hypercholesterolaemic rats. *Journal of the Science of Food and Agriculture*, **90**: 245–251.

- Schlemmer, U., Frolich, W., Prieto, R.M., and Grases, F. (2009) Phytate in foods and significance for humans, Food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition and Food Research*, **53**: S330–S375.
- Seki, T., Nagase, R., Torimitsu, M., Yanagi, M., Ito, Y., Kise, M., Mizukuchi, A., Fujimura, N., Hayamizu, K., and Ariga, T. (2005) Insoluble fiber is a major constituent responsible for lowering the post-prandial blood glucose concentration in the pre-germinated brown rice. *Biological and Pharmaceutical Bulletin*, **28**: 1539–1541.
- Sen, C.K., Khanna, S., and Roy, S. (2006) Tocotrienols, Vitamin E beyond tocopherols. *Life Sciences*, **78**: 2088–2098.
- Shao, Y.F., Jin, L., Zhang, G., Lu, Y., Shen, Y., and Bao, J.S. (2011) Association mapping of brown rice color, phenolic content, flavonoid content and antioxidant capacity in rice grain. *Theoretical and Applied Genetics*, **122**: 1005–1016.
- Shen, Y., Jin, L., Xiao, P., Lu, Y., and Bao, J.S. (2009) Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science*, **49**: 106–111.
- Shewry, P.R. (2007) Improving the protein content and composition of cereal grain. *Journal of Cereal Science*, **46**: 239–250.
- Shin, Y.M., Park, H.J., Yim, S.D., Baek, N.I., Lee, C.H., An, G., and Woo, Y.M. (2006) Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. *Plant Biotechnology Journal*, **4**: 305–315.
- Silva, C.R., De Oliveira, J.E.D., De Souza, R.A.H.G., and Silva, H.C. (2005) Effect of a rice bran fiber diet on serum glucose levels of diabetic patients in Brazil. *Archivos Latinoamericanos de Nutricion*, **55**: 23–27.
- Somasundar, P., Riggs, D.R., Jackson, B.J., Cunningham, C., Vona-Davis, L., and McFadden, D.W. (2005) Inositol hexaphosphate (IP6), A novel treatment for pancreatic cancer. *Journal of Surgical Research*, **126**: 199–203.
- Sookwong, P., Makagawa, K., Murata, K., Kojima, Y., and Miyazawa, T. (2007) Quantitation of tocotrienol and tocopherol in various rice brans. *Journal of Agricultural and Food Chemistry*, **55**: 461–466.
- Srinivasan, M., Sudheer, A.R., and Menon, V.P. (2007) Ferulic acid, Therapeutic potential through its antioxidant property. *Journal of Clinical Biochemistry and Nutrition*, **40**: 92–100.
- Storozhenko, S., De Brouwer, V., Volckaert, M., Navarrete, O., Blancquaert, D., Zhang, G.F., Lambert, W., and Van Der Straeten, D. (2007) Folate fortification of rice by metabolic engineering. *Nature Biotechnology*, **25**: 1277–1279.
- Sun, W., Xu, W., Liu, H., Liu, J., Wang, Q., Zhou, J., Dong, F., and Chen, B. (2009)  $\gamma$ -Tocotrienol induces mitochondria-mediated apoptosis in human gastric adenocarcinoma SGC-7901 cells. *Journal of Nutritional Biochemistry*, **20**: 276–284.
- Suwannalert, P., Rattanachitthawat, S., Chaiyasut, C., and Riengrojpitak, S. (2010) High levels of 25-hydroxy-vitamin D3 [25(OH)D3] and  $\alpha$ -tocopherol prevent oxidative stress in rats that consume Thai brown rice. *Journal of Medicinal Plant Research*, **4**: 120–124.
- Takahashi, S., Ohtani, T., Iida, S., Sunohara, Y., Matsushita, K., Maeda, H., Tanetani, Y., Kawai, K., Kawamukai, M., and Kadowaki, K.I. (2009) Development of CoQ10-enriched rice from giant embryo lines. *Breeding Science*, **59**: 321–326.
- Tan, J., Baisakh, N., Oliva, N., Parkhi, V., Rai, M., Torrizzo, L., Datta, K., and Datta, S.K. (2005) The screening of rice germplasm, including those transgenic rice lines which accumulate  $\beta$ -carotene in their polished seeds, for their carotenoid profile. *International Journal of Food Science and Technology*, **40**: 563–569.
- Topping, D. (2007) Cereal complex carbohydrates and their contribution to human health. *Journal of Cereal Science*, **46**: 220–229.
- Traber, M.G. (1999) Vitamin E. In: M.E. Shils, J.A. Olson, M. Shike, and A.C. Ross (eds.), *Modern Nutrition in Health and Disease*, 10th ed. Baltimore, Williams & Wilkins, pp. 347–362.
- Verschoyle, R.D., Greaves, P., Cai, H., Edwards, R.E., Steward, W.P., and Gescher, A.J. (2007) Evaluation of the cancer chemopreventive efficacy of rice bran in genetic mouse models of breast, prostate and intestinal carcinogenesis. *British Journal of Cancer*, **96**: 248–254.
- Villegas, R., Liu, S., Gao, Y.T., Yang, G., Li, H., Zheng, W., and Xiao, O.S. (2007) Prospective study of dietary carbohydrates, glycemic index, glycemic load, and incidence of type 2 diabetes mellitus in middle-aged Chinese women. *Archives of Internal Medicine*, **167**: 2310–2316.
- Vitaglione, P., Napolitano, A., and Fogliano, V. (2008) Cereal dietary fibre, a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*, **19**: 451–463.



- Wang, Q., Han, P., Zhang, M., Xia, M., Zhu, H., Ma, J., Hou, M., Tang, Z., and Ling, W. (2007) Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pacific Journal of Clinical Nutrition*, **16** (Suppl.): 295–301.
- Welch, R.M. and Graham, R.D. (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, **55**: 353–364.
- Wilson, T.A., Nicolosi, R.J., Woolfrey, B., and Kritchevsky, D. (2007) Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. *Journal of Nutritional Biochemistry*, **18**: 105–112.
- Xia, M., Ling, W.H., Ma, J., Kitts, D.D., and Zawistowski, J. (2003) Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein e deficient mice. *Journal of Nutrition*, **133**: 744–751.
- Xia, X., Ling, W., Ma, J., Xia, M., Hou, M., Wang, Q., Zhu, H., and Tang, Z. (2006) An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E-deficient mice. *Journal of Nutrition*, **136**: 2220–2225.
- Xu, Z. and Godber, J.S. (2001) Antioxidant activities of major components of  $\gamma$ -oryzanol from rice bran using a linoleic acid model. *Journal of the American Oil Chemists' Society*, **78**: 645–649.
- Yamagishi, T., Yamaguchi, A., Saito, T., and Seguchi, M. (2008) Complement-modulating water-soluble polysaccharide fractions in two global staple cereals: rice and wheat. *Journal of the Science of Food and Agriculture*, **88**: 805–812.
- Yang, L. and Kadowaki, M. (2009) Effects of rice proteins from two cultivars, Koshihikari and shunyo, on hepatic cholesterol secretion by isolated perfused livers of rats fed cholesterol-enriched diets. *Annals of Nutrition and Metabolism*, **54**: 283–290.
- Yao, Y., Sang, W., Zhou, M., and Ren, G. (2010) Antioxidant and  $\alpha$ -glucosidase inhibitory activity of colored grains in China. *Journal of Agricultural and Food Chemistry*, **58**: 770–774.
- Yawadio, R., Tanimori, S., and Morita, N. (2007) Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. *Food Chemistry*, **101**: 1616–1625.
- Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., and Potrykus I. (2000) Engineering the provitamin A ( $\beta$ -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, **287**: 303–305.
- Yean, J.N., Seok, H.N., and Mi, Y.K. (2008) Cholesterol-lowering efficacy of unrefined bran oil from the pigmented black rice (*Oryza sativa* L cv. Suwon 415) in hypercholesterolemic rats. *Food Science and Biotechnology*, **17**: 457–463.
- Yu, S., Nehus, Z.T., Badger, T.M., and Fang, N. (2007) Quantification of vitamin E and  $\gamma$ -oryzanol components in rice germ and bran. *Journal of Agricultural and Food Chemistry*, **55**: 7308–7313.
- Zha, X.Q., Wang, J.H., Yang, X.F., Liang, H., Zhao, L.L., Bao, S.H., Luo, J.P., Xu, Y.Y., and Zhou, B.B. (2009) Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. *Carbohydrate Polymers*, **78**: 570–575.
- Zhang, C.Y., Shen, Y., Chen, J., Xiao, P., and Bao, J.S. (2008) Nondestructive prediction of total phenolics and flavonoid contents, and antioxidant capacity of rice grain using near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, **56**: 8268–8272.
- Zhang, J., Zhang, H., Wang, L., Guo, X., Wang, X., and Yao, H. (2009) Antioxidant activities of the rice endosperm protein hydrolysate, Identification of the active peptide. *European Food Research and Technology*, **229**: 709–719.
- Zhou, Z., Robards, K., Helliwell, S., and Blanchard, C. (2004) The distribution of phenolic acids in rice. *Food Chemistry*, **87**: 401–406.
- Zhu, F., Cai, Y.Z., Bao, J.S., and Corke, H. (2010) Effect of r-irradiation on phenolic compounds in rice grain. *Food Chemistry*, **120**: 74–77.

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# 5 Hypolipedemic effects of rice bran oil

John Parry and Haiwen Li

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## 5.1 Introduction

Rice is the second most produced grain in the world behind wheat ([http://nue.okstate.edu/crop\\_information/world\\_wheat\\_production.htm](http://nue.okstate.edu/crop_information/world_wheat_production.htm)). In 2009, the world's production of wheat was 681,915,830 metric tonnes (MT), and the production of rice was very similar at 678,688,289 MT ([http://nue.okstate.edu/crop\\_information/world\\_wheat\\_production.htm](http://nue.okstate.edu/crop_information/world_wheat_production.htm)). In most of the world, whole rice (*Oryza sativa*) is milled to make white rice. This process requires the removal of the bran, which is the exocarp, primarily consisting of cellulose, lignins, and the germ containing oil, lipid soluble components such as waxes, and vitamins.

## 5.2 Chemical composition of rice bran oil (RBO)

In the germ of whole rice, as well as other grains such as wheat and barley, there are oils that consist of triglycerides (Table 5.1), tocopherols, and other vitamins and minerals. A study by Lichtenstien *et al.* (2009) compared rice bran oil (RBO), canola oil, corn oil, and olive oil for three sterols including  $\beta$ -sitosterol, campesterol, and stigmasterol. The results showed that RBO was highest in all three tested sterols. The content of  $\beta$ -sitosterol was 1,259 mg/100 g, campesterol was 223 mg/100 g, and stigmasterol was 159 mg/100 g. As a note, corn oil was the second highest in concentration of the three tested sterols, but the amount in the corn oil was only one-half of the concentration that was in the RBO. In a follow-up study by Berger *et al.* (2005), RBO was tested for sterol concentrations. The results showed that RBO contained concentrations of  $\beta$ -sitosterol at 422–626 mg/100 g, campesterol at 160–332 mg/100 g, and stigmasterol at 118–192 mg/100 g. There were also other sterols detected, including  $\Delta^5$ -avenosterol at 26–32 mg/100 mg,  $\Delta^7$  avenosterol at 30–32 mg/100 gm, campestanol at 24 mg/100 gm and others. Alpha-,  $\beta$ -, and  $\gamma$ -tocopherols and  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocotrienols were found to be present in the RBO having a total concentration of 32–34 mg/100 g (Berger *et al.*, 2005).

In RBO there is also a group of phenolics (mainly ferulic acid) bound to sterols and they are called  $\gamma$ -oryzanol. The  $\gamma$ -oryzanol compounds have been determined to include cycloartenyl ferulate, campesteryl ferulate, 24-methylene cycloartenyl ferulate, cycloartenyl

**Table 5.1** Fatty acid composition of rice bran oil<sup>1</sup> and peanut oil<sup>2</sup> (g/100g oil)

Fatty Acid	RBO g/100g oil <sup>a,b</sup>	Peanut oil g/100g oil <sup>a</sup>
16:0	15.3 <sup>a</sup> –21.5 <sup>b</sup>	15.2 <sup>a</sup>
18:0	1.7 <sup>a</sup> –2.9 <sup>b</sup>	1.7 <sup>a</sup>
18:1	38.4 <sup>b</sup> –43.3 <sup>a</sup>	43.2 <sup>a</sup>
18:2	34.4 <sup>b</sup> –36.3 <sup>a</sup>	36.4 <sup>a</sup>
18:3	1.1 <sup>a</sup> –2.2 <sup>b</sup>	1.1 <sup>a</sup>
20:0	0.5 <sup>b</sup> –0.6 <sup>a</sup>	0.9 <sup>a</sup>
20:1	0.2 <sup>b</sup> –0.5 <sup>a</sup>	0.8 <sup>a</sup>

<sup>1</sup>RBO represents rice bran oil extracted using hexane.

<sup>2</sup>Peanut oil was from the whole peanut and was obtained from Oleificio Sabo (Manno, Switzerland).

<sup>a</sup>Berger *et al.*, 2005.

<sup>b</sup>Cosmetic Review Expert Panel, 2006.

**Table 5.2**  $\gamma$ -Oryzanol contents in rice bran oil (RBO)<sup>a,b</sup>

$\gamma$ -Oryzanol	ppm
cycloartenyl ferulate <sup>ab</sup>	45–232
campesteryl ferulate <sup>ab</sup>	39–342
24-methylene cycloartanyl ferulate <sup>ab</sup>	30–314
cycloartanyl ferulate <sup>ab</sup> & $\beta$ -sitosteryl ferulate <sup>a</sup> were combined	15–84
$\beta$ -sitosteryl ferulate <sup>b</sup>	NA
sitostanyl ferulate <sup>b</sup>	NA
stigmasteryl ferulate <sup>b</sup>	NA
$\Delta^7$ -campesterol ferulate <sup>b</sup>	NA
$\Delta^7$ -sitostanyl ferulate <sup>b</sup>	NA
campestanyl ferulate <sup>b</sup>	NA

<sup>a</sup>Rogers *et al.*, 1993; the results are from five different manufacturers of RBO.

<sup>b</sup>Xu and Godber, 1999; these results were from one sample only and were not quantified.

NA: not available. The presented range is from Rogers *et al.*, 1993 and was qualified by Xu and Godber, 1999.

ferulate  $\beta$ -sitosteryl ferulate and others (Rogers *et al.*, 1993). Later in 1999, Xu and Godber examined the  $\gamma$ -oryzanol components of rice bran oil using HPLC-MS and identified five more  $\gamma$ -oryzanol compounds. The five additional  $\gamma$ -oryzanol compounds included sitostanyl ferulate, stigmasteryl ferulate,  $\Delta^7$ -campesterol ferulate,  $\Delta^7$ -sitostanyl ferulate, and campestanyl ferulate (Table 5.2). Berger *et al.* (2005) demonstrated that  $\gamma$ -oryzanol was present in RBO from 100 to 1,600 mg/100g oil while peanut oil was determined to have zero  $\gamma$ -oryzanol.

The fatty acid composition of the studied RBO was very similar to the fatty acid composition of peanut oil (Berger *et al.*, 2005), and they were compared in a clinical human study for cholesterol lowering effects. The results of the fatty acid comparison are as shown in Table 5.1.

### 5.3 Hypolipidemic effect of rice bran oil

Tocotrienols isolated from RBO were tested for cholesterol lowering effects in hyperlipidemic rats that were fed an atherogenic diet for three weeks (Minhajuddin *et al.*, 2005). The

atherogenic diet included 5% hydrogenated oil, 0.5% cholic acid (bile acid), and 1% cholesterol. Before tocotrienol treatments were initiated, all lipid profiles in the treatment group had increased to be significantly higher compared to the control rats. Plasma triglycerides had increased 3.3 times, LDL (low-density lipoprotein) increased 5 times, HDL increased 1.2 times, and total cholesterol increased 2.4 times. After treatment with RBO tocotrienols, there were significant decreases in triglycerides (38–46%), LDL (39–62%), and total cholesterol (48–54%), and the results were shown to be dose-dependent (Minhajuddin *et al.*, 2005). Unfortunately, the study did not include rat subjects that were fed the high cholesterol diet while taking the RBO tocotrienols, which would have made the study results much more solid.

In a study by Wilson *et al.* (2007), RBO,  $\gamma$ -oryzanol and ferulic acid were tested for cholesterol lowering effects using Golden Syrian hamsters. The results showed that after ten weeks of treatment, total and non-HDL cholesterol levels were reduced in all three test models compared to control. The RBO diet decreased the total and non-HDL cholesterols by 64 and 70%, the ferulic acid diet decreased both by 22 and 24%, respectively, and the  $\gamma$ -oryzanol diet decreased them by 70 and 77%, respectively. In another study using a hamster model, researchers found that  $\gamma$ -oryzanol reduced fatty streaks in the aorta (Rong *et al.*, 1997).

Sharma and Rukmini (1986) performed an interesting study in which they compared RBO to peanut oil in the diets of rats fed cholesterol or no cholesterol. The results showed that rats fed RBO at 10% for eight weeks in the cholesterol feeding group had significantly lower LDL cholesterol and significantly higher HDL than the rats in the peanut oil feeding group. In the groups fed no cholesterol, RBO and peanut oil were similar in their cholesterol results. This study was followed up with a study by Seetharamaiah and Chandrasekhara (1989), who found similar results in their rat model.

Sugano and Tsuji (1997) suggested that the cholesterol lowering effect of RBO was mainly due to the non-saponifiable compounds and not the fatty acids. The non-saponifiable compounds mainly included  $\gamma$ -oryzanol and tocotrienols.

A study of eight healthy mares, (female horses aged 5–17 years old) were tested using RBO treatment for plasma cholesterol (Frank *et al.*, 2005). The results of the study showed that after five weeks of daily supplementation of 240 mL RBO, corn oil, or water, there was a significant decrease in tryglycerides and an increase in HDL in the RBO group, but there was also a significant increase in LDL compared with the water and corn oil treatment groups.

RBO was compared to peanut oil for cholesterol lowering effects in mildly hypercholesterolemic men 5.1 to 8.4 mmol/L (197mg/dL to 325mg/dL) (Berger *et al.*, 2005). The two oils were also tested for other chemical compositions including tocopherols, sterols, and sterols that were bound to ferulic acid as  $\gamma$ -oryzanol (Berger *et al.*, 2005). The fatty acid compositions between the RBO and peanut oils were very similar (see Table 5.1). The experimental design consisted of a high dose of oryzanol (800 mg/50 mL), the low dose contained 50 mg/50 mL in the RBO, and the peanut oil had 0 mg/mL oryzanol/50 mL. Subjects consumed approximately 50 g oil per day. After 15 and 30 days of treatment, results showed that  $\gamma$ -oryzanol in the RBO was able to significantly reduce total cholesterol and LDL compared to the peanut oil treatment.

Defatted rice bran and rice bran oil were examined for their cholesterol lowering effects in moderately hypertensive humans (Most *et al.*, 2005). Subjects were 18–50 years old and had total cholesterol between the 25th and 90th percentile after age, sex, and race adjustments were made. A comparison of the ability to lower cholesterol was tested in defatted rice bran and rice bran oil. All subjects consumed approximately the same amount of total

fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrate, and protein. Both rice bran and RBO had separate control groups, but they were very similar in macronutrient composition. The primary difference between the two diets was the amount of fiber, where the rice bran group consumed a much higher amount of fiber.

In the defatted rice bran study, interestingly and contradictorily, the human subjects consuming the defatted rice bran had significantly higher LDL cholesterol than those consuming the control diet. While in the RBO study, the researchers found that the subjects had significantly lower total cholesterol and LDL cholesterol levels compared to the control group even though the cholesterol content in the rice bran oil diet was 12.5% higher than the control diet. The researchers speculated that other phytosterols, such as  $\gamma$ -oryzanol, in the oil had the cholesterol lowering effect (Most *et al.*, 2005).

The effect of RBO, canola oil, corn oil, and olive oil on lowering plasma lipids and apolipoproteins (ApoB and Apo-I) was investigated by Lichtenstein *et al.* (2009). A 32-day, double-blind human study was ordered in a Latin square design with 15 middle-aged and elderly subjects with elevated LDL cholesterol from 133 to 219 mg/dL. Diets were enriched with RBO, canola oil, corn oil, or olive oil. Each of the test oils were consumed by all subjects. All foods and drinks were provided by the metabolic research team. The results showed that plasma LDL cholesterol concentrations were similar and statistically indistinguishable when the subjects consumed RBO, canola oil, and corn oil-enriched diets, yet all were lower than the olive oil-enriched diet. These data suggest that those who have moderately elevated levels of LDL may consume diets enriched with RBO to lower their LDL, which is a predictive measure of cardiovascular risk. These results of the RBO were similar to those of more commonly consumed vegetable oils in the United States (Lichtenstein *et al.*, 2009).

Cicero and Gaddi (2001) reported several studies on rats, rabbits, hamsters, monkeys, and humans, regarding the relationship of RBO and  $\gamma$ -oryzanol supplementation to the improvement of blood lipid profiles. In all but one of the 16 animal experiments, testing RBO or  $\gamma$ -oryzanol results showed a decrease in total plasma cholesterol, and there was also an increase in HDL levels in six of the nine experiments that tested for HDL.

The effect of plant sterols from RBO and sheanut on the reduction of triglycerides and cholesterol in humans was tested by Vissers *et al.* (2000). Margarine was used as the vehicle. The researchers found that the RBO treatment significantly reduced total cholesterol and LDL cholesterol levels compared to the control and sheanut oil groups.

## 5.4 Other beneficial effects of rice bran oil

### 5.4.1 Increased suppression of keratin

It was shown that  $\gamma$ -oryzanol had a strong affinity for human skin and had a suppressive effect against the increase in keratin (Ueda *et al.*, 1976). Keratin is the primary protein component that forms a triple helix of amino acids in finger nails, toe nails, and hair and has the possibility to form rigidity among cells in other parts of the body. Suppression of keratin formation can be important for those individuals with hyperkeratinosis that is marked by redness of the skin and blistering, which can lead to infection.

It makes sense that a lipophilic compound such as  $\gamma$ -oryzanol would be associated with the skin because of the lipophilic plasma membranes of the cell. Other plasma membranes include all other cells and their internal organelle membranes such as the nuclear membrane, endoplasmic reticulum, golgi apparatus, mitochondria, peroxisomes,

lysosomes, and so on. Reducing or preventing rigidity of the cell membranes may reduce the occurrence of cell death due to necrosis.

#### **5.4.2 Protection against gastric ulcers**

In 1987, Jayaraj *et al.* showed that fresh RBO decreased the ulceration in rats, whereas stored oil was ulcerogenic. Interestingly, it was demonstrated that cysteine was able to restore the gastric protective effect. Lloris *et al.* (1991) reported the RBO on gastric juices using a rat feeding study. The rats were subjected to stress induced ulcers. RBO showed a significant decrease ( $P < 0.05$ ) of protons in the gastric juice in rats that were pretreated with RBO compared to the control group.

#### **5.4.3 Reduction of platelet aggregation**

RBO and sesame seed oil (SESO) were compared with coconut oil (CNO) for their ability to reduce platelet aggregation in male Wistar rats (Reena *et al.*, 2010). The diets included 10% oil from RBO, SESO, and CNO individual oils, blends of CNO with RBO and CNO with SESO, and interesterified combinations of CNO and RBO or SESO. Interesterification was accomplished by mixing the two blended oil combinations in lipase. Platelet aggregation was induced by either (Adenosine di Phosphate) ADP or collagen. The results of the study showed that RBO, SESO, blended combinations, and interesterified combinations had significantly lower rates and percentages of platelet aggregation compared to CNO. The RBO interesterified with CNO and SESO interesterified with CNO reduced the rate of platelet aggregation by 37 and 34%, respectively compared to CNO alone.

#### **5.4.4 Cancer inhibition**

A study by Shih *et al.* (2011) tested RBO for its effect on inhibiting the formation of colon cancer in F344 rats by measuring aberrant crypt foci (ACF) and mucin-depleted foci (MDF). The rats were administered with 1,2-dimethylhydrazine to induce colon cancer. Soybean oil was used as the negative control. RBO was given at low, medium, and high doses, and the results showed that all RBO groups had significantly less formation of ACF and MDF compared to the soybean oil. Additionally, the RBO groups had higher glutathione, catalase, and superoxide dismutase levels and lower thiobarbituric reactive substances compared to the soybean oil group (Shih *et al.*, 2011).

### **5.5 Future studies**

With regard to using RBO as a food supplement for frying or eating as a salad dressing etc., it would seem reasonable that it could be a possible choice to replace other oils due to its high content of  $\gamma$ -oryzanol. With its high content of palmitic and oleic acids (see Table 5.1), it should be relatively stable to oxidative rancidity.

RBO can also possibly be used as a biodiesel fuel, which would be a renewable energy source. The remaining cellulose and lignans can also be used for the production of ethanol, which is an energy source for automobiles and other types of equipment that require a similar fuel source such as gasoline, diesel and ethanol (Zullaikah *et al.*, 2005).

## References

- Berger, A., Rein, D., Schafer, A., Monnard, I., Gremaud, G., Lambelet, P., and Bertoli, C. (2005) Similar cholesterol-lowering properties of rice bran oil, with varied  $\gamma$ -oryzanol, in mildly hypercholesterolemic men. *European Journal of Nutrition*, **44**: 164–173.
- Cicero, A.F.G. and Gaddi, A. (2001) Rice bran oil and  $\gamma$ -oryzanol in the treatment of hyperlipoproteinemias and other conditions. *Phytotherapy Research*, **15**: 277–289.
- Cosmetic Ingredient Review Expert Panel. (2006).
- Frank, N., Andrews, F.M., Elliot, S.B., Lew, J., and Boston, R.C. (2005) Effects of rice bran oil on plasma lipid concentrations, lipoprotein composition, and glucose dynamics in mares. *Journal of Animal Science*, **83**: 2509–2518.
- Jayaraj, A.P., Tovey, F.I., Clark, C.G., Rees, K.R., White, J.S., and Lewin, M.R. (1987) The ulcerogenic and protective action of rice and rice fractions in experimental peptic ulceration. *Clinical Science*, **72**: 463–466.
- Lichtenstien, A.H., Ausman, L.M., Carrasco, W., Gualtieri, L.J., Jenner, J.L., Ordovas, J.M., Nicolosi, R.J., Goldin, B.R., and Schafer, E.J. (2009) Rice bran oil consumption and plasma lipid levels in moderately hypercholesteremic humans. *Arteriosclerosis, Thrombosis and Vascular Biology*, **14**: 548–566.
- Lloris, J.M., Bolant, B., Gimeno, L., Cejalvo, D., Gimeno, L.O., Calvo, M.A., and Perkins, I. (1991) Gastric juice and analysis of basal and stimulated secretion following treatment with rice bran oil. *Research Communications in Chemical Pathology and Pharmacology*, **74**: 245–248.
- Minhajuddin, M., Beg, Z., and Iqbal, J. (2005) Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in induced hyperlipidemic rats. *Food Chemistry Technology*, **43**: 747–753.
- Most, M.M., Tulley, R., Morales, S., and Lefevre, M. (2005) Rice bran oil, not fiber, lowers cholesterol in humans. *American Journal of Clinical Nutrition*, **81**: 64–68.
- Reena, M.B., Krishnakantha, T.P., and Lokesh, B.R. (2010) Lowering of platelet aggregation and serum eicosanoid levels in rats fed with a diet containing coconut oil blends with rice bran oil or sesame oil. *Prostaglandins, Leukotrienes and Fatty Acids*, **151**: 151–160.
- Rogers, E.J., Rice, S.M., Nicolosi, R.J., Carpenter, D.R., McClelland, C.A., and Romanczyk, Jr. L.J. (1993) Identification and quantification of  $\gamma$ -oryzanol components and simultaneous assessment of tocopherols in rice bran oil. *Journal of the American Oil Chemists' Society*, **70**: 301–307.
- Rong, N., Ausman, L.M., and Nicolosi, R.J. (1997) Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters. *Journal of the American Oil Chemists' Society*, **32**: 302–309.
- Seetharamaiah, G.S. and Chandrakeshara, N. (1989) Studies on hyperlipidemic. *Effects of bran oil. Atherosclerosis*, **78**: 219–223.
- Sharma, R.D. and Rukmini, C. (1986) Rice bran oil and hypocholesterolemia in rats. *Lipids*, **11**: 715–717.
- Shih, C-K., Ho, C-J., Li, S.C., Yang, S-H., Hou, W.C., and Cheng, H-H. (2011) Preventive effects of rice bran oil on 1,2-dimethylhydrazine/dextran sodium sulphate-induced colon carcinogenesis in rats. *Food Chemistry*, **126**: 562–567.
- Sugano, M. and Tsuji, E. (1997) Rice bran oil on cholesterol metabolism. *Journal of Nutrition*, 521S–524S.
- Ueda, H.R., Hayakawa, S., Hoshino, S., and Kobayashi, M. (1976) The effect of topically applied gamma-Oryzanol on sebaceous glands. *Journal of Dermatology*, **3**: 19–24.
- Vissers, M.N., Zock, P.L., Meijeir, G.W., and Katan, M.B. (2000) Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. *Journal of American Clinical Nutrition*, **72**: 1510–1515.
- Wilson, T.A., Nicolosi, R.J., Woolfrey, B., and Kritchevski, D. (2007) Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. *Journal of Nutritional Biochemistry*, **18**: 105–112.
- Xu, Z. and Godber, S. (1999) Purification and identification of components of rice bran. *Journal of Agricultural and Food Chemistry*, **47**: 2724–2728.
- Zullaikah, S., Lai, C-C., Vali, S.J., and Ju, Y-H. (2005) A two-step acid catalyzed process for the production of biodiesel from rice bran oil. *Bioresource Technology*, **96**: 1889–1896.

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## 6 Phenolic phytochemicals from rye (*Secale Cereale L.*)

Devanand Luthria, Ronita Ghatak, and Haiqiu Huang

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### 6.1 Introduction

Grains are good natural sources of complex carbohydrates, vitamins, minerals, and other phytochemicals. The non-refined grains also known as whole grains are even better as they contain higher levels of fiber and other important nutrients such as vitamins, potassium, selenium, iron, zinc, riboflavin, niacin, thiamin, pantothenic acid, and phenolic phytochemicals (Fardet *et al.*, 2008). A whole grain kernel contains three parts: bran (the outer shell that provides fiber, vitamins, minerals, and phenolic phytochemicals), endosperm (the middle part that contains carbohydrates and protein), and the germ (that provides oils and vitamins). There has been increased awareness among scientific professionals, commercial interest groups, and consumers about the health benefits of whole grains during the past decade. This has stemmed from epidemiological studies, which have shown that whole grain consumption reduces the risk of type 2 diabetes, stroke, cardiovascular disease, and obesity. The beneficial effect of whole grains has been attributed to the presence of dietary fiber, micronutrients, and other phytochemicals.

Rye (*Secale Cereale L.*) is considered to be a primitive crop with low yield. However, rye cultivation requires marginal soil with low fertilization and is comparatively more tolerant to a colder climate. On a worldwide production basis, rye is the eighth most important cereal crop after corn, wheat, rice, barley, sorghum, oat, and millet (FAO FAOSTAT database, 2004). Currently, Poland and Germany are the two major rye producers of the world. The main food applications of rye are production of bakery products, breakfast cereals, and alcoholic beverages (Vinkx and Delcour, 1996). Rye is one of the major grains used for the production of bread in Europe. Rye is a recognized source of dietary fiber (arabinoxylan), vitamins, and other phenolic phytochemicals. This chapter describes the phenolic compounds present in rye.

Phenolic phytochemicals are a complex group of secondary metabolites synthesized by plants. Phenolic phytochemicals occupy a unique position in nutrition today due to their ubiquitous presence throughout the plant kingdom (vegetables, fruits, grains, and nuts) and their inclusion in healthcare and nutraceutical products. Phenolic phytochemicals are involved in a variety of roles in the life span of the plants, ranging from structural functions to reproductive, to protection in response to stress conditions such as infection, wounding, and UV radiation



(Beckman, 2000; Nicholson and Hammerschmidt, 1992). Approximately, 8000 naturally occurring phenolic compounds have been extracted and identified from natural sources. These compounds are known to occur as monomers, polymers (tannins), and in conjugation with other acids, sugars, and alkyl groups. All phenolic compounds contain an aromatic ring bearing at least one hydroxyl substituent (a phenol moiety) (Luthria *et al.*, 2006).

Phenolic compounds can be classified into multiple subgroups based on chemical structure as well as its biosynthetic origin (Ferreira *et al.*, 2010). In rye, the three major classes of phenolic compounds are phenolic acids, lignans, and alkylresorcinols.

## 6.2 Three classes of the phenolic compounds

### 6.2.1 Phenolic acids

These are phenols that possess at least one carboxylic acid moiety. The naturally occurring phenolic acids can be categorized into two primary groups: the hydroxylated derivatives of benzoic and cinnamic acids. Both groups are biosynthesized from phenyl alanine by the action of phenylalanine ammonia-lyase (PAL). The major phenolic acids extracted and identified from rye are ferulic, caffeic, *p*-coumaric, sinapic, vanillic, syringic, and *p*-hydroxybenzoic acids. The structure of the common phenolic acids extracted from rye is presented in Figure 6.1.

### 6.2.2 Lignans

These are polyphenolic compounds synthesized by the union of two cinnamic acid residues or their biogenetic equivalents. The common lignans identified in rye are syringaresinol, pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, and medioresinol. The structures of the major lignans are presented in Figure 6.2. Plant lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols. Due to the structural similarity of lignans with estradiol, lignans along with isoflavonoids and coumestans are sometimes classified as phytoestrogens.

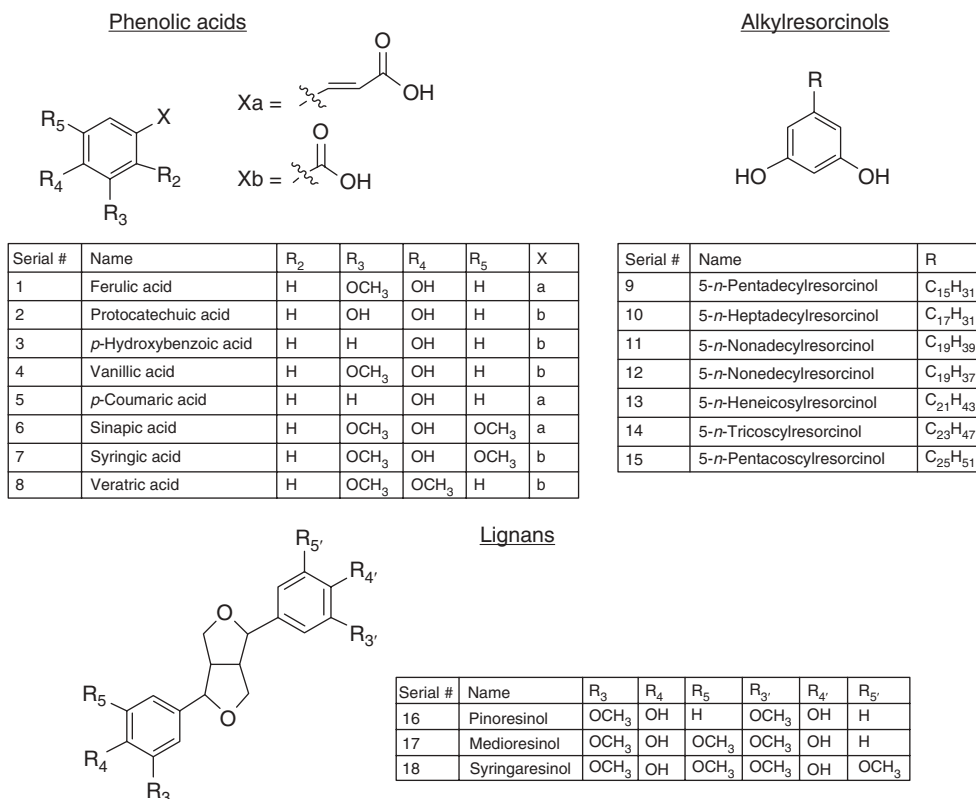
### 6.2.3 Alkylresorcinols

These comprise a group of 1,3-dihydroxy-5-alkylbenzene derivatives. The number of carbons in the alkyl side chain is mainly odd containing 15–25 saturated carbon atoms. Around 85% of the alkylresorcinols have saturated alkyl side chains and the remaining 15% may contain mono or di-unsaturated carbon chains with keto group. The structures of the common alkylresorcinols present in rye are shown in Figure 6.1.

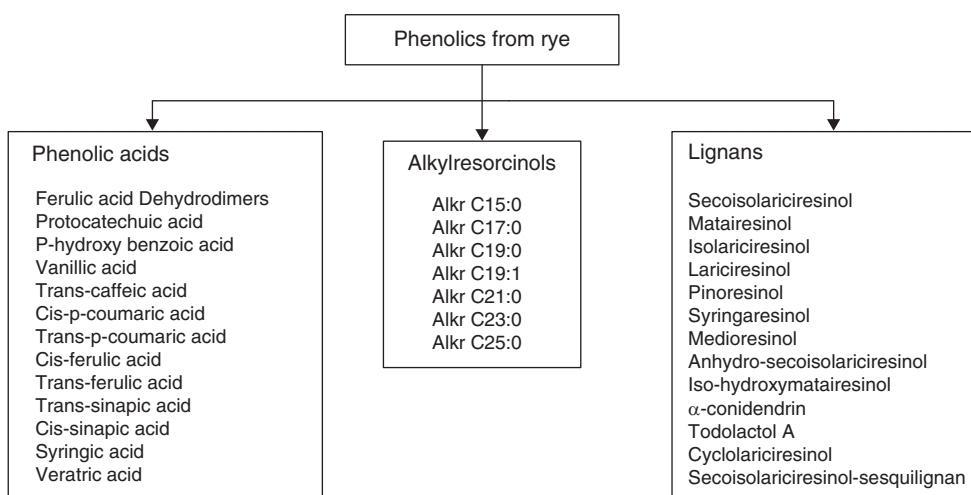
## 6.3 Extraction methodology

### 6.3.1 Phenolic acids

Several researchers have conducted analyses of total phenolic acids from different matrices of rye (Figure 6.2). The solvents used for extraction of phenolic acids are methanol, acetone, or aqueous dilutions of one or both (Table 6.1). The fermentation conditions for extraction of bioactive compounds from rye are presented in Table 6.2. Heiniö *et al.* (2008) used a



**Figure 6.1** Structures of some phenolic phytochemicals extracted and identified from rye (*Secale Cereale L.*).



**Figure 6.2** Major phenolic phytochemicals extracted and identified from rye (*Secale Cereale L.*).

**Table 6.1** Conditions for extraction of bioactive phenolics from rye

Serial no.	Extraction condition							Yield (mg/100 g, dm)	References		
	Class	Matrix	Amount (g)	Moisture content (%)	Solvent	Extraction temperature & time	Supernatant			Residue	
1	Total Phenolic Content	Wholemeal		16.5	Methanol						
	Total Phenolic acid	Wholemeal		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis, liquid-liquid partitioning by diethyl ether (DE)-ethyl acetate (EA) (1:1 v/v), conc., redissolved in MeOH	Alkali Hydrolysis, 20 C, 4 H	65.3	Heiniö <i>et al.</i> (2008)
		Bran		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis, 20 C, 4 H	127.2	
		Shorts		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis, 20 C, 4 H	49.4	
	Free Phenolic acid	Wholemeal		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	1.4	
		Bran		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	9.9	
		Shorts		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	5.2	
	Alkylresorcinols	Wholemeal		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	143	
		Bran		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	196.6	
		Shorts		16.5	Methanol/acetone/water (2:2:1 v/v/v)			Acid Hydrolysis	Alkali Hydrolysis	77.2	
	Lignans	Wholemeal		16.5	Diethyl Ether			Acid Hydrolysis	Alkali Hydrolysis	2.27	
		Bran		16.5	Diethyl Ether			Acid Hydrolysis	Alkali Hydrolysis	4.08	
		Shorts		16.5	Diethyl Ether			Acid Hydrolysis	Alkali Hydrolysis	1.62	

2	Total Phenolic Content	Native Bran	15-16	Methanol, Ultrasonicated EthylAcetate		584	Katrina <i>et al.</i> (2007b)
	Total Phenolic acid	Native Bran	15-16			274	
	Free Phenolic acid	Native Bran	15-16		Acid Hydrolysis	6.8	
	Alkylresorcinols	Native Bran	15-16			285	
	Lignans	Native Bran	15-16			5.8	
	Total Phenolic Content	Peeled Bran	15-16			415	
	Total Phenolic acid	Peeled Bran	15-16			215	
	Free Phenolic acid	Peeled Bran	15-16			2.3	
	Alkylresorcinols	Peeled Bran	15-16			222	
	Lignans	Peeled Bran	15-16			4.9	
3	Alkylresorcinols	Pearled		EthylAcetate	RT, 24 H, Shake	3.73±0.7	Landberg <i>et al.</i> (2008)
	Free Phenolic acid	Defatted Bran	1	80% Ethanol, Chilled	Dried, redissolved EthylAcetate Combined (2), Redissolved Water	12.3±0.9	Hosseinian <i>et al.</i> (2009)
4	Total Phenolic acid					200.4±1.4	
	Lignans			Alkali Hydrolysis	Acid Hydrolysis	0.7	

(continued)

**Table 6.1** (cont'd)

Serial no.	Class	Extraction condition							References	
		Matrix	Amount (g)	Moisture content (%)	Solvent	Extraction temperature & time	Supernatant	Residue		Yield (mg/100 g, dm)
5	Alkylresorcinols	Milled	0.5		Ethyl/Acetate	RT, 24 H	Dried, redissolved Ethyl/Acetate		84.3±2.1 (fm)	Landberg <i>et al.</i> (2007)
		Intact	1		Super Critical-CO2 Ethyl/Acetate	RT, 24 H	Dried, redissolved Ethyl/Acetate		117.6±0.56 83.3±1.2	
		Milled Bran			Ethyl/Acetate	RT, 24 H	Dried, redissolved Ethyl/Acetate		292.1±1.9	
6	Alkylresorcinols	Milled Aleurone			Super Critical-CO2 Ethyl/Acetate	RT, 24 H	Dried, redissolved Ethyl/Acetate		293.2±2.0 123.0±0.5	Landberg <i>et al.</i> (2009)
		Rye	1		Super Critical-CO2 Ethyl/Acetate	RT, 24 H			103.5±5.0 134.3±25.3	
		Native Bran Pearled Bran							200.0–350.0 200.0–350.0	

mixture of methanol, acetone, and water for extraction of free and conjugated phenolic acids from rye flour, shorts, and bran. They found that the free phenolic acids comprised 25%, 10%, and 7.8% in flour, shorts, and bran, respectively, indicating that the relative proportion of phenolic acids decreased in the outer grain layers. They also found that sinapic and ferulic acids were the two most prominent phenolic acids in rye grains. Hosseinian and Mazza (2009) used chilled 80% ethanol to extract phenolic acids from defatted rye bran. They found alkaline hydrolysis was more efficient than acid hydrolysis. Đorđević *et al.* (2010) extracted total phenolics from native and fermented rye using 70% ethanol and found that fermentation with *L. rahmnosus* had increased the yield of total phenolic acids extracted from rye. Similarly, Katina *et al.* (2007) used fermentation techniques to increase the yield of total phenolic acids in rye grain. They found that fermentation enhanced the structural breakdown of cell walls and assisted in the liberation of phenolic acids. They observed that fermentation of native rye with *S. cerevisiae* increased the amount of extractable phenolic acids by three times. Katina *et al.* (2007) studied the effect of fermentation time (6, 13, and 20h), and fermentation temperature (20, 27.5, and 35 °C) on the yield of total extractable phenolic acids from rye bran. They found a linear increase in phenolic acids content with longer fermentation time and higher temperatures. Nyström *et al.* (2008) used aqueous ethanol followed by acid and alkali hydrolysis to estimate phenolic acids in ten different rye varieties. Mattila *et al.* (2005) also used acidified methanol to extract phenolic acids and found that rye bran has higher amounts of phenolic acids than does rye flour. Andreasen *et al.* (2000) used alpha amylase to degrade starch followed by alkali saponification to extract ferulic, sinapic, and *p*-coumaric acids from 17 rye varieties. They found that ferulic acid was the dominant phenolic acid in all rye varieties. The details of the methodology used for the extraction of phenolic acids from rye are summarized in Table 6.1.

### 6.3.2 Alkylresorcinols

Ethyl acetate has been the most commonly used solvent for extraction of alkylresorcinols from different rye matrices. Landberg *et al.* (2007, 2008, 2009) used ethyl acetate to determine content of alkylresorcinol in different rye varieties. They found that most of the alkylresorcinols are located in the outermost layers of the caryopsis. They also found that rye bran has the maximum amount of alkylresorcinols. Nyström *et al.* (2008) used the same solvents to determine alkylresorcinols from ten varieties of rye and found that the range was 79.7–123.1 mg/g dry weight. Heiniö *et al.* (2008) and Mattila *et al.* (2005), unlike others, used methanol to extract alkylresorcinols. Both reported that alkylresorcinols are concentrated in rye bran compared to whole rye flour. Fermentation of rye grain and bran did not bring any substantial increase in measurable alkylresorcinols content when compared to native rye grain and bran, respectively (Katina *et al.*, 2007 a, b). Andreasen *et al.* (2000) used acetone to extract alkylresorcinols from 17 rye varieties. Kulawinek *et al.* (2008) used both ethyl acetate and acetone to extract alkylresorcinols from whole, ground rye grain, whole rye bran, and whole rye flour. Like others, they also reported that alkylresorcinols are more concentrated in the bran fraction.

### 6.3.3 Lignans

Heiniö *et al.* (2008) used diethyl ether to extract lignans from rye. The sample was hydrolyzed enzymatically before HPLC quantification. They found that lignans comprised about 1.6% of the amount of alkylresorcinols present in rye flours, shorts, and brans. The major

**Table 6.2** Fermentation conditions for extraction of bioactive compounds from rye

Serial no.	Class	Extraction condition					Yield (mg/100g, dm)	References
		Matrix	Moisture content (%)	Fermentative organism	Fermentation temperature & time	Solvent		
1	Total Phenolic Content	Wholemeal	16.5	None	20H, 30C	Methanol, Ultrasonicated	340±30	Katina et al. (2007a)
		Wholemeal	16.5	<i>Lb. plantarum</i>	20H, 30C	Methanol	410±1	
		Wholemeal	16.5	<i>Lb. brevis</i>	20H, 30C	Methanol	430±2	
		Wholemeal	16.5	<i>S. cerevisiae</i>	20H, 30C	Methanol	470±3	
		Wholemeal	16.5	<i>S. cerevisiae</i> + <i>Lb. plantarum</i> + <i>Lb. Brevis</i>	20H, 30C	Methanol	430±11	
		Wholemeal	16.5	Spontaneous fermentation (no added microbes).	20H, 30C	Methanol	380±22	
	Total Phenolic acid	Wholemeal	16.5	None	20H, 30C	Methanol	300±13	
		Wholemeal	16.5	<i>Lb. plantarum</i>	20H, 30C	Methanol	319±5	
		Wholemeal	16.5	<i>Lb. brevis</i>	20H, 30C	Methanol	302±8	
		Wholemeal	16.5	<i>S. cerevisiae</i>	20H, 30C	Methanol	329±12	
		Wholemeal	16.5	<i>S. cerevisiae</i> + <i>Lb. plantarum</i> + <i>Lb. Brevis</i>	20H, 30C	Methanol	309±7	
		Wholemeal	16.5	Spontaneous fermentation (no added microbes).	20H, 30C	Methanol	304±8	
	Free Phenolic acid	Wholemeal	16.5	None	20H, 30C	Methanol	3.1±0.4	
		Wholemeal	16.5	<i>Lb. plantarum</i>	20H, 30C	Methanol	0	
		Wholemeal	16.5	<i>Lb. brevis</i>	20H, 30C	Methanol	0.35±0.01	
		Wholemeal	16.5	<i>S. cerevisiae</i>	20H, 30C	Methanol	10.7±0.01	
		Wholemeal	16.5	<i>S. cerevisiae</i> + <i>Lb. plantarum</i> + <i>Lb. Brevis</i>	20H, 30C	Methanol	0.5±0.02	

	Wholemeal	16.5	Spontaneous fermentation (no added microbes).	20H, 30C	Methanol	1.0±0.3	
Alkylresorcinols	Wholemeal	16.5	None	20H, 30C	Methanol	1020±90	
	Wholemeal	16.5	<i>Lb. plantarum</i>	20H, 30C	Methanol	800±50	
	Wholemeal	16.5	<i>Lb. brevis</i>	20H, 30C	Methanol	1140±20	
	Wholemeal	16.5	<i>S. cerevisiae</i>	20H, 30C	Methanol	1280±40	
	Wholemeal	16.5	<i>S. cerevisiae</i> + <i>Lb. plantarum</i> + <i>Lb. Brevis</i>	20H, 30C	Methanol	880±40	
	Wholemeal	16.5	Spontaneous fermentation (no added microbes).	20H, 30C	Methanol	490±90	
2	Total Phenolic Content		Baker's Yeast	20-25C; 6, 13, 20H; Central Composite Design	TPH, TPA, TPAF Increased (NB)		Katina et al. (2007b)



lignan compound was identified as syringaresinol (~80%). They also found that lignans were more concentrated in the bran fraction of the rye grain. Katina *et al.* (2007a) found that the lignans were higher in native bran compared to peeled bran. Hosseinian and Mazza (2009) extracted lignans from defatted rye bran following direct alkaline hydrolysis and found similar results.

## 6.4 Analysis methods

### 6.4.1 Phenolic acids

Most researchers have used high performance liquid chromatography with a diode array detector (HPLC-DAD) for the analysis of individual phenolic acids. Nyström *et al.* (2008) used reversed phase HPLC-DAD using an acetonitrile/acidic water gradient to analyze individual phenolic acids. They identified nine phenolic acids from rye. Hosseinian and Mazza (2009) used reversed phase HPLC-DAD with an acidified methanol gradient to detect nine individual phenolic acids. Michalska *et al.* (2007) used reversed phase HPLC-DAD and eluted five individual phenolic acids with water/acetonitrile/acetic acid gradient. Andreasen *et al.* (2001) detected ferulic acids and different analogs of ferulic acids using reversed phase HPLC-DAD and eluted them using binary solvent (aqueous acetonitrile/methanolic acetonitrile) and a combination of linear and isocratic gradient. Andreasen *et al.* (2000) used reversed phase HPLC-DAD to quantify 13 phenolic acids. They used a combination of linear and isocratic gradient with binary solvents (phosphate buffer/acidified methanol). Mattila *et al.* (2005) used reversed phase HPLC-DAD with a phosphoric acid/acetonitrile gradient to identify nine individual phenolic acids. Heiniö *et al.* (2008) followed the method used by Mattila *et al.* (2005) to quantify seven phenolic acids.

The flow rates varied from 0.4 to 1.0 mL/min for HPLC with an injection volume of 10  $\mu$ L. The phenolic acids were detected at 280 nm. Michalska *et al.* (2007), however, detected the phenolics at 260 and 320 nm. Andreasen *et al.* (2000, 2001) used an injection volume of 40  $\mu$ L and detected the phenolic acids at 280 and 325 nm. Mattila *et al.* (2005) and Heiniö *et al.* (2008) used three different detection wavelengths for phenolics acids which are 254, 280, and 329 nm. A recent review by Robbins (2003) summarized different methods, used for the assay of phenolic acids from different matrices.

### 6.4.2 Alkylresorcinols

Researchers have used gas chromatography (GC) to quantify alkylresorcinols. Ross *et al.* (2001) used a BP-5 capillary column with a flame ionization detector (FID). Helium was used as the carrier gas and the injector and detector temperatures were set at 325 and 350 °C, respectively. They used an injection volume of 1.0  $\mu$ L and a gas flow rate of 1.5 mL/min. However, Kulawinek *et al.* (2005) used reversed phase HPLC-DAD to quantify alkylresorcinols. They used a mobile phase containing aqueous methanol at a flow rate of 1 mL/min with injection volume of 20  $\mu$ L and detected six compounds at 280 nm. Mattila *et al.* (2005), Heiniö *et al.* (2008), and Katina *et al.* (2007) also used reversed phase HPLC-DAD to quantify seven alkylresorcinols with aqueous methanol using a linear gradient and detected at 280 nm. A review by Ross *et al.* (2004) discusses

the details of the methods used for extraction and analyses of alkylresorcinols from cereal grains, food, and biological fluids.

### 6.4.3 Lignans

Researchers used gas chromatography with mass spectral (GC-MS) to quantify lignans. Katina *et al.* (2007b) and Heiniö *et al.* (2008) used BP-1 vitreous silica column with a FID detector using helium as carrier gas to quantify seven lignans. On the other hand, Peñalvo *et al.* (2006) used HPLC with coulometric electrode array detection for the analyses of isoflavones and lignans in biological matrices. Chromatographic methods and procedures used for analysis of lignans in trees and other plants are described in a research article published by Willför *et al.* (2006).

## 6.5 Bioactivity

### 6.5.1 Phenolic acids

Andreasen *et al.* (2001) demonstrated the release of sinapic acid and *p*-coumaric acid from rye bran by human colonic esterase(s) (mostly of microbial origin). They hypothesized that hydrolysis by intestinal esterase(s) is very likely the major route for release of hydroxycinnamic acids *in vivo*. Andreasen *et al.* (2001) also studied the antioxidant activities of purified monomeric and dimeric hydroxycinnamates and of phenolic extracts from rye (whole grain, bran, and flour) using an *in vitro* copper-catalyzed human LDL oxidation assay. They found that ferulic acid dehydro dimer was a better antioxidant compared to other acids. They also determined that rye bran had the most antioxidant potential compared to its other fractions. Harder *et al.* (2004) found that subjects after ingestion of rye bran showed bioavailability of ferulic acid in a cross over study.

### 6.5.2 Alkylresorcinols

Rejman and Kozubek (1997) found that 5-*n*-alk(en)ylresorcinols isolated from rye bran, effectively inhibited glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8), the key enzyme of triacylglycerol synthesis in adipocytes, suggesting the potential of rye alkylresorcinols in reducing the plasma triglycerides. Stasiuk *et al.* (2008) demonstrated that alkylresorcinols from rye grain inhibited acetylcholinesterase activity. This enzyme was shown to participate in the onset of Alzheimer's diseases.

### 6.5.3 Lignans

Glitsø *et al.* (2000) found higher recovery of dietary lignans in pigs after being fed rye bread diets. Juntunen *et al.* (2000) did a crossover study on the effect of rye bread as part of the usual diet on serum and urine enterolactone (ENL). They found that daily urinary ENL excretion increased significantly and was 5- and 10-fold higher in men and women, respectively, in comparison with the amount of plant lignan precursors measured in the rye bread.

## 6.6 Health beneficial effects of rye intake

Vinkx and Delcour (1996) reported that intake of rye was associated with reduction in weight gains, and assisted in better digestion and absorption of all nutrients. They also found that rye arabinoxylans reduced enzymatic digestion rate by forming viscous solutions. McIntosh *et al.* (2003) found that consumption of rye foods was associated with increased plasma enterolactone and fecal butyrate. Leinonen *et al.* (2000) found that intake of rye bread decreased serum cholesterol in men by 8% in a two by four week crossover trial study. Juntunen *et al.* (2003) observed that high fiber rye bread enhanced insulin secretion in a randomized crossover trial study. Laaksonen *et al.* (2005) concluded that consumption of rye bread increased early insulin secretion in persons with metabolic syndrome, and might reduce chances development of type 2 diabetes.

## 6.7 Summary

Researchers have used a wide variety of different methodologies for extraction and analyses of all three groups of phenolic phytochemicals (phenolic acids, alkylresorcinols, and lignans) in rye samples. It is evident that the analysis procedure directly impacts the efficiency of phytochemical extraction, which in turn influences the bioactivity of grain extract. Acid, base, or enzymatic hydrolysis procedures were used to release the bound and conjugated phenolic compounds. Extraction methodology and environmental factors along with variations in cultivars may be the major reasons for the variations in the quantity of phenolic phytochemicals determined by different researchers (see Table 6.1).

## References

- Andreasen, M.F., Christensen, L.P., Meyer, A.S., and Hansn, Å. (2000) Content of Phenolic Acids and Ferulic Acid Dehydrodimers in 17 Rye (*Secale cereale* L.) Varieties. *Journal of Agricultural and Food Chemistry*, **48**: 2837–2842.
- Andreasen, M.F., Landbo, A-K., Christensen, L.P., Hansen, Å., and Meyer, A.S. (2001) Antioxidant Effects of Phenolic Rye (*Secale cereale* L.) Extracts, Monomeric Hydroxycinnamates, and Ferulic Acid Dehydrodimers on Human Low-Density Lipoproteins. *Journal of Agricultural and Food Chemistry*, **49**(8): 4090–4096.
- Beckman, C.H. (2000) Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants. *Physiological and Molecular Plant Pathology*, **57**(3): 101–110.
- Dorđević, T.M., Šiler-Marinković, S., and Dimitrijević-Branković, S.I. (2010) Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry*, **119**: 957–963.
- FAO FAOSTAT data base: archives, <http://faostat.fao.org>, (2004).
- Fardet, A., Rock, E., and Rémésy, C. (2008) Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected in vivo? *Journal of Cereal Science*, **48**: 258–276.
- Ferreira, J.F.S., Luthria, D.L., Sasaki, T., and Heyerick, A. (2010) Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with Artemisinin against Malaria and Cancer. *Molecules*, **15**: 3135–3170.
- Glitsø, L.V., Jensen, B.B., and Knudsen, K.E.B. (2000) In vitro fermentation of rye carbohydrates including arabinoxylans of different structure. *Journal of the Science of Food and Agriculture*, **80**(8): 1211–1218.

- Harder, H., Tetens, I., Let, M.B., and Meyer, A.S. (2004) Rye bran bread intake elevates urinary excretion of ferulic acid in humans, but does not affect the susceptibility of LDL to oxidation *ex vivo*. *European Journal of Nutrition*, **43**(4): 230–236.
- Heiniö, R.-L., Liukkonen, K.-H., Myllymäki, O., Pihlava, J.-M., Adlercreutz, H., Heinonen, S.-M., and Poutanen, K. (2008) Quantities of phenolic compounds and their impacts on the perceived flavor attributes of rye grain. *Journal of Cereal Science*, **47**: 566–575.
- Hosseini, F.S. and Mazza, G. (2009) Triticale bran and straw: Potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Food*, **1**: 57–64.
- Juntunen, K.S., Mazura, W.M., Liukkonen, K.H., Uehara, M., Poutanen, K.S., Adlercreutz, H.C.T., and Mykkänen, H.M. (2000) Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. *British Journal of Nutrition*, **84**: 839–846.
- Juntunen, K.S., Laaksonen, D.E., Poutanen, K.S., Niskanen, L.K., and Mykkänen, H.M. (2003) High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. *American Journal of Clinical Nutrition*, **77**(2): 385–391.
- Katina, K., Laitila, A., Juvonen, R., Liukkonen, K.-H., Kariluoto, S., Piironen, V., *et al.* (2007a) Bran fermentation as a means to enhance technological properties and bioactivity of rye. *Food Microbiology*, **24**: 175–186.
- Katina, K., Liukkonen, K.-H., Kaukovirta-Norja, A., Adlercreutz, H., Heinonen, S.-M., Lampi, A.-M., *et al.* (2007b) Fermentation-induced changes in the nutritional value of native or germinated rye. *Journal of Cereal Science*, **46**: 348–355.
- Kulawinek, M. and Kozubek, A. (2007) Quantitative determination of alkylresorcinols in cereal grains: independence of the length of the aliphatic side chain. *Journal of Food Lipids*, **15**: 251–262.
- Kulawinek, M., Jaromin, A., Kozubek, A., and Zarnowski, R. (2008) Alkylresorcinols in Selected Polish Rye and Wheat Cereals and Whole-Grain Cereal Products. *Journal of Agricultural and Food Chemistry*, **56**(16): 7236–7242.
- Laaksonen, D.E., Toppinen, L., Juntunen, K.S., Autio, K., Liukkonen, K.-H., Poutanen, K.S., Niskanen, L., and Mykkänen, H.M. (2005) Dietary carbohydrate modification enhances insulin secretion in persons with the metabolic syndrome. *American Journal of Clinical Nutrition*, **82**(6): 1218–1227.
- Landberg, R., Szwajcer Dey, E., Francisco, J.D.C., Åman, P., and Kamal-Eldin, A. (2007) Comparison of supercritical carbon dioxide and ethyl acetate extraction of alkylresorcinols from wheat and rye. *Journal of Food Composition and Analysis*, **20**: 534–538.
- Landberg, R., Kamal-Eldin, A., Andersson, A., Vessby, B., and Åman, P. (2008) Alkylresorcinols as biomarkers of whole grain wheat and rye intake: Plasma concentration and intake estimated from dietary records. *American Journal of Clinical Nutrition*, **87**(4): 832–838.
- Landberg, R., Xavier Autio, K., Kamal-Eldin, A., and Åman, P. (2008) Localization of alkylresorcinols in barley, wheat and rye kernels. *Journal of Cereal Science*, **48**: 401–406.
- Landberg, R., Andersson, A.A.M., Åman, P., and Kamal-Eldin, A. (2009) Comparison of GC and colorimetry for the determination of alkylresorcinol homologues in cereal grains and products. *Food Chemistry*, **113**: 1363–1369.
- Leinonen, K.S., Poutanen, K.S., and Mykkänen, H.M. (2000) Rye Bread Decreases Serum Total and LDL Cholesterol in Men with Moderately Elevated Serum Cholesterol. *Journal of Nutrition*, **130**: 164–170.
- Luthria, D.L., Mukhopadhyay, S., and Kwansa, A.L. (2006) A systematic approach for extraction of phenolic compounds using parsley (*Petroselinum crispum*) flakes as a model substrate. *Journal of the Science of Food and Agriculture*, **86**(9): 1350–1358.
- Mattila, P., Pihlava, J.M., and Hellström, J. (2005) Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, **53**: 8290–8295.
- Michalska, A., Ceglinska, A., Amarowicz, R., Piskula, M.K., Szawara-Nowak, D., and Zielinski, H. (2007) Antioxidant Contents and Antioxidative Properties of Traditional Rye Breads. *Journal of Agricultural and Food Chemistry*, **55**(3): 734–740.
- McIntosh, G.H., Noakes, M., Royle, P.J., and Foster, P.R. (2003) Whole-grain rye and wheat foods and markers of bowel health in overweight middle-aged men. *American Journal of Clinical Nutrition*, **77**(4): 967–974.
- Nicholson, R.L. and Hammerschmidt, R. (1992) Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology*, **30**: 369–389.

- Nyström, L., Lampi, A.-M., Andersson, A.A.M., Kamal-Eldin, A., Gebruers, K., Courtin, C.M., Delcour, J.A., Li, L., Ward, J.L., Fraš, A., Boros, D., Rakszegi, M., Bedő, Z., Shewry, P., and Piironen, V. (2008) Phytochemicals and Dietary Fiber Components in Rye Varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, **56**: 9758–9766.
- Peñalvo, J.L., Haajanen, K.M., Botting, N., and Adlercreutz, H. (2005) Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry*, **53**: 9342–9347.
- Peñalvo, J.L. and Nurmi, T. (2006) Application of coulometric electrode array detection to the analysis of isoflavonoids and lignans. *Journal of Pharmaceutical and Biomedical Analysis*, **41**(5): 1497–1507.
- Rejman, J. and Kozubek, A. (1997) Long-chain orcinol homologs from cereal bran are effective inhibitors of glycerophosphate dehydrogenase. *Cellular & Molecular Biology Letters*, **2**: 411–419.
- Robbins, R.J. (2003) Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, **51**: 2866–2887.
- Ross, A.B., Shepherd, M.J., Schupphaus, M., Sinclair, V., Alfaro, B., Kamal-Eldin, A., and Åman P. (2003) Alkylresorcinols in cereals and cereal products. *Journal of Agricultural and Food Chemistry*, **51**: 4111–4118.
- Ross, A.B., Åman, P., Andersson, R., and Kamal-Eldin, A. (2004) Chromatographic analysis of alkylresorcinols and their metabolites. *Journal of Chromatography*, **A 1054**(1–2): 157–164.
- Stasiuk, M., Bartosiewicz, D., and Kozubek, A. (2008) Inhibitory effect of some natural and semisynthetic phenolic lipids upon acetylcholinesterase activity. *Food Chemistry*, **108**(3): 996–1001.
- Vinkx, C.J.A. and Delcour, J.A. (1996) Rye (*Secale cereale* L) arabinoxylans: a critical review. *Journal of Cereal Science*, **24**: 1–14.
- Willför, S.M., Smeds, A.I., and Holmborn, B.R. (2006) Chromatographic analysis of lignans. *Journal of Chromatography*, **A 1112**(1–2): 64–77.

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# 7 Bioactive compounds in corn

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## 7.1 Introduction

A large body of epidemiological, clinical and experimental studies have well demonstrated that consumption of whole grain and derived food products is associated with reduced risk of various chronic and metabolic diseases, such as cardiovascular disease (Tighe *et al.*, 2007; Mellen, Walsh, and Herrington, 2008), obesity (Melanson *et al.*, 2006; Lutsey *et al.*, 2007), type 2 diabetes (de Munter *et al.*, 2007; Fisher *et al.*, 2009) and certain cancers (Schatzkin *et al.*, 2007, 2008). Although the whole grains possess numerous health-promoting effects for human beings, the average intake of whole grains is still much less than the recommended amount per day in many countries, especially in the United States of America where 90% of Americans do not meet the standards (Liu, 2003, 2004).

Corn (*Zea mays L.*), also known as maize, is one of the cereal grains domesticated in Mesoamerica and subsequently cultivated throughout the American continent. As one of the major cereal grains, corn is a staple food for large groups of people in Latin America, Asia, and Africa. The annual global production of corn is about 780 million metric tons, of which the United States and China produce more than 40% and 20%, respectively ([http://en.wikipedia.org/wiki/Maize#Chemicals\\_and\\_medicines](http://en.wikipedia.org/wiki/Maize#Chemicals_and_medicines)). Corn is proverbially utilized directly for human food all over the world. In the United States, corn is widely processed into various types of products, such as cornmeal, grits, starch, flour, snacks, tortillas, and breakfast cereals, and is also generally used for animal feed. However, with increasing attention being drawn to the development of nutraceuticals in this decade, the bioactive compounds derived from corn and their health properties have recently become the major focus of studies on corn. Thus, this chapter aims to discuss the major bioactive compounds in corn and summarize their health-promoting effects, in order to help readers better understand the nutritional and health properties of corn and consequently improve the consumption of corn.

## 7.2 Phytochemicals in corn and their health benefits

Phytochemicals are defined as bioactive nonnutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic

diseases (Liu, 2004). Numerous epidemiological studies have been carried out to reveal the inverse correlation between the consumption of phytochemicals and the development of chronic diseases including cardiovascular disease and cancer. Among these studies, the most attention has been focused on exploring both the *in vitro* and *in vivo* functional value of phytochemicals and antioxidants derived from vegetables, fruits, and other plant materials. However, the phytochemicals and antioxidants in whole grains have received less attention, and sometimes been underestimated by the scientists. Most recent research has suggested that the phytochemicals in grains demonstrate equally, or even more, significant beneficial contribution in reducing the risk of many diseases due to their potent antioxidant activities (Shahidi, 2009; Liu, 2007; Madhujith and Shahidi, 2007).

Beyond serving as a pivotal source of both basic micro- and macronutrients, corn is an essential source of various phytochemicals, such as carotenoids (Kopsell *et al.*, 2009), phenolic compounds (Pedreschi and Cisneros-Zevallos, 2007; Nesci and Etcheverry, 2009; Lopez-Martinez *et al.*, 2009), and phytosterols (Jr Ostlund *et al.*, 2002; Jiang and Wang, 2005). All of these phytochemicals contribute to various beneficial effects of corn.

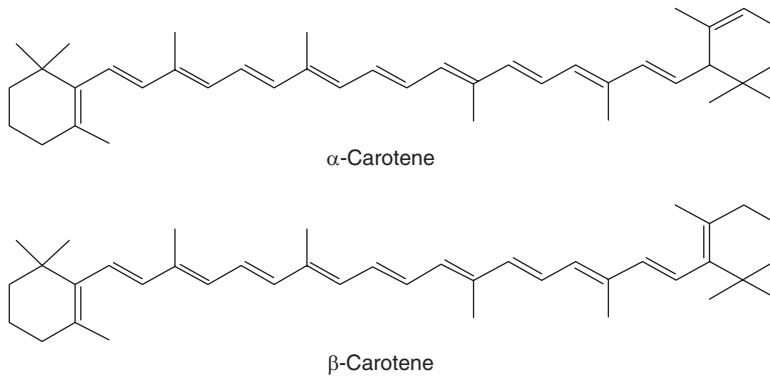
## 7.2.1 Carotenoids

Carotenoids are of a family of yellow, orange, and red organic and natural pigments. They are subcategorized to tetraterpenoids with a polyene chain 40 carbon atoms, and structurally in the form of a polyene chain, which is sometimes terminated by rings. There are more than 600 known carotenoids most widespread via modification of the molecular skeleton by cyclization, substitution, elimination, addition, and rearrangement and these minute structural variations lead to differences in their individual biological activities. Carotenoids can be divided into two classes, carotenes (which are purely hydrocarbons, and contain no oxygen) and xanthophylls (which contain oxygen). Numerous epidemiological studies have well documented that daily consumption of carotenoids-rich foods are beneficial for prevention of various diseases in humans, including cancer and chronic diseases (Kritchevsky, 1999; Michaud *et al.*, 2000). However, human beings are not able to biosynthesize carotenoids and have to assimilate them from diets. Thus, it is of pivotal significance to enhance dietary supplementation of carotenoids from various foods, especially whole grains. Yellow corn is one of the grains containing large quantities of the carotenoid pigments, mostly in the horny and flourey endosperm of the kernel (Liu, 2007).

### 7.2.1.1 A. Carotene

Yellow corn, corn silage, and stalklage are good sources of provitamin, 22, 17.3, and 6.5 mg/kg, respectively (Watson and Ramstad, 1987). Most carotenes, such as  $\alpha$ -carotene and  $\beta$ -carotene (Figure 7.1), possess provitamin A activity, indicating that they are able to be metabolized in the gut and tissues of animals to vitamin A.

The cancer preventive activities of carotenes have been widely reported since the 1990s. The inhibitory effects of  $\beta$ -carotene on preneoplastic lesions induced by resistant hepatocyte model in Wistar rats were reported by Moreno *et al.* (1991). The authors found that oral administration of  $\beta$ -carotene to rats could reduce the incidence, multiplicity, total number, and size of hepatocyte nodules. They suggested that continuous long-term consumption of carotenoids would confer a greater degree of protection against neoplasia. Another study was further carried out to elucidate the inhibitory mechanism of  $\beta$ -carotene against resistant



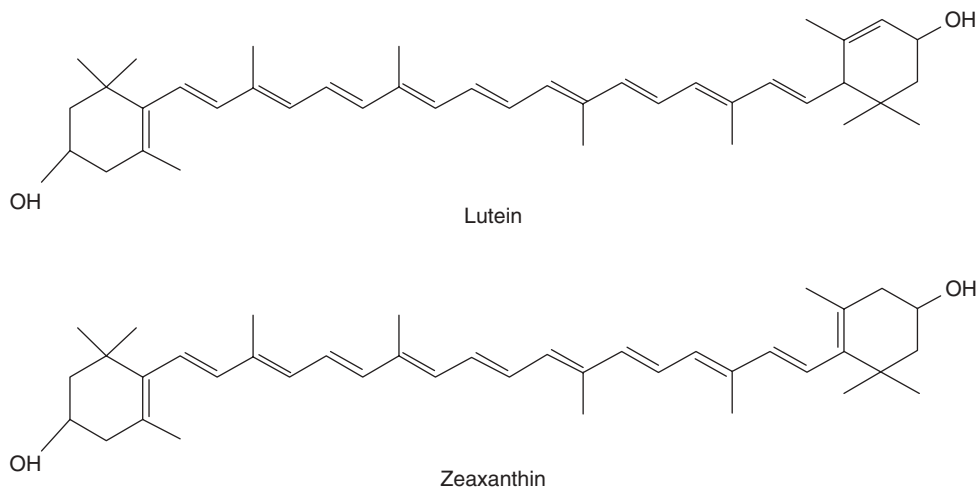
**Figure 7.1** Chemical structure of major carotenes in corn.

hepatocyte model of carcinogenesis (Naves *et al.*, 2001). By observing morphology of livers of Wistar rats treated with  $\beta$ -carotene, it was pointed out that  $\beta$ -carotene reduced not only the preneoplastic lesions but also the ductular (oval) cell reaction, which proliferated in the liver during rat and mouse hepatocarcinogenesis. Recently, a high concentration of  $\beta$ -carotene was observed to act as a pro-antioxidant and was proved to induce apoptosis of certain cancer cells, such as colon cancer cells (Palozza *et al.*, 2001), melanoma cancer cells, leukemia cells (Palozza *et al.*, 2001, 2003), and gastric cancer cells (Jang *et al.*, 2009); thus, it may render potent chemopreventive effect. However, a diet with a high dose of  $\beta$ -carotene might not be appropriate for smokers, since it would increase lung cancer incidence among male smokers (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994; Omenn *et al.*, 1996; Duffield-Lillico and Begg, 2004).

#### 7.2.1.2 B. Xanthophylls (*lutein and zeaxanthin*)

Unlike carotenes, xanthophylls (lutein and zeaxanthin, Figure 7.2) do not possess provitamin A activity. In the early days, xanthophylls did not receive much attention and even reported not making a contribution to any nutritional need of known importance (Watson and Ramstad, 1987). However, increasing epidemiological studies on lutein and zeaxanthin have been carried out to discover some specific and pivotal biological functions. In 1996, Martin *et al.* (1996) performed a series of parallel studies to compare the antioxidant activities of  $\beta$ -carotene and lutein. They discovered that both  $\beta$ -carotene and lutein could significantly protect HepG2 cells against oxidant-induced damage and that the protective effect was independent of provitamin A activity. Chew *et al.* (1996) investigated the biological effects of dietary lutein. Through *in vivo* animal experiments, they found that dietary lutein supplementation in food could significantly increase tumor latency and inhibit mammary tumor growth in a dose-dependent manner, and consequently lower the incidence of palpable tumor and enhance lymphocyte proliferation. Moreover, since lutein and zeaxanthin are found to be the only carotenoids in the macular of the retina that are responsible for sharp and detailed vision, their effects on prevention of age-related macular degeneration has drawn increasing interest in recent decades. By supplementing lutein to their subjects' diets for a period, Landrum and others (1997a, 1997b) observed a significant enhancement in macular pigment optical density and thus notable protection of the macula from light



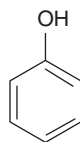


**Figure 7.2** Chemical structure of major xanthophylls in corn.

damage. In order to investigate the response to dietary lutein and zeaxanthin from corn in other tissues, another study (Johnson *et al.*, 2000) was carried out among human subjects who consumed corn containing high lutein and zeaxanthin in a long-term investigation. They found that consumption of lutein and zeaxanthin had a significantly different effect on men and women regarding the correlation between lutein concentrations in adipose tissue and macular pigment density (negative relation for women, positive relation for men). They suggested that this may be caused by different lutein metabolism in men and women.

Recent studies on novel health-promotion effects of lutein and zeaxanthin from corn have been carried out. Because of the polyene chain structure in carotenoids, most studies have focused on the conjugated double bonds with respect to the nutritional properties of carotenoids. In order to target on the activity-structure relationship of lutein and zeaxanthin, Sun and Yao (2007) investigated the role of the hydroxyl groups by di-acetylation in the anti-tumor activities of lutein or zeaxanthin extracted from corn protein residues. They studied the differences of the anti-proliferative effects on the human mouth epithelial cancer line KB cell between structurally modified di-acetylation lutein or zeaxanthin and original counterparts. Through their study, it was suggested that hydroxyl groups were responsible for the anti-tumor activity of lutein and zeaxanthin. Additionally, lutein was also found to act as a cancer chemopreventive suppressing agent, presenting inhibitory actions during promotion but not initiation of hepatocarcinogenesis (Moreno *et al.*, 2007).

Due to the availability of more and more data on their nutritional properties, methods to extract and refine lutein and zeaxanthin from corn are becoming an interesting topic of investigation. During this decade, an increasing number of researchers have focused on some specific concerns on recovering and extracting lutein and zeaxanthin from corn. Corn gluten meal, as the major by-product during the wet milling process of corn starch production, contains a high concentration of carotenoids (200–400 µg/g) with 92% xanthophylls. Since the early days, many researchers have reported processes for recovering xanthophylls from corn gluten meal (Muralidhara, 1997; Cheryan, 2001). Nevertheless, because carotenoids can bind to zein, they cannot be completely and effectively extracted from corn gluten meal. Recently, Lu and others (2005) applied enzymatic treatment to the extraction of lutein and



**Figure 7.3** The basic and simplest structure of phenol.

zeaxanthin from corn gluten meal. With protease pretreatment (Neutrase 0.5L) under appropriate conditions, the yields of crude lutein, crude zeaxanthin, and total carotenoids were increased by approximately 50–100%, compared with traditional extraction processes. To better investigate lutein and zeaxanthin in eye health and their anti-cancer activity, several methods have recently been reported for rapid extraction and HPLC analysis of individual xanthophylls (Moros *et al.*, 2002; Kale and Cheryan 2007; Perry *et al.*, 2009). Furthermore, in corn-based products, the content, structure, and nutritional properties of xanthophylls may vary from case to case, according to their processing and preparation procedures and food formulation and processing conditions. For instance, it was suggested that during the wet milling of corn the xanthophylls would go with the zein protein fraction, which might end up in the corn gluten meal (Moros *et al.*, 2002). To prove this, the study compared the concentration of xanthophylls in different corn products, and discovered that the total concentration of xanthophylls in corn gluten meal was seven times higher than that in whole corn; and it also pointed out that zein protein proportion (dry basis) in corn gluten meal was almost eight times higher than that in whole corn. Another example is that the predominant isomeric xanthophyll form is *trans* for all foods, but processed foods contain more *cis* isomeric than original vegetables and fruits (de Oliveira and Rodriguez-Amaya, 2006; Wang *et al.*, 2008; Perry *et al.*, 2009).

## 7.2.2 Phenolic compounds

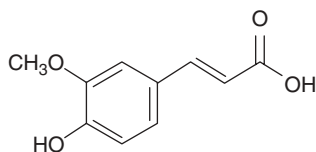
Phenolic compounds are a class of chemical compounds consisting of a hydroxyl group (–OH) attached directly to an aromatic hydrocarbon moiety (Figure 7.3). They are usually specified as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Liu, 2004). Food grains, such as corn, wheat, rice, and barley, among others, are the major sources of polyphenolic compounds. Corn, especially corn bran, a main dietary fibre, is shown to be the most abundant source of polyphenolic compounds among various grains investigated (Adom and Liu, 2002; Zhao *et al.*, 2005). However, the contents and types of phenolic compounds in corn are affected by varieties and the cultivation conditions. The dominant phenolic compounds found in corn and their health benefits are shown in Table 7.1. The following sections will discuss the health benefits of each major phenolic compound from corn in detail.

### 7.2.2.1 A. Ferulic acid (FA)

FA (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous phenolic compound mostly found in plant tissues. It is one of the major bioactive ingredients of many staple foods, including grain bran, and whole grain foods, among others. FA acts in the cross-linking of plant cell walls and is the precursor of numerous compounds that are playing important roles in the defense system of plants. It occurs in two forms: free form and conjugated form, which is covalently linked to lignin and other biopolymers; the total of these two forms represents

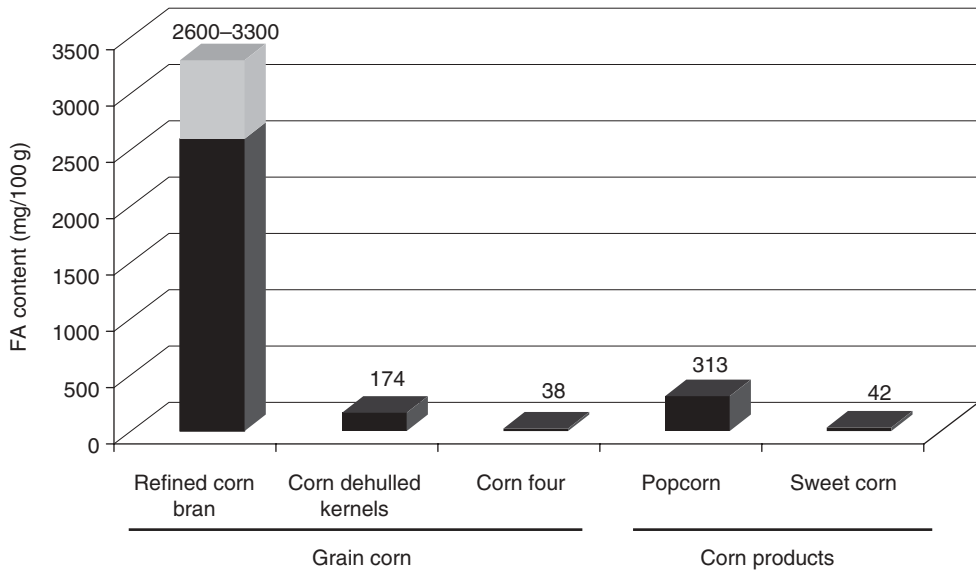
**Table 7.1** The major health compounds in corn and their health benefits

Major health compounds in corn	Health benefits	Corn genotype
Carotenoids (Lutein, zeaxanthin, $\beta$ -cryptoxanthin, $\beta$ -carotene)	Cancer preventive activities (Moreno <i>et al.</i> , 1991), protection against age-related muscular degeneration (Landrum and Bone, 2004; Bone <i>et al.</i> , 2007); inhibitory effect against promotion of hepatocarcinogenesis (Moreno <i>et al.</i> , 2007)	Yellow, red corn
Ferulic acid	Anti-inflammatory (Ou <i>et al.</i> , 2003), anti-colon carcinogenesis (Imaida <i>et al.</i> , 1990), and anti-diabetic effects through stimulating insulin secretion (Noumura <i>et al.</i> , 2003)	Yellow, orange, and white corn
Anthocyanins	Inhibits colorectal carcinogenesis (Hagiwara <i>et al.</i> , 2001); antimutagenic and antioxidant (Pedreschi and Zevallos 2006); prevents obesity and ameliorates hyperglycemia (Tsuda <i>et al.</i> , 2003); antimicrobial (Cevallos-Casals and Cisneros-Zevallos, 2003); gastroprotection (Matsumoto <i>et al.</i> , 2004)	Red, blue, purple, black corn
Phytosterols	Decrease serum total LDL-cholesterol (Jiang and Wang, 2005); Inhibit adsorption of dietary cholesterol and biosynthesis of cholesterol (Jr Ostlund <i>et al.</i> , 2002)	Whole corn kernel (no specific color)

**Figure 7.4** Chemical structure of ferulic acid.

the total FA. It is one of the metabolites of the biosynthesis of lignin from tyrosine and phenylalanine in plants. The structure of FA is illustrated in Figure 7.4. FA possesses potent antioxidant properties, due to its distinctive methoxy and hydroxyl functional groups, both of which are electron donating substituents. Additionally, adjacent to the carboxyl group is the unsaturated C-C double bond that can act as extra attack sites for free radicals and, therefore, protect the membrane against oxidation. The carboxylic acid also plays a role as the anchor of FA so that it can bind to the lipid bilayer, thus preventing lipid peroxidation. All of these distinctive structural properties endow FA a potent antioxidant against various free radicals (Graf, 2000; Kanaski *et al.*, 2002).

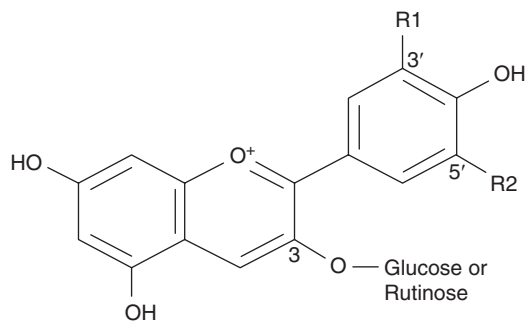
The bioavailability of free FA is of great significance to exert its beneficial bioactivities *in vivo*. Free FA is reported to be more bioavailable than most other dietary phenolic compounds studied so far, since it stays in the blood for a much longer time than other antioxidants and consequently is able to maintain an effective concentration in the body long enough to protect against free radicals attack (Srinivasan *et al.*, 2007). Because of its potent antioxidant properties, considerable public and scientific interests have been drawn to research of health-promoting effects of FA derived from foods in recent years, including anticancer (Kawabata



**Figure 7.5** The content of ferulic acid in corn and related food products. Zhao *et al.* (2005), Mattila *et al.* (2005), Adom and Liu (2002), Saulnier and Thibault (1999), and Nishizawa *et al.* (1998). The data are expressed by mean or a range of mean values reported as total ferulic acid.

*et al.*, 2000), anti-inflammatory (Ou *et al.*, 2003), preventive effects against bone loss (Sassa *et al.*, 2003), antidiabetic (Balasubashini *et al.*, 2004), and hepatoprotective effects (Rukkumani *et al.*, 2004). Among various foods investigated, grains are the major source of FA. Particularly, refined corn bran contains the highest FA content, followed by barley and wheat (Zhao and Moghadasian, 2008). The FA content of corn and related products are summarized in Figure 7.5.

The bioavailability of FA from corn is dependent on many factors, such as the existing forms of FA and daily intake, both of which would further affect the profile of FA in the gastrointestinal tract and its systemic concentrations (van der Logt *et al.*, 2003; Zhao *et al.*, 2003). The bioavailability of FA from corn is lower than free FA because of the conjugation with arabinose or arabinoxylan from corn bran (Rondini *et al.*, 2004). The form of bound-FA, especially with complex polymers, would reduce the effect of hydrolyzing enzymes, resulting in a low absorbability. Therefore, FA in corn bran binding with more complex heteroxylans has lower absorbability than FA in other grains, which binds with simple structure polymers (Adam *et al.*, 2002; Zhao *et al.*, 2005). Since FA is often bound with structural polysaccharides or hemicellulose through ester linkages, both chemical and enzymatic treatments are applied to release FA and then to use it in further transformations. FA can be recovered from corn residues (corn bran and corn fibers) by enzymatic approaches, using certain fungus capable of producing a full complement of enzymes to release FA (Shin *et al.*, 2006). Through the chemical process, FA can also be obtained from corn cobs by NaOH hydrolysis (Torre *et al.*, 2008). Bound-FA was recently used for enzymatic production of vanillin, and the yield of vanillin from corn bran bound-FA was higher than that from wheat bran and flax shrives (Buranov and Mazza, 2009).



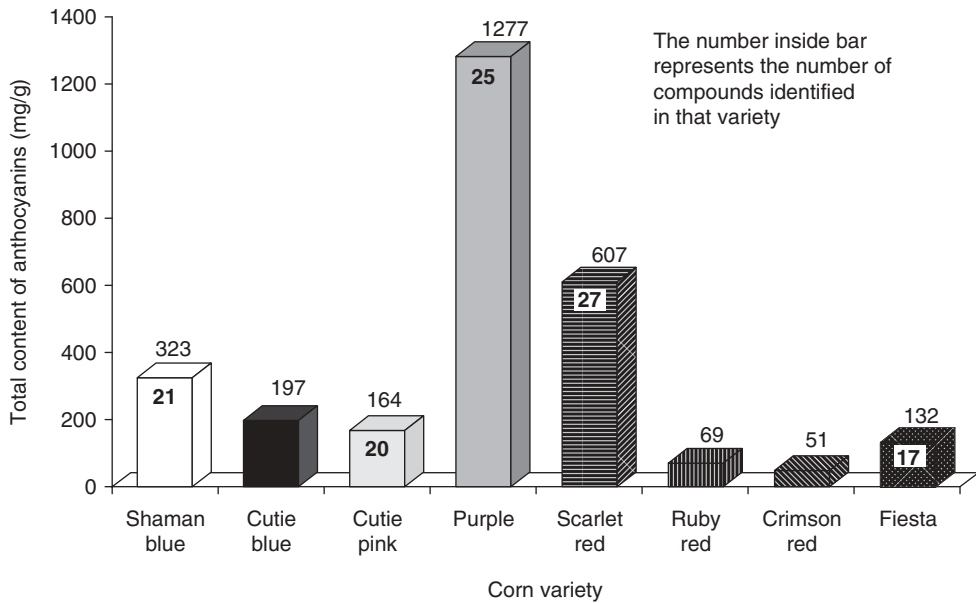
Anthocyanidin	R1	R2	$\lambda$ max (nm)
Pelargonidin-3-glucoside	H	H	520
Cyanidin-3-glucoside	OH	H	535
Peonidin-3-glucoside	OCH <sub>3</sub>	H	532
Delphinidin-3-glucoside	OH	OH	546
Petunidin-3-glucoside	OCH <sub>3</sub>	OH	543
Malvidin-3-rutinoside	OCH <sub>3</sub>	OCH <sub>3</sub>	542

**Figure 7.6** Chemical structure of common anthocyanins in corn.

### 7.2.2.2 B. Anthocyanin

Anthocyanins are the largest group of water-soluble plant pigments from reddish to purple, belonging to the most common class of phenolic compounds, also known collectively as flavonoids. They are water-soluble glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. Due to the different number and position of hydroxyl groups and their degree of methylation on flavan nucleus, six anthocyanidins commonly found in plants have been classified (Figure 7.6). The characteristics of anthocyanidins are also determined by the nature, number, and location of sugars attached to the molecule; and aliphatic or aromatic acids attached to the sugars in the molecules (Ghosh and Konishi, 2007).

Anthocyanidins are the major pigments in colored cereal grains, among which corn has been proven to be the cereal containing the second highest concentration and most abundant types of anthocyanins (Abdel-Aal *et al.*, 2006). The total content and types of anthocyanins vary among different corn varieties with various colors. The anthocyanins profile is summarized in Figure 7.7. Corns with deep color contain a higher total content of anthocyanins. Especially, the purple corn contains the most total content among all corn varieties reported, being two times greater than the scarlet red corn containing second most total content of anthocyanins. Due to the abundant content of anthocyanins, purple and scarlet red corn are the two that are being investigated most, and, until now, 25 and 27 anthocyanins have been identified in these two corns, respectively. The most popular anthocyanin compounds in corn have been identified as, cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, pelargonidin-3-(6''-malonylglucoside), cyanidin-3-(6''-malonylglucoside), and cyanidin-3-(3'',6''-dimalonylglucoside), cyanidin-3-(3'',6''-malonylglucoside), and cyanidin-3-(3'',6''-dimalonylglucoside), among which the cyanidin derivatives are major ones accounting for more than 70% in kernel (pericarp and aleurone layer), seed, and cob of purple



**Figure 7.7** Total content and number of compounds of anthocyanins (mg/g) in various corn varieties according to Abdel-Aal *et al.* Abdel-Aal *et al.* (2006).

corn (Aoki *et al.*, 2002; Moreno *et al.*, 2005). The different varieties, growing conditions as well as extraction procedures of purple corn may also impact the anthocyanin profiles (de Pascual-Teresa *et al.*, 2002; Gonzalez-Manzano *et al.*, 2008; Zhao *et al.*, 2009). For example, the Chinese purple corn hybrids possess greater total anthocyanin content, showing 2.5-, 3-, and 10-fold higher than the pigmented corns from Canada, Mexico, and the USA, respectively (Zhao *et al.*, 2009).

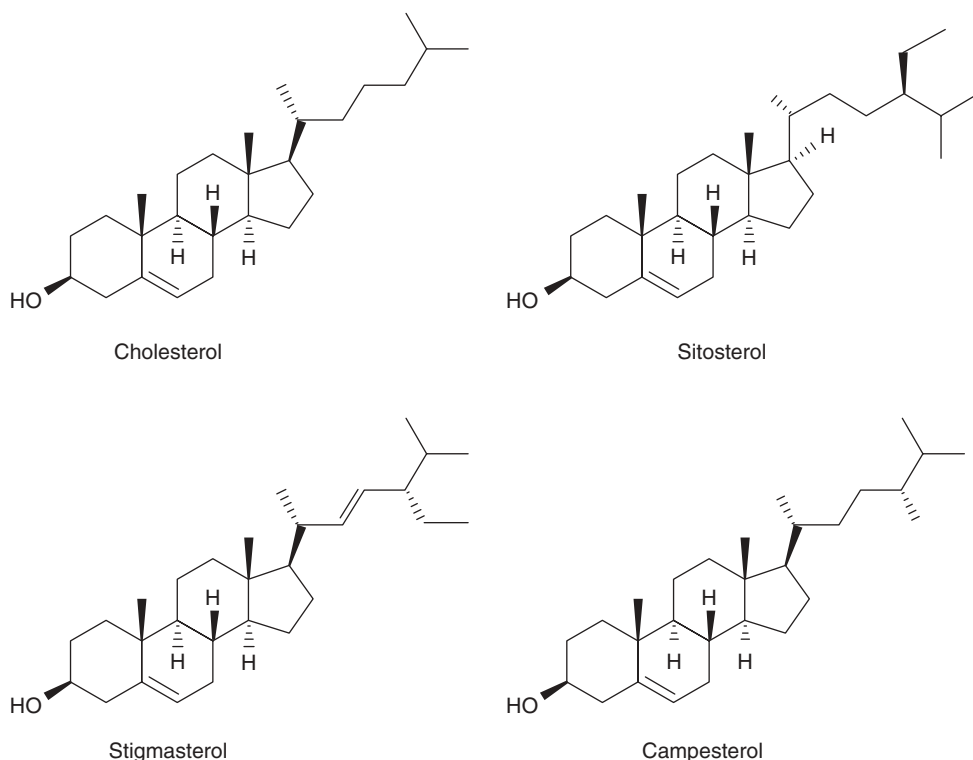
Not only considered as desirable natural colorants and value-added ingredients, anthocyanins have also been well known for their health-promoting properties. Briefly, anthocyanins from various colored vegetables and fruits are well documented to have antineoplastic, anticarcinogenic, antiatherogenic, lipid lowering, antidiabetic, antimicrobial, and anti-inflammatory properties, and they are also able to decrease capillary permeability and fragility, inhibit platelet aggregation and immune stimulation, all of which are mainly attributed to the potent antioxidant properties (Stintzing and Carle, 2004; Ghosh and Konishi, 2007). To be more specific, the beneficial effects of anthocyanins derived from purple corn, have been well addressed in recent years. A study investigated antimicrobial activity and antiproliferative capacity of the anthocyanin extracts from five purple corn hybrids (*Zea mays L.*) from China (Zhao *et al.*, 2009). The study found that these purple corns possess antimicrobial activity against *S. enteritidis*, *S. aureus*, and *C. albicans* except for *E. coli*, and demonstrated inhibitory effect against proliferation of colon cancer cell (HT-29). Both of the activities were dose dependent and related to the total anthocyanins content. The antiproliferative effect against HT-29 of anthocyanins from purple corn was most potent among seven fruits and vegetables investigated by Jing *et al.* (2008), including three berries, two carrots, and one grape. The authors found a significant relationship between the

chemoprotective activities of anthocyanins and multiple characteristics of anthocyanins in these fruits and vegetables, such as the type of aglycones, sugars, and acylated acids, and the position and degree of glycosylation and acylation. Some other researchers have suggested that the effects of anthocyanins structures on their biological activities are also dependent on different experimental models employed (Yoshimoto *et al.*, 2001; Zhang *et al.*, 2005). Besides the *in vitro* investigation, the *in vivo* studies have also been extensively carried out. The consumption of the anthocyanins from purple corn at a dietary level of 5% demonstrated a pronounced inhibition of PhIP-associated colorectal carcinogenesis in male F344 rats, showing that the lesion development of colon was significantly suppressed during 36-week administration (Hagiwara *et al.*, 2001). In another *in vivo* study, the obesity and hyperglycemia model was induced by a high fat diet in male C57BL/6 mice, and the cyanidin 3-glucoside-rich purple corn color was added to diets at a cyanidin 3-glucoside concentration of 2 g/kg diet in a 12-week experiment (Tsuda *et al.*, 2003). The results suggested that dietary supplementation of purple corn pigment might ameliorate high fat diet-induced insulin resistance in mice, via suppressing the fatty acid and triacylglycerol biosynthesis and hence reducing the triacylglycerol accumulation in white adipose tissue. The dietary administration of purple corn pigment has also been recently reported to have anti-hypertensive effects on spontaneously hypertensive male rats, through lowering the systolic blood pressure (Shindo *et al.*, 2007). The pigments from black glutinous corn cob have been recently demonstrated to possess potent anti-hyperlipidemia effects in high-fat-fed mice, through improving the serum lipids profile and reducing the atherogenic index (Zhang *et al.*, 2010).

### 7.2.3 Phytosterols

Phytosterols, known as plant sterols, are the minor constituents of vegetable oils, but they are also the essential components of the plant cell walls and membranes (Piironen *et al.*, 2000). Until now, more than 250 different sterols and related compounds have been found in various plant and marine materials. Based on the number of methyl groups at the C-4 position, phytosterols can be categorized into three classes: the 4-desmethylsterols or simple sterols, the 4,4-dimethylsterols, and the 4-monomethylsterols (Grunwald, 1975). Several studies have pointed out that the oil extracted from whole corn kernel is very rich in phytosterols (Piironen *et al.*, 2000; Verleyen *et al.*, 2002). The distributions of phytosterols and phytostanols vary in different fractions of corn kernel, such as endosperm, pericarp, and germ (Harrabi *et al.*, 2008). The most commonly consumed phytosterols from corn oils are sitosterol, stigmasterol, and campesterol, all of which belong to the class of 4-desmethylsterols. The chemical structures of these phytosterols are very similar to cholesterol, only differing in the side chain. As shown in Figure 7.8, at the C-24 position, sitosterol and stigmasterol have an ethyl group while campesterol has a methyl group.

The beneficial effects of phytosterols have already been noted and dietary consumption of phytosterol is shown to be negatively related to cholesterol absorption and serum total and LDL cholesterol (Moghadasian and Frohlich, 1999; Jiang and Wang, 2005). Due to similar structure with cholesterol, the major underlying mechanism involved in the health benefits of dietary phytosterols is the inhibition of cholesterol absorption through intestine and subsequent compensatory stimulation of the synthesis of cholesterol, resulting in the enhanced elimination of cholesterol in stools. To test the contribution of phytosterols in corn



**Figure 7.8** The chemical structures of cholesterol and phytosterols.

oil on cholesterol-lowering effect, one study compared cholesterol absorption between the human subjects who consumed original and phytosterol-removed commercial corn oil (Jr Ostlund *et al.*, 2002). The study reported that the cholesterol absorption of healthy subjects was 38% higher in the group consuming the phytosterol-removed commercial corn oil than the group consuming the original commercial corn oil for two weeks. When corn oil phytosterols were added back to phytosterol-removed corn oil, the cholesterol absorption was reduced significantly again. Thus, consumption of corn oil in a long-term period can reduce cholesterol concentrations and prevent atherosclerotic disease.

## 7.2.4 Other phytochemicals in corn

Besides the major phytochemicals discussed above, many other phytochemicals also exist in corn, which may also contribute to the beneficial effects. These compounds include the phenolic compounds distributed in corn leaf and silk, such as *p*-hydroxybenzoic acid, vanillic, protocatechuic, syringic, *p*-coumaric, sinapic, and chlorogenic acids, among others (Sosulski *et al.*, 1982; Gueldner *et al.*, 1992; Pedreschi and Cisneros-Zevallo, 2007). However, research on the beneficial effects of these phytochemicals, which are specifically derived from corn, are still very limited, partly due to their low content in corn.



### 7.3 Corn resistant starch and bioactivities

Resistant starch (RS) is the starch that may escape digestion in the small intestine and potentially acts as major contributor of fermentable carbohydrate in the large intestine. RS is also early defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Asp, 1992). So far, four major subtypes of RS have been recognized based on their nature, structure, and source (Brown *et al.*, 1995), as shown in Table 7.2. Consumption of RS has been well documented to have various beneficial effects, such as lowering cholesterol, enhancing fecal excretion of cholesterol, altering microbial populations, increasing the fermentation and short-chain fatty acid production in large intestine, and reducing symptoms of diarrhea, all of which will lead to the reduced risk of cecal cancer, atherosclerosis, and obesity-related complications. (Annison and Topping, 1994; Asp, 1997; Murphy *et al.*, 2008). The recommended intake of dietary fiber in the United States varies from 19 to 38 g per day for the population older than one year, however, fewer than 5% of Americans consume this much fiber daily; more specifically, the intake of RS by Americans is approximately only 3–8 g per person per day (Murphy *et al.*, 2008). These current data indicate a huge gap between recommended and actual intakes. Therefore, the research on RS has never ceased and much effort should be given to advocate the positive relationship between consumption of RS and health benefits. Among various RS investigated, RS from corn, also called high-amylose maize, has been the most extensively studied one so far.

The consequences of consuming RS from high-amylose corn in clinical studies have been well established. Murphy *et al.* (2008) summarized nine clinical trials and compared the effects of RS from high-amylose corn on colonic fermentation. Half of these studies found that the fecal weight was significantly increased and the fecal pH significantly decreased, partly due to the higher production of short chain fatty acids, especially the butyrates. However, all of these nine clinical studies focused on RS2 from high-amylose, and the results varied with the different subjects involved in the clinical trials. Regarding the beneficial effects of RS3, an early study on human flora-associated rats fed on diets containing RS3, the retrograded amylose starch, suggested that consumption of RS3 provided the greatest protection against DNA damage in the colonic mucosa induced by colon carcinogen (Rowland *et al.*, 1998). These authors also carried out another study and pointed out that the retrograded amylose starch RS3 performed different protective roles in the colon environment if human flora-associated rats were colonized with microfloras of subjects from different countries (Silvi *et al.*, 1999). The amylose starch has also been shown to enhance the desirable composition of colonic bacteria in mice, suggesting that it might possess potential prebiotic properties (Wang *et al.*, 2002). However, this effect was dependent on the type of RS as well as the modification methods (acetylation and carboxymethylation). A recent study found that the hydrothermal treatment (or heat-moisture) of high-amylose corn starch can result in more pronounced effects on promoting fermentation throughout the large bowel (in the distal region), compared to untreated starch (Bird *et al.*, 2007).

Another important functional property of RS is that consumption of dietary RS (from amylose corn) can influence cholesterol metabolism as well as lower the body fat storage, and hence reduce the risk of hyperlipidemia, atherosclerosis, diabetes, and obesity (Higgins, 2004). In 1997, the influence of raw (RS2) and retrograded (RS3) high amylose corn starch on cholesterol metabolism in both normal and hypercholesterolemic rats has been first studied (Vanhoof and de Schrijver, 1997). This study proved that RS exerted no effect on

**Table 7.2** The subtypes of resistant starch and their nature and source

Resistant subtype	Nature	Source
Resistant starch 1	Entrapped within food matrix, and physically inaccessible to digestive enzyme (amylase)	Whole or partly milled grains and seeds
Resistant starch 2	Resistant to digestion due to their granular structure	Raw potato, unripe banana, some legumes, and in high amylase starches ( from corn)
Resistant starch 3	Retrograded amylose and amylopectin formed during food processing	Cooked foods such as potatoes, breads, and cornflakes
Resistant starch 4	Produced by chemical modifications	With a wide variety of structures, not found in nature

(Murphy *et al.*, 2008).

cholesterol metabolism in normal rats; on the contrary, both types of RS showed significant influence on cholesterol metabolism in hyperlipidemic rats, via decreasing plasma total cholesterol concentrations and increasing fecal neutral steroid excretion. However, only RS3 was able to decrease liver cholesterol concentrations based on their results. The cholesterol-lowering effect of RS from corn has also been demonstrated in diabetic rats. In addition to improving plasma total lipid and cholesterol concentrations in this model, the RS from corn also shortened the intestinal transit time significantly (Kim *et al.*, 2003), resulting in a quicker time to eliminate the waste material through feces. As dietary fiber, RS can also be considered an alternative dietary carbohydrate for developing weight control. The two major mechanisms to explain the decreased body fat after consumption of RS could be that RS dilutes the energy density of the diet and reduce the food intake by modulating certain gene expressions. Some new studies have further developed and explained the underlying mechanisms in animal models. Inclusion of RS from corn in the diet of rats can affect energy balance through its effect as a fiber and a stimulator of gut peptide YY and glucagon-like peptide-1 expressions as well as other genes in the brain hypothalamic area (neuropeptide messenger RNA); all of which are the key factors for regulating energy homeostasis and reducing the food intake by increasing satiety (Keenan *et al.*, 2006; Shen *et al.*, 2009).

In addition to the above laboratory studies, a recent investigation examined the effects of different high-fiber foods on the satiety of healthy human subjects (with a visual analog scale questionnaire method) (Willis *et al.*, 2009). The results showed that eating muffins containing resistant starch and corn bran had the most impact on satiety compared with foods containing other fibers. What's more, the RS from amylose corn has recently been suggested to be potentially beneficial for improving insulin sensitivity in both animal and human subjects (Deng *et al.*, 2010; Johnston *et al.*, 2010). Based on all the above studies, there is no doubt that RS from amylose corn starch can be considered as a bioactive functional food for the treatment of obesity.

## 7.4 Future studies

When talking about the health benefits of corn, there is another important component derived from corn that should be highly considered: zein. Found in the endosperm of corn, zein is an alcohol soluble prolamine. It is a GRAS (generally recognized as safe) ingredient, and

possesses unique solubility and film forming properties. Thus, it has received increasing attention and become a popular research topic in recent years. Being a non-toxic and biodegradable corn protein, zein possesses great potential to act as a nano-scale biomaterial to provide various health benefits to human beings. The novel applications of zein in the pharmaceutical and nutraceutical areas are to coat nanoparticles (Luo *et al.*, 2010), develop promising antimicrobial agent in form of nano-composite (Zhang *et al.*, 2010), produce novel food packaging (Sanchez-Garcia *et al.*, 2010), encapsulate nutrients (Fernandez *et al.*, 2009; Jin *et al.*, 2009; Luo *et al.*, 2011), and provide target delivery with controlled release, (Lai and Guo, 2011). However, most of the studies on zein are conducted at a lab-scale level, due to the high cost of zein manufacture. With growing recognition of the health benefits provided by zein, more commercial ventures will be explored in the near future.

Nutritional properties and health benefits of nutraceuticals derived from corn have been receiving more and more interest around the world; however, we are still faced with various future problems. First, as mentioned above, the components of corn bran and corn fiber, both of which are low-value by-products during corn product development, have been known as good sources of nutraceuticals, while there are numerous technical challenges to be solved in order to commercialize the processes for the isolation or production from corn bran and corn fibre (Rose *et al.*, 2010). Thus, more knowledge is needed to promote utilization of the health-promoting effects of every part of corn. Second, although the evaluation of these nutraceuticals has been well established in various cell lines (*in vitro*) and animal models (*in vivo*), some of the underlying mechanisms are still inconclusive. Further evaluations should be carried out and compared in the same or similar experimental models, which are close to the human body system. More clinical trials in human subjects of these nutraceuticals are needed before any conclusion can be made.

## References

- Abdel-Aal, E.M., Young, C., and Rabbalski, I. (2006) Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, **54**: 4696–4704.
- Adam, A., Crespy, V., Levrat-Verny, M.A., Leenhardt, F., Leuillet, M., Demigne, C., and Remesy, C. (2002) The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. *The Journal of Nutrition*, **132**: 1962–1968.
- Adom, K.K., and Liu, R.H. (2002) Antioxidant activity of grains. *Journal of Agricultural Food Chemistry*, **50**: 6182–6187.
- Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine*, **330**: 1029–1035.
- Asp, N.G. (1992) *European Journal of Clinical Nutrition*, **46** (Suppl 2): S1.
- Annisson, G., and Topping, D.L. (1994) Resistant Starch - Proceedings from the 2nd Plenary Meeting of Eureka – European Flair Concerted Action 11 on Physiological Implications of the Consumption of Resistant Starch in Man. *Annual Review Nutrition*, **14**: 297–320.
- Aoki, H., Kuze, N., and Kato, Y. (2002) Anthocyanins isolated from purple corn (*Zea mays* L.). *Foods and Food Ingredients Journal of Japan*, **199**: 41–45.
- Asp, N.G. (1997) Resistant starch-an update on its physiological effects. *Advanced Experimental and Medicinal Biology*, **427**: 201–210.
- Balabusashini, M.S., Rukkumani, R., Viswanathan, P., and Menon, V.P. (2004) Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research*, **18**: 210–214.
- Bird, A.R., Vuaran, M., Brown, I., and Topping, D.L. (2007) Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. *British Journal of Nutrition*, **97**: 134–144.

- Brown, I.L., McNaught K.J., and Moloney R. (1995) Hi-maize: new directions in starch technology and nutrition. *Food Australia*, **47**: 272–275.
- Buranov, A.U., and Mazza, G. (2009) Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents. *Food Chemistry*, **114**: 1542–1548.
- Cheryan, M. (2001). Method for extracting xanthophylls from corn. Patent US6169217.
- Chew, B.P., Wong, M.W., and Wong, T.S. (1996) Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. *Anticancer Research*, **16**: 3689–3694.
- de Munter, J.S.L., Hu, F.B., Spiegelman, D., Franz M., and van Dam, R.M. (2007) Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. *Plos Medicine*, **4**: 1385–1395.
- de Pascual-Teresa, S., Santos-Buelga, C., and Rivas-Gonzalo, J.C. (2002) LC–MS analysis of anthocyanins from purple corn cob. *Journal of the Science and Food and Agriculture*, **82**: 1003–1006.
- de Oliveira, G.P.R., and Rodrigues-Amaya, D.B. (2006) Antioxidant Capacity of Extracts from Wild and Crop Plants of the Mediterranean Region. *Journal of Food Science*, **72**: S79–S85.
- Deng, J., Wu, X., Bin, S., Li, T.J., Huang, R., Liu, Z., Ruan, Z., Deng, Z., Hou, Y., and Yin, Y.L. (2010) Dietary amylose and amylopectin ratio and resistant starch content affects plasma glucose, lactic acid, hormone levels and protein synthesis in splanchnic tissues. *Journal of Animal Physiology and Animal Nutrition*, **94**: 220–226.
- Duffield-Lillico, A.J., and Begg, C.B. (2004) Reflections on the landmark studies of  $\beta$ -carotene supplementation. *Journal of the National Cancer Institute*, **96**: 1729–1731.
- Fernandez, A., Torre-Giner, S., and Lagaron, J.M. (2009) Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloids*, **23**: 1427–1432.
- Fisher, E., Boeing, H., Fritsche, A., Doering, F., and Joost, H.G. (2009) Whole-grain consumption and transcription factor-7-like 2 (TCF7L2) rs7903146: gene-diet interaction in modulating type 2 diabetes risk. *British Journal of Nutrition*, **101**: 478–481.
- Ghosh, D., and Konishi, T. (2007) Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition*, **16**: 200–208.
- Gonzalez-Manzano, S., Perez-Alonso, J.J., Salinas-Moreno, Y., Mateus, N., Silva, A.M.S., de Freitas, V., and Santos-Buelga, C. (2008) Flavanol-anthocyanin pigments in corn: NMR characterisation and presence in different purple corn varieties. *Journal of Food Composition and Analysis*, **21**: 521–526.
- Graf, E. (2000) Antioxidant potential of ferulic acid. *Free Radical Biology and Medicine*, **28**: 1249–1256.
- Grunwald, C. (1975) Plant sterols. *Annual Reviews Plant Physiology*, **26**: 209–236.
- Guedner, R.C., Snook, M.E., Widstrom, N.W., and Wiseman, B.R. (1992) TLC screen for maysin, chlorogenic acid, and other possible resistance factors to the fall armyworm and the corn earworm. *Journal of Agricultural and Food Chemistry*, **40**: 1211–1213.
- Hagiwara, A., Miyashita, K., Nakanishi, K., Sano, M., Tamano, S., Kadota, T., Koda, T., Nakamura, M., Imaida, K., Ito, N., and Shirai, T. (2001) Pronounced inhibition by a natural anthocyanin, purple corn color of 2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. *Cancer Letters*, **171**: 17–25.
- Harrabi, S., St-Amand, A., Sakouhi, F., Sebei, K., Kallel, H., Mayer P.M., and Boukhchin, S. (2008) Phytosterols and phytosterols distributions in corn kernel. *Food Chemistry*, **111**: 115–120.
- Higgins, J.A. (2004) Resistant starch: metabolic effects and potential health benefits. *Journal of AOAC International*, **87**: 761–768.
- Jang S.H., Lim, J.W., and Kim, H. (2009)  $\beta$ -carotene at high concentrations induces apoptosis by enhancing oxy-radical production in human adenocarcinoma cells. *Annual New York Academic Science*, **1171**: 156–162.
- Jiang, Y.Z., and Wang, T. (2005) Phytosterols in cereal by-products. *Journal of the American Oil Society*, **82**: 439–444.
- Jin, M.F., Davidson, P.M., Zivanovic, S., and Zhong, Q.X. (2009) Production of corn zein microparticles with loaded lysozyme directly extracted from hen egg white using spray drying: extraction studies. *Food Chemistry*, **115**: 509–514.
- Jing, P., Bomser, J.A., Schwartz, S.J., He, J., Magnuson, B.A., and Giusti, M.M. (2008) Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. *Journal of Agricultural and Food Chemistry*, **56**: 9391–9398.
- Johnson, E.J., Hammond, B.R., Yeum, K.Y., Qing, J., Wang, X.D., Castaneda, C., Snodderly, D.M., and Russell, R.M. (2000) Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *The American Journal of Clinical Nutrition*, **71**: 1555–1562.

- Johnston, K.L., Thomas, E.L., Bell, J.D., Frost, G.S., and Robertson, M.D. (2010) Resistant starch improves insulin sensitivity in metabolic syndrome. *Diabetic Medicine*, **27**: 391–397.
- Jr Ostlund, R.E., Racette, S.B., Okeke, A., and Stenson, W.F. (2002) Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. *American Journal of Clinical Nutrition*, **75**: 1000–1004.
- Kale, A.V., and Cheryan, M. (2007) Rapid analysis of xanthophylls in ethanol extracts of corn by HPLD. *Journal of Liquid Chromatography and Related Technologies*, **30**: 1093–1104.
- Kanaski, J., Aksenova, M., Stoyanova, A., and Butterfield D.A. (2002) Ferulic acid antioxidant protection against hydroxyl and peroxy radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure activity studies. *Journal of Nutritional Biochemistry*, **13**: 273–281.
- Kawabata, K., Yamamoto, T., Hara, A., Shimizu, M., Yamada, Y., Matsunaga, K., Tanaki, T., and Mori, H. (2000) Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Letters*, **157**: 15–21.
- Keenan, M.J., Zhou, J., McCutcheon, K.L., Raggio, A.M., Bateman, H.G., Todd, E., Jones, C.K., Tulley, R.T., Melton, S., Martin, R.J., and Hegsted, M. (2006) Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity*, **14**: 1523–1534.
- Kim, W.K., Chung, M.K., Kang, N.E., Kim, M.H., and Park, O.J. (2003) Effects of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations in steptozotocin-induced diabetic rats. *Journal of Nutrition Biochemistry*, **14**: 166–172.
- Kopsell, D.A., Arnel, G.R., Mueller, T.C., Sams, C.C., Deyton, D.E., Mcelroy, J.S., and Kopsell, D.E. (2009) Increase in nutritionally important sweet corn kernel carotenoids following mesotrione and atrazine applications. *Journal of Agricultural and Food Chemistry*, **57**: 6362–6368.
- Kritchevsky, S.B. (1999)  $\beta$ -carotene, carotenoids and the prevention of coronary heart disease. *Journal of Nutrition*, **128**: 5–8.
- Lai, L.F., and Guo, H.X. (2011) Preparation of new 5-fluorouracil-loaded zein nanoparticles for liver targeting. *International Journal of Pharmaceutics*, **404**: 317–323.
- Landrum, J.T., Bone, R.A., and Kilburn, M.D. (1997a) The macular pigment: a possible role in protection from age-related macular degeneration. *Advanced Pharmacology*, **38**: 537–556.
- Landrum, J.T., Bone, R.A., Kilburn, M.D., Moore L.L., and Sprague K.E. (1997b) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Experimental Eye Research*, **65**: 57–62.
- Liu, R.H. (2003) Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *American Journal of Clinical Nutrition*, **78**: 517S–520S.
- Liu, R.H. (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*, **134**: 3479S–3485S.
- Liu, R.H. (2007) Whole grain phytochemicals and health. *Journal of Cereal Science*, **46**: 207–219.
- Lopez-Martinez, L.X., Oliart-Ros, R.M., Valerio-Alfaro, G., Lee, C.H., Parkin, K.L., and Garcia, H.S. (2009) Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. *LWT-Food Science and Technology*, **42**: 1187–1192.
- Lu, Y., Yao, H., Gong, Y., and Sheng, Q. (2005) Effects of enzymatic treatment of corn gluten meal on lutein and zeaxanthin extraction. *International Journal of Food Properties*, **8**: 161–169.
- Luo, Y.C., Zhang, B.C., Cheng, W.H., and Wang, Q. (2010) Preparation, characterization and evaluation of selenite-loaded chitosan/TPP nanoparticles with or without zein coating. *Carbohydrate Polymers*, **82**: 942–951.
- Luo, Y.C., Zhang, B.C., Whent, M., Yu, L., and Wang, Q. (2011) Preparation and characterization of zein/chitosan complex for encapsulation of  $\alpha$ -tocopherol, and its in vitro controlled release study. *Colloids and Surfaces B: Biointerfaces*, **85**: 145–152.
- Lutsey, P.L., Jacobs, D.R.J., Kori, S., Mayer-Davis E., Shea S., Steffen L.M., Szklo M., and Tracy R. (2007) Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA study. *British Journal of Nutrition*, **98**: 297–405.
- Madhujith, T., and Shahidi, F. (2007) Antioxidative and antiproliferative properties of selected barley cultivars and their potential of inhibition of copper induced LDL cholesterol oxidation. *Journal of Agricultural and Food Chemistry*, **55**: 2018–2024.
- Martin, K.R., Eailla, M.L., and Smith, J.C. (1996)  $\beta$ -carotene and lutein protect HepG2 human liver cells against oxidant-induced damage. *Journal of Nutrition*, **126**: 2098–2106.
- Melanson, J., Angelopoulos, T.J., Nguyen, V.T., Martini, M., Zukley, L., Lowndes, J., Dube, T.J., Fiutem, J.J., Yount, B.W., and Rippe, J.M. (2006) Consumption of whole-grain cereals during weight loss: effects

- on dietary quality, dietary fiber, magnesium, vitamin B-6, and obesity. *Journal of the American Dietetic Association*, **106**: 1380–1388.
- Mellen, P.B., Walsh, T.F., and Herrington, D.M. (2008) Whole grain intake and cardiovascular disease: A meta-analysis. *Nutrition, Metabolism, and Cardiovascular Diseases*, **18**: 283–290.
- Michaud, D.S., Feskanich, D., Rimm, E.B., Colditz, G.A., Speizer, F.E., Willett, W.C., and Giovannucci, E. (2000) Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *American Journal of Clinical Nutrition*, **72**: 990–997.
- Moghadasian, M.H., and Frohlich, J.J. (1999) Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. *American Journal of Medicine*, **107**: 588–594.
- Moreno, F.S., Rizzi, M.B.S.L., Dagli, M.L.Z., and Penteadó, M.V.C. (1991) Inhibitory effects of  $\beta$ -carotene on preneoplastic lesions in Wistar rats induced by resistant hepatocyte model. *Carcinogenesis*, **12**: 1817–1822.
- Moreno, F.S., Toledo, L.P., de Conti, A., Heidor, R., Jr Jordao, A., Vannucchi, Helio., Cardozo T.M., and Ong, R.T. (2007) Lutein presents suppressing but not blocking chemopreventive activity during diethylnitrosamine-induced hepatocarcinogenesis and this involves inhibition of DNA damage. *Chemico-Biological Interactions*, **168**: 221–228.
- Moreno, Y.S. Sánchez, G.S. Hernández, D.R., and Lobato, N.R. (2005) *Journal of Chromatographic Science*, **43**: 483–487.
- Moros, E.E., Darnoko, D., Cheryan, M., Perkins, E.G., and Jerrell, J. (2002) Characterization of anthocyanin extracts from maize kernels. *Journal of Agriculture and Food Chemistry*, **50**: 5787–5790.
- Muralidhara, H.S. 1997. Process for recovering xanthophylls from corn gluten. United States Patent US5602286.
- Murphy, M.M., Douglass, J.S., and Biekett, A. (2008) Resistant starch intakes in the United States. *Journal of the American Dietetic Association*, **108**: 67–78.
- Naves, M.M.V., Silveira, E.R., Dagli, M.L.Z., Moreno, F.S. (2001) Effects of  $\beta$ -carotene and vitamin A on oval cell proliferation and connexin 43 expression during hepatic differentiation in the rat. *Journal of Nutritional Biochemistry*, **12**: 685–692.
- Nesci, A., and Etcheverry, M. (2009) Effect of natural maize phytochemicals on *Aspergillus* section *Flavi* sclerotia characteristics under different conditions of growth media and water potential. *Fungal Ecology*, **2**: 44–51.
- Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S., and Hammar, S. (1996) Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine*, **334**: 1150–1155.
- Ou, L., Kong, L.Y., Zhang, X.M., and Niwa, M. (2003) Oxidation of ferulic acid by momordica charantia peroxidase and related anti-inflammation activity changes. *Biological and Pharmaceutical Bulletin*, **26**: 1511–1516.
- Palozza, P., Calviello, G., Serini, S., Maggiano, N., Lanza, P., Ranelletti, F.O., and Bartoli, G.M. (2001)  $\beta$ -carotene at high concentrations induces apoptosis by enhancing oxy-radical production in human adenocarcinoma cells. *Free Radical Biology and Medicine*, **30**: 1000–1007.
- Palozza, P., Serini, S., Torsello, A., Di Nicuolo, F., Maggiano, N., Ranelletti, F.O., Wolf, F.I., and Calviello, G. (2003) Mechanism of activation of caspase cascade during beta-carotene-induced apoptosis in human tumor cells. *Nutrition and Cancer*, **47**: 76–87.
- Pedreschi, R., and Cisneros-Zevallo, L. (2007) Pheonolic profiles of Andean purple corn (*Zea mays* L.). *Food Chemistry*, **100**: 956–963.
- Perry, A., Rasmussen, H., and Johnson, E.J. (2009) Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. *Journal of Food Composition and Analysis*, **22**: 9–15.
- Piironen, V. Lindsay, D.G., Miettinen, T.A., Toivo, J., and Lampi, A. (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture*, **80**: 839–966.
- Rondini, L., Peyrat-Maillard, M.N., Marsset-Baglieri, A., Fromentin, G., Durand, P., Tome, D., Prost, M., and Berset, C. (2004) Bound ferulic acid from bran is more bioavailable than the free compound in rat. *Journal of Agricultural and Food Chemistry*, **52**: 4338–4343.
- Rose, D.J., Inglett, G.E., and Liu, S.X. (2010) Utilisation of corn (*Zeamays*) bran and corn fiber in the production of food components. *Journal of the Science of Food and Agriculture*, **90**: 915–924.
- Rowland, I.R., Rumney, C., Silvi, S., Cresci, A., and Pool-Zobel, B. (1998) Effect of non-digestible oligosaccharides and resistant starch on biochemical and mucosal biomarkers of colonic neoplasia in rats.

- In: *Functional Properties of Non-Digestible Carbohydrates*, Guillon, F., Amado, R., Amaral-Collaco, M.T., Asp, N.G., BachKnudsen, K.E., Champ, M., Mathers, J., Robertson, J.A., Rowland, I., and Loo, J.V. (eds) pp, 219–221. Nantes: INRA.
- Rukkumani, R., Aruna, K., Varma, P.S., and Menon, V.P. (2004) Influence of ferulic acid on circulatory prooxidant antioxidant status during alcohol and PUFA induced toxicity. *Journal of Physiology and Pharmacology*, **55**: 551–561.
- Sanchez-Garcia, M.D., Hillious, L., and Lagaron, J.M. (2010) Nanobiocomposites of carrageenan, zein, and mica of interest in food packaging and coating applications. *Journal of Agricultural and Food Chemistry*, **58**: 6884–6894.
- Sassa, S., Kikuchi, T., Shinoda, H., Suzuki, S., Kudo, H., and Sakamoto, S. (2003) Preventive effect of ferulic acid on bone loss in ovariectomized rats. *In Vivo*, **17**: 277–280.
- Schatzkin, A., Mouw, T., Park, Y., Subar, A.F., Kipnis, V., Hollenbeck, A., Leitzmann, M.F., and Thoppson, F.E. (2007) Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. *American Journal of Clinical Nutrition*, **85**: 1353–1360.
- Schatzkin, A., Park, Y., Leitzmann, M.M., Hollenbeck, A.R., and Cross, A.J. (2008) Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. *Gastroenterology*, **135**: 1163–1167.
- Shahidi, F. (2009). Nutraceuticals and functional foods: whole versus processed foods. *Trends in Food Science and Technology*, **20**, 376–387.
- Shen, L., Keenan, M.J., Martin, R.J., Tulley, R.T., Raggio, A.M., McCutcheon, K.L., and Zhou, J. (2009) Dietary resistant starch increases hypothalamic POMC expression in rats. *Obesity*, **17**: 40–45.
- Shin, H.D., McClendon, S., Le, T., Taylor, F., and Chen, R.R. (2006) A complete enzymatic recovery of ferulic acid from corn residues with extracellular enzymes from *Neosartorya spinosa* NRRL185. *Biotechnology and Bioengineering*, **95**: 1108–1115.
- Shindo, M., Kasai, T., Abe, A., and Kondo, Y. (2007) Effects of dietary administration of plant-derived anthocyanins-rich colors to spontaneously hypertensive rats. *Journal of Nutritional Science and Vitaminology*, **53**: 90–93.
- Silvi, S., Rumney, C.J., Cresci, A., and Rowland, I.R. (1999) Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. *Journal of Applied Microbiology*, **86**: 521–530.
- Sosulski, F., Krygier, K., and Hogge, L. (1982) Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereals and potato flours. *Journal of Agricultural and Food Chemistry*, **30**: 337–340.
- Srinivasan, M., Sudheer, A.R., and Menon, V.P. (2007) Ferulic acid: therapeutic potential through its antioxidant. *Journal of Clinical and Biochemical Nutrition*, **40**: 92–100.
- Stintzing, F.C., and Carle, R. (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science and Technology*, **15**: 19–38.
- Sun, Z., and Yao, H.Y. (2007) The influence of di-acetylation of the hydroxyl groups on the anti-tumor-proliferation activity of lutein and zeaxanthin. *Asian Pacific Journal of Clinical Nutrition*, **16** (Suppl 1): 447–452.
- Tighe, P., Vaughan, N., Brittenden, J., Simpson, W.G., Mutch, W., Horgan, G., Duthie, G., and Thies, F. (2007) Effect of increased consumption of whole grain foods on markers of cardiovascular disease risk in middle-aged healthy volunteers. *Arteriosclerosis Thrombosis and Vascular Biology*, **27**: E56–E56.
- Torre, P., Aliakbarian, B., Rivas, B., Dominguez, J.M., and Converti, A. (2008) Release of ferulic acid from corn cobs by alkaline hydrolysis. *Biochemical Engineering Journal*, **40**: 500–506.
- Tsuda, T., Horio, F., Uchida, K., Aoki, H., and Osawa, T. (2003) Dietary cyanidin 3-O-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition*, **133**: 2125–2130.
- van der Logt, E.M., Roelofs, H.M., Nagengast, F.M., and Peters, W.H. (2003) Induction of rat hepatic and intestinal UDP-glucuronosyltransferases by naturally occurring dietary anticarcinogens. *Carcinogenesis*, **24**: 1651–1656.
- Vanhoof, K.M.Sc., and De Schrijver, R. (1997) Consumption of enzyme resistant starch and cholesterol metabolism in normo- and hypercholesterolemic rats. *Nutrition Research*, **17**: 1331–1340.
- Verleyen, T., Forcades, M., Verhe, R., Dewettinck, K., Huyghebaert, A., and De Greyt, W. (2002) Analysis of free and esterified sterols in vegetable oils. *Journal of American Oil Chemist's Society*, **79**: 117–122.
- Wang, X., Brown, I.L., Khaled, D., Mahoney, M.C., Evans, A.J., and Conway, P.L. (2002) Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. *Journal of Applied Microbiology*, **93**: 390–397.

- Wang, Y., King, J.M., Xu, Z., Losso, J., and Prudente, A. (2008) Lutein from ozone-treated corn retains antimutagenic properties. *Journal of Agriculture and Food Chemistry*, **56**: 7942–7949.
- Watson S.A., and Ramstad P.E. (1987) *Corn: Chemistry and Technology*. (1st ed.) pp. 453–455. Minnesota, USA: American Association of Cereal Chemists Inc.
- Willis, H.J., Eldridge, A.L., Beiseigel, J., Thomas, W., and Slavin, J. (2009) Greater satiety response with resistant starch and corn bran in human subjects. *Nutrition Research*, **29**: 100–105.
- Yoshimoto, M., Okuno, S., Yamaguchi, M., and Yamakawa, O. (2001) Antimutagenicity of deacylated anthocyanins in purple-fleshed sweet potato. *Bioscience, Biotechnology and Biochemistry*, **65**: 1652–1655.
- Zhang, B., Luo, Y., and Wang, Q. (2010) Development of silver-zein composites as a promising antimicrobial agent. *Biomacromolecules*, **11**: 2366–2375.
- Zhang, Y., Vareed, S.K., and Nair, M.G. (2005) Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life Sciences*, **76**: 1465–1472.
- Zhang, Z., Yang, L., Ye, H., Du, X.F., Gao, Z.M., and Zhang, Z.L. (2010). Effects of pigment extract from black glutinous corn cob in a high-fat-fed mouse model of hyperlipidemia. *European Food Research and Technology*, **230**, 943–946.
- Zhao, X.Y., Zhang, C., Guigas, C., Ma, Y., Corrales, M., Tauscher, B., and Hu, X.S. (2009) Composition, antimicrobial activity, and antiproliferative capacity of anthocyanin extracts of purple corn (*Zea mays* L.) from China. *European Food Research and Technology*, **228**: 1438–2385.
- Zhao, Z., Egashira, Y., and Sanada, H. (2003) Digestion and absorption of ferulic acid sugar esters in rat gastrointestinal tract. *Journal of Agricultural and Food Chemistry*, **51**: 5534–5539.
- Zhao, Z., Egashira, Y., and Sanada, H. (2005) Phenolic antioxidants richly contained in corn bran are slightly bioavailable in rats. *Journal of Agricultural and Food Chemistry*, **53**: 5030–5035.
- Zhao, Z.H., and Moghadasian, M.H. (2008) Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry*, **109**: 691–702.



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## 8 Nutraceutical and health properties of adlay

Junjie Hao and Liangping Yu

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### 8.1 Introduction

Adlay (*Coix lacryma-jobi* var. *ma-yuen*), also known as soft-shelled Job's Tears or Chinese pearl barley, is a minor cereal crop primarily grown in Southern Asia (Pattanagul *et al.*, 2008). Aside from serving as a source of starch, adlay has also been associated with medicinal properties in traditional medicine. Within the species *Coix lacryma-jobi*, there is also the var. *lacryma-jobi* L., which has a harder shell, and is used to make beads and other ornamental jewelry. Both varieties have been significantly involved with the historical culture and society of the region (Jiang *et al.*, 2008).

Because of its use in traditional remedies, adlay, its various milling fractions, and some of its processed products, have been under investigation recently for their possible health-beneficial properties. Studies have found it to be a good source of many bioactive compounds. Adlay has also been shown to exhibit antioxidant, hypolipidemic, anticarcinogenic, and anti-inflammatory effects in a variety of *in vitro* and *in vivo* studies. In addition, both chemical composition and the health beneficial properties have been found to differ significantly among milling fractions, suggesting that post-harvest treatment and processing conditions may significantly impact the nutraceutical properties of adlay in food.

### 8.2 Health components of adlay

Polished adlay grains from Laos, Thailand, Vietnam, and Taiwan were reported to contain 10.6–12.4% moisture, 6.3–7.2% crude fat, 12.1–14.2% protein, 1.8–2.4% crude fiber, 1.6–2.3% ash, and a trace amount of unsaponifiable matter (Wu *et al.*, 2007). According to another report by the Korean Nutrition Society, adlay may contain 16.2% protein, 4.65% lipid, 79.17% carbohydrate, and a small amount of vitamin B1 (Kim *et al.*, 2004). It was also reported that the bran and hull of adlay might differ from the polished adlay in their approximate composition. For instance, adlay bran from the Laos had a much higher fat content of 23.5%, almost four times that of the polished adlay; the hull contained 27% fiber and 15% ash, about ten and seven times that of the polished adlay, respectively (Wu *et al.*,

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2007). In addition, adlay bran oil was reported to contain 12.1% 16:0, 0.44% 16:1, 2.3% 18:0, 49.98% 18:1n-9, 33.8% 18:2n-6, 1.38% 18:3n-3, with a total of 50.42% monounsaturated fatty acids, and 35.18% polyunsaturated fatty acids, and 14.4% saturated fatty acids (Huang *et al.*, 2005). Adlay bran oil is a highly unsaturated edible oil, which is rich in monounsaturated fat.

Polished adlay grains from different countries including Laos, Taiwan, Thailand, and Vietnam differed in their policosanols contents, and contained 14.2–18.5 mg/kg  $C_{22}$ , 119.9–22.6 mg/kg  $C_{24}$ , 18.0–23.9 mg/kg  $C_{26}$ , 21.5–25.1 mg/kg  $C_{28}$ , and 74.9–90.1 mg/kg total policosanols (Wu *et al.*, 2007). Policosanols distribute differently in the polished adlay, and are particularly rich in the hull and bran fractions. Adlay bran from Laos contained the greatest  $C_{22}$  (50.7 mg/kg),  $C_{24}$  (65.5 mg/kg),  $C_{26}$  (76.7 mg/kg), and total policosanols (246 mg/kg), while the hull had the greatest content of  $C_{28}$  (71.5 mg/kg) policosanols (Wu *et al.*, 2007). Policosanols are a group of aliphatic primary alcohols containing 20–36 carbon atoms. Policosanols may reduce total and LDL cholesterol levels, inhibit platelet aggregation, modulate blood pressure, and have liver protective effects (Wu *et al.*, 2007; Aneiros *et al.*, 1995). Adlay may serve as an excellent dietary source of policosanols.

Phytosterols and tocopherols were also detected in Adlay grains and fractions. Polished adlay grains from Thailand, Vietnam, Taiwan, and Laos contained 36.4–51.9 mg/kg tocopherols, 41.0–61.2 mg/kg squalene, 58.0–106 mg/kg campesterol, 53.4–88.8 mg/kg stigmata-5, 22-dien 3-ol, 232–638 mg/kg  $\beta$ -sitosterol, 112–213 mg/kg ergosterol, and 3.92–6.44 mg/kg friedelin (Wu *et al.*, 2007). Adlay grains with greater level of one selected phytosterol did not necessarily contain higher amount of another phytosterol. It was also shown that the bran fraction of adlay contained greater amount of tocopherols, individual phytosterols, and total phytosterols (Wu *et al.*, 2007). The phytosterol concentration in adlay was comparable to that in other cereal grains including rye, barley, wheat, and oats, but was much lower than that of 13,250 mg/kg in rice bran (Wu *et al.*, 2007). Phytosterols are believed to suppress the absorption of cholesterol and reduce the risk of coronary heart disease.

In addition, adlay grains from different locations differed in their oleamide, *cis*-9-octadecenamide content, ranging from 19.3 mg/kg to 28.1 mg/kg (Wu *et al.*, 2007). Oleamide, a primary fatty acid amide, was more concentrated in the bran fraction, which was more than double the amount of the polished adlay grains and the hull (Wu *et al.*, 2007). Primary fatty acid amides are a group of bioactive compounds involved in many biological functions. Oleamide has been recognized for its activity in regulating sleep and as an anticonvulsant, and may also alter food intake and sexual behavior by affecting many biological targets, such as gap junction communication, serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A/2C</sub>, 5-HT<sub>7</sub>, and cannabinoid and GABA receptors (Wu *et al.*, 2007). Adlay grains and bran may serve as a dietary source for oleamide.

In 2008, five  $\gamma$ -lactam compounds, including coixspirolactams A-C, coixlactam, and methyl dioxindole-3-acetate, were isolated from the bran of adlay grown in Taiwan (Lee *et al.*, 2008). These compounds showed antiproliferative activities in A549, HT-29, and COLO205 cancer cells with IC<sub>50</sub> values of 28.6–72.6  $\mu$ g/mL. Lactams are widely accepted and utilized as antibacterial agents with no significant safety concerns. Some lactams also showed activities in inducing apoptosis and blocking the G2 portion of the cell cycle, suggesting a potential for these compounds to be further developed as anticancer agents. In 2002, phenolic compounds including ferulic and syringic acids, coniferyl alcohol, syringaresinol, 4-ketopinosinol, and mayuenolide, a lignin compound, were identified in adlay hull and exhibited strong DPPH radical scavenging activities (Kuo *et al.*, 2002). In a recent

study, 15 compounds were identified from the ethanol extract of adlay bran and tested for their potential anti-inflammatory effects in RAW 254.7 cells (Chen *et al.*, 2011). Among them, isoliquiritigenin, tangeretin, and 3,3',4',5,6,7,8-heptamethoxyflavone showed significant inhibition of nitric oxide (NO<sup>•</sup>) production at 50  $\mu$ M in RAW 254.7 cells stimulated with LPS. In addition, it was reported that adlay starch could contain about 18% resistant starch (Kim *et al.*, 2008). Resistant starches are so named for their resistance to digestion in the small intestine, but could be used by the microbial flora in the large intestine, resulting in health beneficial effects such as formation of short chain fatty acids and calorie reduction.

## 8.3 Potential health beneficial properties

### 8.3.1 Antioxidant properties

Adlay was compared to several other cereal grains including rice, sorghum, millet and mung-beans for its antioxidant properties (Choi *et al.*, 2007). Adlay had a total phenolic content of 43 mg gallic acid equivalents (GAE)/100 g, which is greater than that of 18–29 mg GAE/100 g in white rice and proso millet, comparable to that of 45–54 mg GAE/100 g in barley, mung-bean, foxtail millet, and brown rice, but much lower than that of 313 and 733 mg GAE/100 g determined in the black rice and sorghum, respectively (Choi *et al.*, 2007). Among the grain samples, adlay had the second lowest total carotenoid and tocopherol contents measured using a spectrometric method, while the lowest tocopherol and carotenoid contents were observed in white rice under the same experimental conditions. The methanol extract of adlay was evaluated for scavenging capacity against DPPH and ABTS cation radicals, chelating activity, reducing power, and inhibitory effect against lipid peroxidation (Choi *et al.*, 2007). The DPPH and ABTS cation radical scavenging capacities of adlay were comparable to that of brown rice, mungbean, foxtail millet and proso millet, and were greater than that of white rice but less than a third of that of black rice and sorghum and two thirds to one third of that of barley. Adlay also showed significant inhibitory effect on lipid peroxidation, as well as some Fe(II) chelating activity and significant reducing power, although these activities were the weakest among all tested grain extracts (Choi *et al.*, 2007).

Methanol extracts of polished, dehulled, monascal polished, and monascal dehulled adlay were also investigated for their capability to suppress lipid peroxidation, DPPH radical scavenging, and Fe(II) chelating properties (Tseng *et al.*, 2006). The results indicated that monascal adlay products had higher antioxidant activity and greater total phenolic contents. Ascorbic acid, phenols, and tocopherols were detected in all adlay samples, and the phenol concentration was 3.05 and 9.73 mg GAE/g for polished and dehulled adlay and 25.45 and 29.75 mg GAE/g for the monascal polished and dehulled adlay, suggesting that processing can affect the antioxidant components and properties in adlay (Tseng *et al.*, 2006). In addition, adlay oil significantly reduced the plasma TBARS values in a rat feeding study, and the reduction in TBARS was not dose dependent (Huang *et al.*, 2005). TBARS stands for thiobarbituric acid reactive substances, which is a measurement of lipid peroxidation where a greater TBARS value corresponds to a higher level of lipid peroxidation. Later, in 2009, Huang and others reported that the ethanol extractable components of adlay seed testa, especially the ethyl acetate and butanol soluble components, were able to suppress the formation of conjugated dienes in LDL (Huang *et al.*, 2009a). At the 25  $\mu$ g/mL concentration, the ethyl

acetate and butanol soluble components of adlay testa resulted in 89 and 92 inhibition of TBARS. All together, these data confirmed the antioxidant properties of adlay components.

### 8.3.2 Hypolipidemic properties

In 2004, Kim and others investigated the effects of crude adlay seed extract on serum lipids and the expressions of TNF- $\alpha$  and leptin mRNA in obese rats fed a high fat diet. Oral intake of the adlay crude extract (*Coix lachrymajobi* var. *mayuen*) was found to significantly reduce body weight, food intake, food efficiency, wet weight of adipose tissues, and size of adipocytes after four weeks at a daily dose of 50 mg/100 g body weight. Compared to the vehicle group, the rats with adlay extract injection had about 90% reduction in serum triglyceride level and 59% reduction in serum leptin concentration, but no difference in serum cholesterol level (Kim *et al.*, 2004). In addition, adlay extract intake resulted in a 30% decrease in TNF- $\alpha$  and a 48% reduction in leptin mRNA expressions in the white adipose tissues. It was concluded that intake of adlay could have hypolipidemic effect through altering TNF- $\alpha$  and leptin mRNA expressions and modulating plasma lipids, therefore, result in potential benefits to human health.

The hypolipidemic effect of adlay extract was also observed in another rat feeding study using adlay bran oil prepared by extrusion of a *C. lachrymal-jobi* L. var. *ma-yuen* Stapf grown in Taichung, Taiwan (Huang *et al.*, 2005). Intake of adlay bran oil at 5 and 10% significantly reduced abdominal adipose tissue and relative adipose tissue, without affecting body weight, food intake, and liver weight in the three-week-old Wistar rats fed the oil for 28 days. Adlay bran oil also reduced plasma insulin and lectin levels, and plasma total and VLDL triglycerides, as well as total VLDL and LDL cholesterol concentrations (Huang *et al.*, 2005). Increasing the adlay bran oil intake from 5 to 10% was not necessary to warrant a greater hypolipidemic effect under the experimental conditions. The reduced lipid oxidation was also detected in the liver and plasma of the rats fed adlay bran oil (Huang *et al.*, 2005), confirming the presence of antioxidants in the oil and bran of adlay. It was suggested that the oil soluble phytochemicals such as phytosterol, phenolics, and diglycerides might be the contributors of the hypolipidemic effects of adlay seeds and its fractions.

In addition, adlay carbohydrates may contribute to its overall hypolipidemic effects. In 2005, water-soluble polysaccharides were isolated from the dehulled adlay, and the extract was further fractionated into 40% ethanol soluble and insoluble fractions (Yu *et al.*, 2005). The 40% ethanol soluble, 40% ethanol insoluble, and total water extractable adlay polysaccharides at levels of 6.17, 8.04, and 6.68 g/100 g diet, in substitution of 5 g cellulose/100 g diet, were able to significantly reduce serum triglyceride, and LDL and total cholesterol concentrations in male Golden Syrian hamsters, and the 40% ethanol soluble fraction was significantly more effective than the 40% ethanol insoluble fraction but not necessarily more effective than the water extractable polysaccharides (Yu *et al.*, 2005). All three polysaccharide preparations also significantly enhanced fecal excretion of total lipids, cholesterol, and bile acids, and the 40% ethanol soluble fraction and the total water extractable polysaccharide were more effective than the 40% ethanol insoluble fraction under the same experimental conditions. Further investigation also showed that the 40% ethanol insoluble fraction was the most effective in reducing the liver total cholesterol and triglyceride concentrations, without altering the ratio of liver to body weights (Yu *et al.*, 2005). All three adlay polysaccharide preparations were fermentable and produced

significantly more short chain fatty acids, but the 40% ethanol insoluble polysaccharide produced more acetic, propionic, and total short chain fatty acids, whereas the 40% ethanol soluble fraction produced the highest level of butyric acid. Taken together, the hypolipidemic effect of adlay could be attributed to more than a single group of chemical components, and the overall mechanism remains interesting.

### 8.3.3 Anticarcinogenic effects

Several previous studies have demonstrated that both acetone and methanol extracts of adlay may have anti-tumor activities in mice and rats, and methanol extracts also showed anticarcinogenic effects in cultured cancer cell lines including lymphoma U937, A549 lung, and human breast cancer MCF-7, T-47D, and MDA-MB-231 cell lines (Chang *et al.*, 2003; Shih *et al.*, 2004; Chung *et al.*, 2011; Li *et al.*, 2011). In 2004, Shih and others concluded from an azoxymethane-induced colon carcinogenesis using male F344 rats that intake of dehulled adlay may suppress the early events in colon carcinogenesis but not the tumor formation (Shih *et al.*, 2004). Shih and others conducted five-week and 52-week feeding studies using rats. It was found that five weeks feeding dehulled adlay at levels of 10, 20, and 40% in the diet significantly reduced the numbers of azoxymethane-induced aberrant crypt foci (ACF) and aberrant crypts, but had no influence on crypt multiplicity. The 52-week feeding study showed that dehulled adlay did not inhibit colon tumors (Shih *et al.*, 2004). The anticarcinogenic effect of the dehulled adlay was stronger in the middle colon. Also noted was that intake of dehulled adlay at a 20% level reduced the expression of cyclooxygenase-2 (COX-2) protein in both proximal and distal colon tumors (Shih *et al.*, 2004). The anticarcinogenic effect of adlay in colon was also observed in a recent study (Li *et al.*, 2011). Adlay bran, its ethanol extract and the residue were able to reduce the number of chemically induced ACF and modify their mucin composition in rats, and the inhibitory effect of the ethanol extract of bran was dose-dependent (Li *et al.*, 2011). An early study reported that methanol extract, its fractions, and the five lactam compounds isolated from the extract were tested for their antiproliferative effects in human colorectal carcinoma HT-29 and COLO 205 cells, and human lung cancer A549 cells (Lee *et al.*, 2008). The methanol extract, its ethyl acetate-soluble fractions, and the five lactam compounds all showed significant inhibitory effects on HT-29, COLO 205, and A549 cancer cell growth, and the ethyl acetate-soluble fractions had stronger antiproliferative activity than the methanol extract. The  $IC_{50}$  values of the 5 lactam compounds ranged 28.57–65.00  $\mu\text{g/mL}$  for A549 cells, 32.70–52.63  $\mu\text{g/mL}$  for HT-29 cells, and 35.94–72.57  $\mu\text{g/mL}$  for COLO 205 cells (Lee *et al.*, 2008). The stronger inhibitor against one of the cancer cell lines did not necessarily show stronger anti-proliferative activity in another cell line, suggesting that each cancer cell line may respond to the anticarcinogenic lactam differently and the selection of cell line may alter the estimation of anticarcinogenic activity of a selected compound, which suggest that use of more than one cell line is preferred for screening and evaluation of antiproliferative properties of a selected compound or extract.

Early in 2003, methanol extract of adlay seeds was examined for its effects on COX-2 expression in A549 human lung cancer cells and in tumor tissue of nude mice (Hung and Chang, 2003). The methanol extract of adlay at 11 and 33  $\mu\text{g/mL}$  concentrations significantly suppressed basal and TPA-stimulated COX-2 protein expression of A549 cells in a dose dependent manner, but had no effect on COX-1 protein expression under the same

experimental conditions, suggesting the presence of selective COX-2 inhibitors in the adlay seeds. The inhibition was found with the methanol extract of adlay at a level of 3 mg/kg by intraperitoneal injection, which dose-dependently suppressed the COX-2 expression in the tumor tissues of the BALB/c-nu nude mice inoculated with human lung cancer cells by SC injection (Hung and Chang, 2003). The adlay extract also significantly reduced blood PGE<sub>2</sub> level in the nude mice. Findings from another study by Chang and others also showed that methanol extract, but not the water extract, of adlay seeds inhibited A549 human lung cancer cell proliferation and suppressed tumor growth in A/J mice induced by a tobacco specific carcinogen (Chang *et al.*, 2003). Feeding with diet containing 30% adlay seed powder reduced the number of surface lung tumors by approximately 50%. The inhibition of cell cycle progress at the G1/S transition was observed in the study, and the methanol extract of adlay suppressed the expression of cell cycle regulation proteins including cyclin D1 and E without influence on CDK expression in A549 cells. In addition, treatment with the adlay extract at 300 µg/mL concentration led to degradation of PARP, a typical marker of apoptosis (Chang *et al.*, 2003), indicating the potential of adlay components in inducing apoptosis.

In summary, adlay seeds, its milling fractions, and its chemical components have been shown to have anticarcinogenic effects. The mechanisms involved may include suppressing cell cycle, inhibiting COX-2 gene expression and inducing apoptosis, or the combination of all. Lactams, spiroenone, coixenolide, fatty acids, and neutral lipid extract might be the significant contributors to the anticarcinogenic effects of adlay.

### 8.3.4 Anti-inflammatory effect

From an early study in 1998, feeding 20% dehulled adlay to BALB/c mice, an animal model sensitive to allergic responses, was found to significantly reduce the serum anti-OVA IgE concentration and to increase serum IgG2a level induced by intraperitoneal injection of ovalbumin and aluminum hydroxide (Shyu *et al.*, 1998), suggesting an anti-inflammatory activity of adlay. Chronic inflammation has been associated with the development of several diseases, including Alzheimer's atherosclerosis and certain cancers. The causes of these illnesses have partly been attributed to the up-regulation of a number of protein pathways in the body, such as the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) resulting from inflammation. iNOS and COX-2 lead to production of NO radical and prostaglandins including prostaglandin E2 (PGE<sub>2</sub>), respectively.

Two recent studies have evaluated the anti-inflammatory effects of adlay seed hull and testa extracts, and identified their active chemical components (Huang *et al.*, 2009a, 2009b). The adlay seed hull extract was further partitioned into ethyl acetate, n-butanol, and water soluble fractions (Huang *et al.*, 2009a). The fractions were subjected to silica gel chromatography, and 3 out of the 15 chromatographic sub-fractions of the ethyl acetate fraction had significant inhibitory effects on lipopolysaccharide (LPS) induced production of cellular nitric oxide production and PGE<sub>2</sub> in RAW 264.7 macrophages, possibly through suppressing inducible expression of nitric oxide synthase and COX-2 and with eriodictyol and ceramide as the possible primary active components (Huang *et al.*, 2009a). It was also reported by the same group that the 95% ethanol extract of adlay testa and its ethyl acetate and n-butanol subfractions down-regulated the expression of LPS induced nitric oxide synthase (iNOS) and COX-2 genes in RAW 264.7 macrophages (Huang *et al.*, 2009b).

Chlorogenic, vallinnic, caffeic, p-coumaric, and ferulic acids were presented in the ethyl acetate and n-butanol subfractions, along with 2-O- $\beta$ -glucopyranosyl-7-methoxy-4-(<sup>2</sup>H)-benzoxazin-3-one. Further experiments showed that chlorogenic, caffeic, and ferulic acids are the major components contributing to the overall anti-inflammatory activities of adlay testa extract (Huang *et al.*, 2009b).

### 8.3.5 Other health beneficial effects

Adlay has been used for improving human health for thousands of years to treat warts, chapped skin, rheumatism, and neuralgia (Wang *et al.*, 2010). In addition to the health properties discussed above, adlay hull extracts have been reported to inhibit PGF<sub>2 $\alpha$</sub>  induced uterine contractions, Ca<sup>2+</sup> channel activator Bay K 8644, and high K<sup>+</sup> in a dose-dependent manner *in vitro*, suggesting the potential utilization of adlay and its components as an alternative treatment for dysmenorrheal (Hsia *et al.*, 2008). Adlay components were also evaluated for their potential in reducing the risk of osteoporosis using cultured neonatal rat calvaria tissues or adult femoral metaphyseal tissues of bone isolated from adult normal or ovariectomized female rats (Yang *et al.*, 2008). The results suggested that adlay components might be able to reverse osteoporosis in rats. In addition, adlay derived food ingredients and food products, such as Lactobacillus-fermented adlay-based milk, may have health benefits (Wang *et al.*, 2010).

## 8.4 Summary

Adlay contains significant levels of diverse chemical components. It may have antioxidant, anti-inflammation, anti-cancer, and hypolipidemic effects. It could also be used for reducing dysmenorrheal and risk of osteoporosis. More study is required to further investigate the active components, the mechanisms involved in their biological actions, and to develop novel products for improved health benefits.

## References

- Aneiros, E., Más, R., Calderon, B., Illnait, J., Fernández, L., Castaño, G., and Fernández, J.C. (1995) Effect of policosanol in lowering cholesterol levels in patients with type II hypercholesterolemia. *Current Therapeutic Research*, **56**: 176–182.
- Chang, H.-C., Huang, Y.-C., and Hung, W.-C. (2003) Antiproliferative and chemopreventive effects of adlay seed on lung cancer in vitro and in vivo. *Journal of Agricultural and Food Chemistry*, **51**: 3656–3660.
- Chen, H.-J., Cheng, P.-C., Chiang, W., and Lin, Y.-L. (2011) Anti-inflammatory effects and chemical study of a flavonoid-enriched fraction from adlay bran. *Food Chemistry*, **126**: 1741–1748.
- Choi, Y., Jeong, H., and Lee J. (2007) Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chemistry*, **103**: 130–138.
- Chung, C.-P., Hsu, C.-Y., Lin, J.-H., Kuo, Y.-H., Chiang, W., and Lin, Y.-L. (2011) Antiproliferative lactams and spiroenone from adlay bran in human breast cancer cell lines. *Journal of Agricultural and Food Chemistry*, **59**: 1185–1194.
- Hsia, S.-M., Kuo, Y.-H., Chiang, W., and Wang, P.S. (2008) Effects of adlay hull extracts on uterine contraction and Ca<sup>2+</sup> mobilization in the rat. *Translational Physiology*, **295**(11): E719–E726.
- Huang, B.-W., Chiang, M.-T., Yao, H.-T., and Chiang, W. (2005) The effect of adlay oil on plasma lipids, insulin, and leptin in rat. *Phytomedicine*, **12**: 433–439.

- Huang, D.-W., Chung, C.-P., Kuo, Y.-H., Lin, Y.-L., and Chiang, W. (2009a) Identification of Compounds in Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) Seed Hull Extracts That Inhibit Lipopolysaccharide-Induced Inflammation in RAW 264.7 Macrophages. *Journal of Agricultural and Food Chemistry*, **57**: 10651–10657.
- Huang, D.-W., Kuo, Y.-H., Lin, F.-Y., Lin, Y.-L., and Chiang, W. (2009b) Effect of Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) Testa and its phenolic components on Cu<sup>2+</sup>-treated low-density lipoprotein (LDL) oxidation and lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry*, **57**: 2259–2266.
- Hung, W.-C. and Chang, H.-C. (2003) Methanolic extract of adlay seed suppresses COX-2 expression of human lung cancer cells via inhibition of gene transcription. *Journal of Agricultural and Food Chemistry*, **51**: 7333–7337.
- Jiang, H.-E., Wang, B., Li, X., Lü, E.-G., and Li, C.-S. (2008) A consideration of the involucre remains of *Coix lacryma-jobi* L. (Poaceae) in the Sampula Cemetery (2000 years BP), Xinjiang, China. *Journal of Archaeological Science*, **35**: 1311–1316.
- Kim M.J., Choi S.J., Shin S.I., Sohn M.R., Lee C.J., Kim Y., Cho W.I., and Moon T.W. (2008) Resistant glutarate starch from adlay: preparation and properties. *Carbohydrate Polymers*, **74**: 787–796.
- Kim, S.O., Yun, S.J., Jung, B., Lee, E.H., Hahm, D.H., Shim, I., and Lee, H.J. (2004) Hypolipidemic effects of crude extract of adlay seed (*Coix lachrymajobi* var. *mayuen*) in obesity rat fed high fat diet: relations of TNF-alpha and leptin mRNA expressions and serum lipid levels. *Life Sciences*, **75**: 1391–1404.
- Kuo, C.-C., Chiang, W., Liu, G.-P., Chien, Y.-L., Chang, J.-Y., Lee, C.-K., Lo, J.-M., Huang, S.-L., Shih, M.-C., and Kuo, Y.-H. (2002) 2,2'-diphenyl-1-picrylhydrazyl radical-scavenging active components from adlay (*Coix lachrymal-jobi* L. var. *ma-yuen* Stapf) hulls. *Journal of Agricultural and Food Chemistry*, **50**: 5850–5855.
- Lee, M.Y., Lin, H.Y., Cheng, F., Chiang, W., and Kuo Y.H. (2008) Isolation and characterization of new lactam compounds that inhibit lung and colon cancer cells from adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) bran. *Food and Chemical Toxicology*, **46**: 1933–1939.
- Li, S.-C., Chen, C.-M., Lin, S.-H., Chiang, W., and Shih, C.-K. (2011) Effects of adlay bran and its ethanolic extract and residue on preneoplastic lesions of the colon in rats. *Journal of the Science of Food and Agriculture*, **91**: 547–552.
- Pattanagul, P., Pinthong, R., Phianmongkhol, A., and Tharatha S. (2008) Mevinolin, citrinin and pigments of adlay angkak fermented by *Monascus* sp. *International Journal of Food Microbiology* **126**: 20–23.
- Shih, C.-K., Chiang, W., and Kuo, M.-L. (2004) Effects of adlay on azoxymethane-induced colon carcinogenesis in rats. *Food and Chemical Toxicology*, **42**: 1339–1347.
- Shyu, M.L., Lin, B.F., and Chiang, W. (1998) Effect of dehulled adlay on allergic responses of sensitized mice. *Nutritional Sciences Journal*, **23**: 161–170.
- Tseng, Y.-H., Yang, J.-H., Chang, H.-L., Lee, Y.-L., and Mau, J.-L. (2006) Antioxidant properties of methanolic extracts from monascal adlay. *Food Chemistry*, **97**: 375–381.
- Wang C.-Y., Wu, S.-C., Ng, C.-C., and Shyu, Y.-T. (2010) Effect of Lactobacillus-fermented adlay-based milk on lipid metabolism of hamsters fed cholesterol-enriched diet. *Food Research International*, **43**: 819–824.
- Wu, T.-T., Charles, A.L., and Huang, T.H. (2007) Determination of the contents of the main biochemical compounds of Adlay (*Coxi lachrymal-jobi*). *Food Chemistry*, **104**: 1509–1515.
- Yang, R.S., Chiang, W., Lu, Y.H., and Liu, S.H. (2008) Evaluation of osteoporosis prevention by adlay using a tissue culture model. *Asia Pacific Journal of Clinical Nutrition*, **17**: 143–146.
- Yu, Y.-T., Lu, T.-J., Chiang, M.-T., and Chiang, W. (2005) Physicochemical properties of water-soluble polysaccharide enriched fractions of adlay and their hypolipidemic effect in hamsters. *Journal of Food and Drug Analysis*, **13**: 361–367.



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# 9 Antioxidant and health promoting properties of wheat (*Triticum spp.*)

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## 9.1 Introduction

Wheat (*Triticum spp.*) has historically played an important role in human nutrition as a dietary staple. Studies since the late twentieth century have suggested that consumption of wheat, specifically whole-grain wheat, may also play an important role in human health by reducing the risk for several chronic diseases. Whole-grain wheat constituents, such as secondary metabolites with antioxidant properties and insoluble fiber, are thought to play an important role in its health promoting properties. This chapter discusses available evidence from epidemiological, clinical, animal, *ex-vivo*, and *in-vitro* studies on whole-grain wheat's health promoting properties, and explores the health promoting constituents of wheat and its milling fractions with a focus on antioxidants. The chapter also discusses information available on the bioavailability of antioxidative phenolic acids from wheat, and recent studies aimed at improving the antioxidant health promoting properties of wheat through post-harvest treatments and optimization of food processing conditions.

## 9.2 Evidence of wheat's health promoting properties

The health promoting aspects of whole-grain foods have been recognized since the fourth century BC (Slavin, 2004). It was not until the 1970s, however, that researchers attributed these properties to fiber and other bioactive constituents present in wheat bran and germ (Slavin, 2004). This “fiber hypothesis” was developed from observational studies in the 1970s of African populations. These populations consumed mostly whole plant based diets high in fiber, and were free of many Western diseases such as cardiovascular disease and colon cancer (Jones, 2006; Trowell, 1972, 1978).

Since that time, numerous epidemiological and clinical studies have presented strong evidence that consumption of whole-grain foods significantly reduces the risks for numerous chronic conditions (Slavin, 2004; Jones, 2006). Animal and cohort studies have suggested that the bran fraction of wheat is the key factor responsible for wheat's protective effects against cancer (Jensen *et al.*, 2004; Barbolt and Abraham, 1978, 1980; Reddy and Mori,

1981; Alabaster *et al.*, 1993, 1995; Jenab and Thompson, 1998; Reddy *et al.*, 2000; Zile *et al.*, 1998). The “fiber hypothesis” of the 1970s hypothesized that fiber was the active component in wheat bran responsible for these effects. Recent epidemiological studies and a pooled analysis of cohort studies, however, have refuted the role that dietary fiber alone plays in reduced risk of chronic disease (Anderson, 2003; Park *et al.*, 2005; Santana-Rios and Dashwood, 2005; Liu *et al.*, 1999; Fuchs *et al.*, 1999; Slavin *et al.*, 2000). This suggests that another wheat bran component besides the fiber component is responsible (Anderson, 2003; Park *et al.*, 2005; Santana-Rios and Dashwood, 2005; Liu *et al.*, 1999; Fuchs *et al.*, 1999; Slavin *et al.*, 2000). One hypothesis is that antioxidants, which are known to be present in higher concentrations in wheat bran compared to endosperm, may be a key bioactive factor for this relationship (Slavin *et al.*, 2000; Slavin, 2004; Anderson, 2004). This is supported in a study by Carter and others (Carter *et al.*, 2006), which found that the *in-vitro* antioxidant potential of wheat brans was correlated with their *in-vivo* antitumor activities.

### 9.3 The antioxidant contents of wheat

A simple and well accepted definition of an antioxidant is a molecule that can significantly delay or prevent the oxidation of a substrate, when present in low concentrations compared to that of the oxidizable substrate (Halliwell and Gutteridge, 1989). The purported role of antioxidants in human health and disease prevention is explained by their reactions with reaction oxygen and nitrogen species (ROS and RNS). These highly reactive species are generated in living systems and can damage important biomolecules such as DNA, lipids, and proteins. Such damage is thought to contribute to numerous diseases such as cardiovascular disease, cancer, and neurodegenerative conditions such as Alzheimer's and Parkinson's. Antioxidants can prevent ROS and RNS from damaging biomolecules by scavenging free radicals, preventing the formation of reactive oxygen species, chelating metal ions, and inducing antioxidative enzyme systems that already exist in living systems.

Studies on antioxidants in wheat have investigated its antioxidant composition, as well as its antioxidant properties such as free radical scavenging and chelating properties. It is known today that wheat naturally contains numerous classes of antioxidant compounds including phenolic acids, alk(en)ylresorcinols, phytic acid, steryl ferulates, plant lignans, carotenoids, tocopherols, and tocotrienols (Zhou and Yu, 2004a; Moore *et al.*, 2005; Adom *et al.*, 2003, 2005; Panfili *et al.*, 2003; Nyström *et al.*, 2005; Li *et al.*, 2005; Kim *et al.*, 2006; Crosby, 2005; Graf and Easton, 1990; Mattila *et al.*, 2005).

Although knowledge of the antioxidant properties of wheat bioactive compounds did not occur until later, actual identification of these compounds in wheat dates back to the early twentieth century. Vitamin E was discovered in wheat in the 1920s, and its antioxidant properties in wheat germ were studied in 1936 in lipid systems (Evans and Burr, 1925; Olcott and Mattill, 1936). Work 50 years later identified several vitamers of vitamin E in wheat (Slover *et al.*, 1969). More recent studies have confirmed the presence of  $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\gamma$ -tocopherols and  $\alpha$ -T3 and  $\beta$ -T3 tocotrienols and reported the total concentration of tocols in soft wheat to be approximately 75 mg/kg dry weight (Zhou and Yu, 2004a; Moore *et al.*, 2005).

Carotenoids were first isolated from wheat in the early twentieth century, but it was debated early on whether these carotenoids had provitamin A activity (Zechmeister and Cholnoky, 1940). A study by Bowden and Moore in 1933 reported xanthophylls, a group of carotenoids lacking provitamin A activity, to be the predominate type of carotenoids in

wheat concentrated in the germ fraction (Bowden and Moore, 1933). More recent studies have confirmed this, showing the major carotenoids present in wheat grain to be lutein and zeaxanthin with concentrations of 0.5–144  $\mu\text{g/g}$  and 2.2–27  $\mu\text{g/g}$  grain respectively (Zhou and Yu, 2004a; Moore *et al.*, 2005; Adom *et al.*, 2003; Humphries and Khachik, 2003). Carotenoids with provitamin A activity are also present in wheat grain at low levels including  $\beta$ -cryptoxanthin at 0.18–13  $\mu\text{g/g}$ , and  $\beta$ -carotene at 0.09–0.40  $\mu\text{g/g}$  (Zhou and Yu, 2004a; Moore *et al.*, 2005; Olcott and Mattill, 1936; Humphries and Khachik, 2003). Zhou and others (2005) examined the carotenoid contents of two varieties of hard red wheat bran, and reported lutein to be the predominant carotenoid with concentrations ranging from 0.97 to 1.43  $\mu\text{g/g}$  wheat bran. This study also found smaller concentrations of  $\beta$ -carotene, zeaxanthin, and  $\beta$ -cryptoxanthin ranging from 0.03 to 0.40  $\mu\text{g/g}$  wheat bran (Zhou *et al.*, 2005). An investigation by Adom and others (Adom *et al.*, 2005) evaluated carotenoid concentrations in different wheat fractions. They found 12-, 4-, and 2-fold higher concentrations of zeaxanthin, lutein, and  $\beta$ -cryptoxanthin respectively in the bran/germ fraction of wheat compared to endosperm. Together, these studies have shown wheat to contain low amounts of antioxidative carotenoids, mostly concentrated in the germ and bran fractions.

Secoisolariciresinol diglycoside is a diphenolic lignan compound that is converted to the mammalian lignans enterodiol and enterolactone by intestinal microflora. It has been found in wheat bran in relatively low concentrations at 1.1  $\mu\text{g/g}$  (Crosby, 2005; Qu *et al.*, 2005). Steryl ferulates are ferulic acid esters of plant sterols with reported antioxidant properties. They have been detected in wheat bran in concentrations ranging from 30 to 39 mg/100 g (Nyström *et al.*, 2005). Phytic acid has been reported in whole wheat flour in the range of 8.5 mg/g (Febles *et al.*, 2002). Alk(en)ylresorcinols are antioxidant compounds containing long nonisoprenoid side chains attached to phenolic acids. They have been detected in wheat bran with total concentrations of 3.2 g/kg (Mattila *et al.*, 2005). Lastly, seven hydrophilic hydroquinone substituted oligosaccharides were recently isolated from wheat germ and reported to have antioxidant properties (Zhokhov *et al.*, 2009). Although present in wheat, lignans, steryl ferulates, phytic acid, and alk(en)ylresorcinols and other minor constituents are not thought to be the predominate compounds responsible for its antioxidant properties.

Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids and in wheat include including vanillic, syringic, *p*-hydroxybenzoic, gallic, *p*-coumaric, caffeic, ferulic, sinapic acids, and dehydromers of ferulic acid (Moore *et al.*, 2005; Garcia-Conesa *et al.*, 1997). They are thought to be one of the primary groups of compounds responsible for the total antioxidant and health promoting properties of wheat (Slavin *et al.*, 2000). Table 9.1 presents data on the phenolic acid contents of whole grain wheat and wheat fractions, and indicates that ferulic acid is the predominate phenolic acid found in wheat. The presence of ferulic acid in its free and protein-bound states in wheat has been known since the 1960s from the studies of el-Basyouni and Towers (El-Basyouni and Towers, 1964) and Fausch and others (1963). Gallus and Jennings, in 1971, reported the majority of wheat phenolics in an ester bound state, and Fulcher and others studying the localization of wheat phenolics found ferulic acid to be most concentrated in the aleurone layer of wheat bran (Fulcher *et al.*, 1972; Gallus and Jennings, 1971). It is now known that phenolic acids exist in wheat in three primary states – soluble free, soluble conjugated, and insoluble bound. A recent study by Moore and others found the total phenolic content of soft wheat grains including soluble free, soluble conjugated, and insoluble bound fractions measured with HPLC with UV/vis detection to range from 455 to 621  $\mu\text{g/g}$  in wheat grain (Moore *et al.*, 2005). This study also found the insoluble bound fraction to comprise the majority (91%) of wheat phenolics, with soluble conjugated and soluble free fractions comprising 8.7% and 0.58%, respectively

**Table 9.1** Reported phenolic acid contents (total) of wheat grain and its fractions

	<b>p-Hydroxybenzoic acid (µg/g)</b>	<b>Vanillic acid (µg/g)</b>	<b>Syringic acid (µg/g)</b>	<b>p-Coumaric acid (µg/g)</b>	<b>o-Coumaric acid (µg/g)</b>	<b>Ferulic acid (µg/g)</b>	<b>Caffeic acid (µg/g)</b>	<b>References</b>
Whole grain	1.24–2.72	2.75–12.7	4.71–16.1	2.67–145.1	146.1–229.2	204–621	7.6–12.9	Moore <i>et al.</i> , 2005, Adom <i>et al.</i> , 2003, Mpofu <i>et al.</i> , 2006, Zhou <i>et al.</i> , 2007
Bran	24.6–39.2	29.27–90.0	3.97–182	35.0–477	NA	838–2330	NA	Kim <i>et al.</i> , 2006, Irmak <i>et al.</i> , 2008, Verma <i>et al.</i> , 2009
Aleurone	NA	NA	NA	NA	NA	2376–3214	NA	Anson <i>et al.</i> , 2008

NA = data not available.

(Moore *et al.*, 2005). As shown in Table 9.1, phenolics have been found to be most concentrated in the bran fraction, specifically the aleurone layer. Of the phenolic acids reported in wheat, ferulic acid has the highest content with total ferulic acid concentrations ranging from 838 to 3214  $\mu\text{g/g}$  in the aleurone layer. Ferulic acid has also been reported as the primary compound responsible for the antioxidant potency of wheat grain (Anson *et al.*, 2008).

## 9.4 Reported antioxidant and other health promoting properties of wheat

Given the hypothesis that the antioxidant compounds in wheat may be responsible for its health promoting properties, many recent studies have evaluated wheat's antioxidant capacity and properties *in-vitro* and *ex-vivo*. These studies have included scavenging capacities against numerous ROS and non-physiologically relevant free radicals, chelating capacities against reactive transition metals, inhibition of lipid oxidation, and reducing capacity.

Table 9.2 summarizes results from numerous studies on commonly reported *in-vitro* antioxidant properties of wheat grain and its fractions measured. These include scavenging capacity against three physiologically relevant radicals, the superoxide anion radical, peroxyl radical (measured with the ORAC assay), and hydroxyl radical (measured with the HOSC assay). Scavenging capacity against two non-physiologically relevant radicals, the ABTS cation radical and DPPH radical, have also been reported. Lastly iron (II) chelating capacity and total phenolic contents (TPC), which measures reducing capacity, have been reported. These data demonstrate that wheat and its fractions do exhibit significant *in-vitro* antioxidant properties. These data also demonstrate that the bran fraction generally exhibits greater antioxidant properties than the whole grain, supporting the notion that the bran fraction plays an important role in the health promoting properties of wheat.

As shown in Table 9.2, the analytical method used to carry out antioxidant capacity estimation for wheat and its fractions significantly affects the result of these measurements. This is due to the complex and multifaceted nature of wheat antioxidants and how they interact with different free radical species. Data presented in Table 9.2 also indicate significant variability for the same wheat fraction measured using the same method. For example, ORAC results reported for the wheat bran fraction range from 15.0 to 310.0  $\mu\text{moles trolox equivalents per gram of sample}$ . Several factors explain this variability including genotype, growing environment, sample preparation conditions, and conditions used to carry out the antioxidant capacity method. All of these factors have been shown to significantly impact antioxidant capacity values, and must be considered when comparing data reported for wheat and its fractions (Mpofu *et al.*, 2006; Moore *et al.*, 2006; Zhou *et al.*, 2004b; Moore and Yu, 2008a, 2008b).

Several *ex-vivo* studies have evaluated the ability of antioxidant rich wheat extracts to protect biomolecules such as LDL and supercoiled DNA from oxidative damage. Yu *et al.* (2005) evaluated the ability of hard wheat brans to inhibit the Cu(II) ion induced oxidation of LDL. This report measured LDL oxidation using the thiobarbituric acid reactive substance (TBARS) lipid oxidation method. It reported results ranging from 1.03 to 1.56 mg TBARS reduction per gram bran per 100  $\mu\text{g}$  protein. Another recent investigation by Liyana-Pathirana and others used the conjugated dienes method to assess the ability of wheat bran to inhibit LDL oxidation (Liyana-Pathirana and Shahidi, 2006a). They reported results of

**Table 9.2** Reported antioxidant properties of wheat grain and its fractions

	<b>Wheat grain</b>	<b>Wheat bran</b>	<b>Aleurone</b>	<b>References</b>
O <sub>2</sub> <sup>-</sup> (% scavenged)	22.0	21.0–44.0	41.0–57.0	Zhou <i>et al.</i> , 2004a, 2004b, Moore <i>et al.</i> , 2006a
TPC (mg GAE/g)	0.23–9.28	2.1–3.5	3.0–4.0	Moore <i>et al.</i> , 2005, Adom <i>et al.</i> , 2003, Mpofu <i>et al.</i> , 2006, Zhou <i>et al.</i> , 2007, 2004b, Yu <i>et al.</i> , 2002a, Zhou and Yu, 2004a, Gelinias and McKinnon, 2006
TPC (mg FAE/g)	0.35–2.06	0.85–6.56	NA	Alabaster <i>et al.</i> , 1993, Velioglu <i>et al.</i> , 1998, Zielinski <i>et al.</i> , 2000, Liyana-Pathirana and Shahidi, 2007a
DPPH EC <sub>50</sub> (mg/ml)	2.0–27.0	5.0–12.0	6.0–8.0	Moore <i>et al.</i> , 2005, Cheng <i>et al.</i> , 2006a, Zhou <i>et al.</i> , 2004b, Yu <i>et al.</i> , 2002
RDSC (μmol TE/g)	0.9–1.53	1.8–3.2	NA	Zhou <i>et al.</i> , 2007, Cheng <i>et al.</i> , 2006a
ABTS (μmol TE/g)	1.1–36	5.0–36	23.0–24.0	Zhou <i>et al.</i> , 2004a, 2005, 2004b, Moore <i>et al.</i> , 2005, 2006a, Yu <i>et al.</i> , 2002a, Zhou and Yu, 2004a, Liyana-Pathirana and Shahidi, 2006a, Yu and Zhou, 2004, Yu <i>et al.</i> , 2003, Zielinski <i>et al.</i> , 2000, Iqbal <i>et al.</i> , 2007
ORAC (μmol TE/g)	16.0–100.0	15.0–310.0	125.0–136.0	Zhou <i>et al.</i> , 2004a, 2005, 2007, 2001b, Moore <i>et al.</i> , 2005, 2006a, Miller <i>et al.</i> , 2000, Iqbal <i>et al.</i> , 2007, Liyana-Pathirana and Shahidi, 2007a
HOSC (μmol TE/g)	15.7–39	75	NA	Zhou <i>et al.</i> , 2007, Moore <i>et al.</i> , 2006
TEAC (μmol TE/G)	4.24–4.99	10.32–70	70.0–80.0	Anson <i>et al.</i> , 2008, Liyana-Pathirana and Shahidi, 2007a
Iron(II) Chelating (mg EDTA eq/g)	0.11–5.4	0.375–2.04	1.25–1.75	Zhou <i>et al.</i> , 2004a, 2005, 2004b, Moore <i>et al.</i> , 2005, 2006a, Zhou and Yu, 2004a, Yu and Zhou, 2004, Yu <i>et al.</i> , 2003, Moore <i>et al.</i> , 2006, Liyana-Pathirana and Shahidi, 2007a, Iqbal <i>et al.</i> , 2007

O<sub>2</sub><sup>-</sup> stands for superoxide anion radical scavenging, TPC stands for total phenolic contents using Folin-Ciocalteu reagent, GAE stands for gallic acid equivalents, FAE stands for ferulic acid equivalents, DPPH<sup>\*</sup> stands for DPPH radical scavenging capacity, EC<sub>50</sub> is the required concentration of a sample to scavenge 50% of DPPH radicals present in a reaction mixture. EC<sub>50</sub> values in this table are expressed as mg wheat equivalents per ml of final reaction mixture. RDSC stands for relative DPPH<sup>\*</sup> scavenging capacity, TE stands for trolox equivalents, ABTS<sup>+</sup> stands for cation ABTS radical scavenging capacity, ORAC stands for oxygen radical absorbing capacity, HOSC stands for hydroxyl radical scavenging capacity, TEAC stands for trolox equivalent antioxidant capacity, EDTA stands for ethylenediaminetetraacetic acid, NA stands for data not available.

3315–3795 and 4845–4978 µg LDL protein protected per gram of defatted wheat bran for soft and hard wheat grains and brans, respectively. Liyana-Pathirana and Shahidi (2006a, 2006b) also reported the dose-dependent ability of wheat extracts to inhibit the hydroxyl radical induced cleavage of supercoiled DNA. They found wheat pearling fractions to be more effective than pearled wheat. Krings and others (2006) demonstrated the ability of roasted wheat germ extract to inhibit damage to DNA induce by a Fe(II)/H<sub>2</sub>O<sub>2</sub> ROS generating system.

Cheng and others, in 2008, investigated the effects of antioxidant-rich wheat extracts on oxygen diffusion-concentration products *ex-vivo* in liposomes prepared with egg yolk and rat liver phosphatidylcholines using electron spin resonance (ESR) oximetry method (2008). Oxidation was initiated in this study using a water-soluble APPH or a fat-soluble AMVN radical generators. Both water-soluble and lipophilic wheat antioxidant extracts were able to suppress oxygen diffusion-concentration product induced by either AAPH or AMVN. The effectiveness of the water-soluble and lipophilic wheat antioxidants were altered by the radical generator and the fatty acid composition of the liposome system. Interestingly, this study also showed that wheat antioxidants could down-regulate the mRNA of HMG-CoA reductase, the key enzyme catalyzing the rate-limiting step during cholesterol biosynthesis, and up-regulate the mRNA of cholesterol 7 $\alpha$ -hydroxylase, a key enzyme for converting cholesterol to bile acids in rat primary hepatocytes. The mRNA of HMG-CoA reductase was less stable in the hepatocytes treated with wheat antioxidants, but the mRNA of cholesterol 7 $\alpha$ -hydroxylase was more stable in the cells treated with wheat antioxidants, compared to the control hepatocytes. Wheat antioxidants did not alter the expression of LDL receptor gene in primary rat hepatocytes. Results from this study suggest the potential of wheat antioxidants in reducing the plasma and liver cholesterol levels and also indicate that wheat antioxidants may benefit human health through mechanisms independent from their antioxidant properties (Cheng *et al.*, 2008).

## 9.5 Bioavailability of phenolic acids in wheat

Bioavailability can be defined as the portion of an oral dose that reaches systemic circulation. In reference to food-based bioactives, bioavailability includes: 1) availability of bioactive for absorption in the gastrointestinal (GI) system also referred to as “bioaccessibility”, 2) physical absorption of bioactive through small or large intestinal epithelium, 3) metabolism before during or after metabolism by phase I and II enzymes, 4) tissue distribution, and 5) bioactivity (Stahl *et al.*, 2002; Kroon *et al.*, 2004). Given the importance of bioavailability on the potential ability of bioactive wheat antioxidant compounds to impart their beneficial effects *in-vivo* numerous groups have investigated the bioavailability of phenolic acids, a class of compounds thought to be highly involved in wheat's antioxidant and health promoting properties.

Several studies have evaluated the absorption characteristics of both soluble free forms of several phenolic acids present in wheat, as well as soluble conjugated forms. Three animal studies have demonstrated efficient absorption of soluble free forms of ferulic, *p*-coumaric, and caffeic acids in rats with up to 50% recovery in urine of the conjugated forms of these phenolic acids (Konishi *et al.*, 2004, 2005; Adam *et al.*, 2002). Two human studies evaluated the absorption of ferulic acid after ingesting soluble free ferulic acid in different food matrices by measuring urinary excretion (Bourne and Rice-Evans, 1998; Bourne *et al.*, 2000). These human investigations found relatively high absorption efficiency with up to

25% of ferulic acid absorbed and peak urinary excretion between seven and nine hours. In addition, significant amounts of the ferulic acid absorbed were found to be glucuronidated derivatives of ferulic acid indicating phase II metabolism (Bourne and Rice-Evans, 1998; Bourne *et al.*, 2000). Results from a rat investigation by Zhao and others (2003) have also shown that 5-*O*-feruloyl-L-arabinofuranose, a soluble conjugated form of ferulic acid, was absorbed in small the intestines of rats, suggesting that soluble conjugated phenolic acids may be absorbable in humans. Lastly, *in-vitro* studies by Konishi and others using intestinal Caco-2 cells to determine absorption mechanisms for phenolic acids have shown transepithelial transport of pure ferulic and *p*-coumaric acids across monolayers by monocarboxylic acid transporters (Konishi and Shimizu, 2003; Konishi *et al.*, 2003). Together, these studies have shown that soluble free forms and potentially soluble conjugated forms of phenolic acids present in wheat might be absorbed in humans.

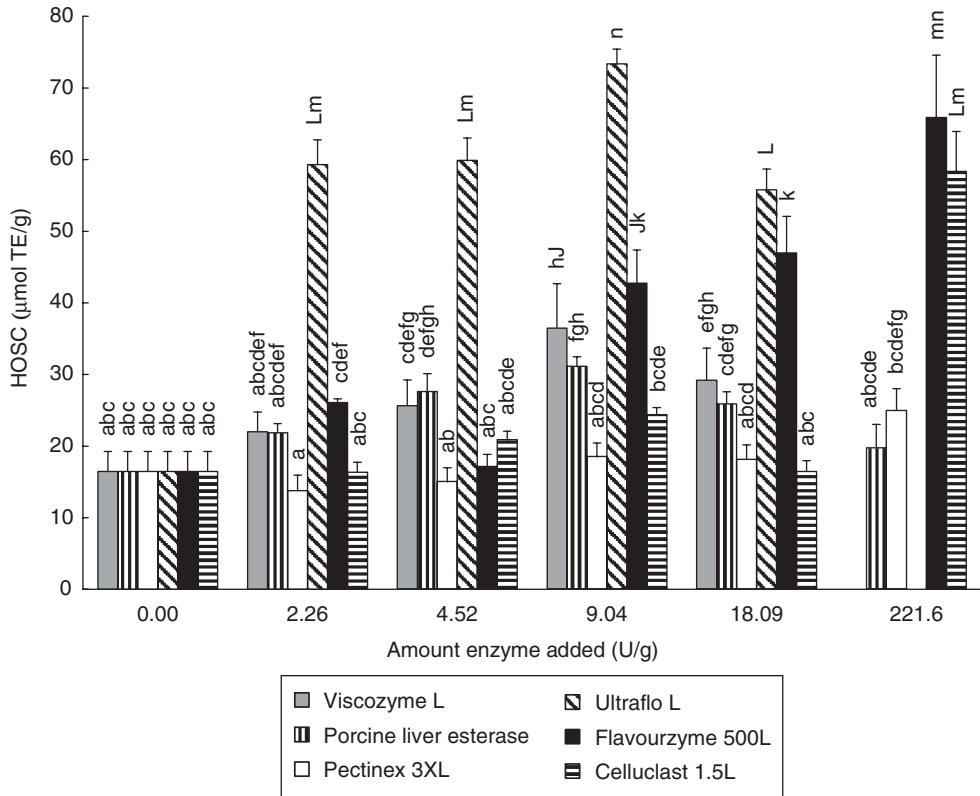
One study has reported on the effect of the wheat matrix on the bioavailability of wheat phenolics since the majority of phenolic acids present in wheat grain are in an insoluble bound form, esterified to cell wall materials. This human study by Kern and others (2003) evaluated the recovery of phenolic acids in plasma and urine after wheat bran consumption found only 3% of the total phenolics (soluble free, soluble conjugated, and insoluble bound) were absorbed after 24 hours. This 3% corresponded approximately to the soluble free phenolic contents of the wheat bran consumed. This study also concluded that the site of absorption was primarily the small intestine (SI), with maximum absorption one to three hours after ingestion.

It is well accepted that soluble free and conjugated wheat phenolics can be absorbed in the human small intestine. The majority of wheat phenolics, however, are in an insoluble bound form impairing their absorption and hence their bioavailability. This has stimulated research on techniques for releasing insoluble bound phenolics from wheat prior to consumption to increase its bioavailable antioxidant contents.

## 9.6 Use of post-harvest treatments to improve the bioaccessibility of antioxidant in wheat-based ingredients

One possible strategy to improve the bioavailability of wheat phenolics is to release the insoluble bound phenolic acids from their matrix prior to consumption using post-harvest treatments. The polysaccharide composition of wheat bran aleurone and pericarp cell walls includes mostly arabinoxylans with some  $\beta$ -glucans and cellulose (Rhodes *et al.*, 2002; Mathew and Abraham, 2004). Phenolic acids are primarily esterified to the C-5 hydroxyl group of  $\alpha$ -L-arabinofuranosyl substituents, which are linked to C-2 or C-3 on the xylopyranosyl backbone (Mathew and Abraham, 2004; Kroon *et al.*, 1999). In 2006, Moore and others evaluated the potential of solid-state enzyme treatments to release insoluble bound antioxidants such as phenolic acids from wheat bran, thereby improving its extractable and potentially bioaccessible antioxidant properties including total phenolic contents, individual phenolic acids, and scavenging capacities against peroxy (ORAC), ABTS cation, DPPH and hydroxyl radicals (Moore *et al.*, 2006c). The results showed that commercial food grade enzyme preparations including Viscozyme L, Pectinex 3XL, Ultraflo L, Flavourzyme 500L and Celluclast 1.5L, as well as a purified non-food grade porcine liver esterase, could significantly increase extractable antioxidant properties, and some were in a dose-dependent





**Figure 9.1** Effects of different enzyme treatments on the hydroxyl radical scavenging capacities (HOSC) of Akron hard wheat bran.

Enzyme treatments of bran were carried out at a moisture content of 35% for all treatments. Initial reaction enzyme dosages are expressed as enzyme activity units per gram of wheat bran on a dry weight basis, using manufacturer declared major activity for each enzyme preparation. Results expressed as  $\mu\text{mol}$  trolox equivalents per gram of wheat bran on a dry weight basis. All tests were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

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matter (Figure 9.1). The Ultraflo L enzyme preparation was the most efficient enzyme and able to convert as much as 50% of the insoluble bound ferulic acid in wheat bran to soluble free form. It was also reported that the effects of moisture content on these solid-state enzyme reactions were dependent on enzyme concentration. These data suggested that solid-state enzyme treatments of wheat grain and fractions may be a commercially viable post-harvest approach to improve the bioavailability of wheat antioxidants.

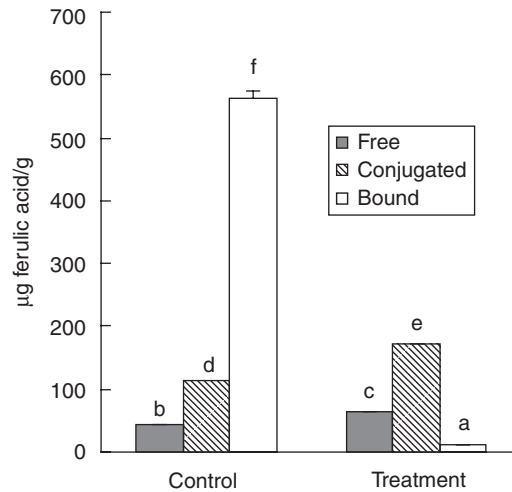
Findings from the solid-state study reported by Moore and others in 2006 were supported by several previous studies. In brief, the enzymatic approach has been shown to be effective using xylanases,  $\beta$ -glucanases, and cellulases to break up wheat bran cell wall material combined with enzymes specifically to hydrolyze the ester linked phenolic acids such as cinnamoyl or feruloyl esterases (Mathew and Abraham, 2004; Kroon *et al.*, 1999;

Sørensen *et al.*, 2003; Bartolomé *et al.*, 1995; Lequart *et al.*, 1999; Faulds *et al.*, 2004; Yuan *et al.*, 2006). Commercial enzyme preparations containing mixtures of these enzymes have been reported including Ultraflo-L, Viscozyme-L, Celluclast 1.5L, Termamyl, and Lallzyme and shown to effectively release phenolic acids (Sørensen *et al.*, 2003; Faulds *et al.*, 2004). One study by Faulds and others (2004) reported release of 90% of insoluble bound ferulic acid by treatment using the Ultraflo-L enzyme preparation. All these previous studies, however, have conducted enzyme reactions in aqueous systems. These systems may prove impractical for commercial scale post-harvest treatments for food ingredients because products must be separated from the aqueous reaction phase. In contrast to aqueous reactions, solid-state reactions are practical for food ingredient production because they require no expensive equipment, are environmentally friendly, and require little post-reaction processing to recover products because there is no need to remove free liquid at the end of the process to isolate or use products.

A solid-state yeast treatment was also investigated for its potential to improve the health properties of wheat bran in 2007 (Moore *et al.*, 2007). Three commercial food grade yeast preparations were compared for their potential in the release of beneficial components from wheat bran. Solid-state yeast treatments were able to significantly increase releasable antioxidant properties ranging 28–65, 0–20, 13–19, 0–25, 50–100, and 3–333% for scavenging capacities against peroxy (ORAC), ABTS cation, DPPH and hydroxyl radicals, total phenolic contents (TPC), and phenolic acids, respectively. Additional studies on the effects of yeast treatments on the composition of four phenolic acids in wheat bran suggest that the studied yeast may have produced enzymes capable of releasing all four measured insoluble bound phenolic acids in wheat bran, thereby increasing its soluble free and/or soluble conjugated phenolic acid contents as shown in Figure 9.2 for ferulic acid (Moore *et al.*, 2007). Results from this series of studies suggest that solid-state yeast treatment may be a commercially viable post-harvest procedure to improve the health properties of wheat bran and other wheat-based food ingredients.

Findings from the 2007 study by Moore and others were supported by the observations from a recent study evaluating the effects of fermentation and a combination of enzymatic treatment and fermentation on *in vitro* bioaccessibility and colonic metabolism of wheat phenolics (Anson *et al.*, 2009). The combination of enzyme treatment and fermentation was shown to be more effective in enhancing the bioaccessibility of ferulic acid from bread samples containing the bioprocessed wheat bran. The bioprocessing procedure was also able to increase the colonic end metabolite of ferulic acid, 3-phenylpropionic acid, under the *in vitro* digestion test conditions (Anson *et al.*, 2009).

The effects of post-harvest treatments including fractionation and reduction in particle size, and heat stress on the availability of wheat antioxidants were recently reported (Cheng *et al.*, 2006b). In this study, grain, bran, and 40-mesh bran samples of both Ankor and Trego wheat varieties stored at 25, 60, and 100 °C for zero, one, two, three, five, and nine days were ground and extracted with pure ethanol and examined for total phenolic content (TPC), phenolic acid composition, and the scavenging activities against peroxy (ORAC), cation ABTS and 2, 2-diphenyl-1-picrylhydrazyl (DPPH<sup>\*</sup>) radicals. The results showed that both heat stress and post-harvest treatments significantly altered the antioxidant properties of wheat grain fractions. The ORAC values of the Ankor bran and the corresponding 40-mesh bran samples kept at 100 °C for nine days reduced to 61 and 40% of that at day zero on a per dry weight basis, respectively, while the ORAC values of the grain samples showed no significant change. The 40-mesh bran samples of both Ankor and Trego varieties also lost more DPPH<sup>\*</sup> scavenging capacity during the storage. The results from this study suggested



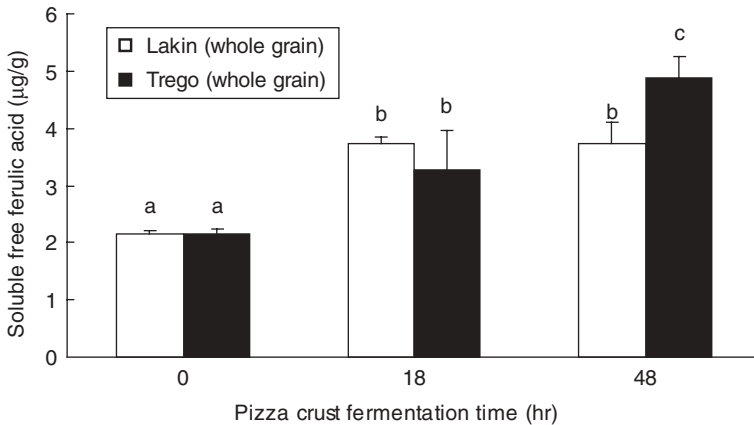
**Figure 9.2** Effect of solid-state yeast fermentation ferulic acid contents of Lakin wheat bran. Solid-state fermentation conditions included yeast concentration of 0.1 g yeast per gram of bran, fermentation time of 48 hours, and temperature of 32 °C. Free stands for soluble free; conjugated stands for soluble conjugated; bound stands for insoluble bound. Results expressed µg individual phenolic acids per gram of wheat bran on a dry weight basis. All measurements were conducted in duplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ). (Reproduced from Moore *et al.*, copyright 2007, with permission of the American Chemical Society.)

that reduction of particle size may accelerate the loss of natural antioxidants in wheat bran during storage and thermal processing, and whole grain as opposed to its fractions is a preferred form of long-term storage for better preserving natural antioxidants although reduction of particle size may enhance the release of wheat antioxidants from bran.

Findings from this study were supported by the observations of two independent studies indicating that pearling and polishing altered the antioxidant properties of wheat flours (Liyana-Pathirana *et al.*, 2006; van Hung *et al.*, 2009). In 2006, Liyana-Pathirana and others investigated the effect of pearling on the phenolic contents and antioxidant properties of Canada Western Amber Durum and Red Spring wheat. The unprocessed whole wheat grains had the greatest antioxidant properties, and the antioxidant properties of the pearled grains and byproducts were inversely associated with the degree of pearling (Liyana-Pathirana *et al.*, 2006). Later, in 2009, graded flours of whole wheat grain, prepared by gradual milling method using a modified Japanese rice-polisher, showed higher phenolic content and antioxidant activities than that of the white flours (van Hung *et al.*, 2009). The results from these two studies also confirmed that phenolic components and antioxidants are concentrated in the bran, which may be used as a natural source of antioxidants for improving human health.

## 9.7 Effects of processing on antioxidants in wheat-based food systems

Given the widespread use of wheat in processed food products it is of interest to understand how individual processing steps may alter antioxidant properties. This information can be



**Figure 9.3** Effects of fermentation time on the soluble free ferulic acid contents of whole-grain pizza dough for two hard wheat varieties. Dough fermentations were carried out at 4°C. Results expressed in micrograms of ferulic acid per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ). (Reproduced from Moore *et al.*, copyright 2009, with permission of the American Chemical Society.)

used to develop optimal processes capable of delivering the maximum antioxidant benefits to consumers through finished food products. Most types of wheat-based food products undergo a simple series steps such as mixing of dry ingredients with water, kneading, fermentative proofing, and baking. Together, the results of these steps have the potential to significantly alter wheat antioxidant contents and bioavailability as a result of thermal, mechanical, chemical, physical, and enzymatic changes. A number of studies have investigated the effects of different processing steps on the antioxidant contents and properties wheat-based food products, mostly using leavened bakery type products.

A 2006 study by Gelinias and McKinnon (2006) found the bread-baking process to slightly increase the total phenolic contents of refined wheat bread. A more recent study by Moore and others in 2009 investigated the effects of different dough fermentation and baking conditions on the antioxidant properties and phenolic acid contents of whole-wheat pizza crust (Moore *et al.*, 2009). Results from this study indicate that increasing dough fermentation time from zero to 48 hours could increase the contents of soluble free ferulic acid as much as 130%, as shown in Figure 9.3. Baking conditions were also shown to increase some antioxidant properties in this study. For example, relative DPPH<sup>•</sup> scavenging capacity (RDSC) of the studied pizza crusts were increased by 50 to 82% as a result of increasing thermal treatment from 7 to 14 minutes at 204°C or from 204 to 288°C for seven minutes. Changes in phenolic acid composition from the altered baking conditions were also studied, but changes overall were minor and did not account for the changes in antioxidant capacity observed in this study. This indicates that other chemical changes beyond alterations in phenolic acid composition may be occurring during the baking of wheat-based foods that alter antioxidant properties.

The formation of Maillard reaction products in wheat-based food systems has been implicated in the changes in antioxidant properties observed in at least two studies. Bressa

and others (1996) reported that enhanced browning reactions in baked cookies, from added sugar and amino acids, increased their oxidative stability. Another study by Lindenmeier and Hoffman (2004), among others, found increasing concentrations of the antioxidant Maillard reaction product 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonylpentyl)-3-oxo-2H-pyrrol (pronyl-L-lysine) in bread crust as a result of increasing thermal treatments from 220 to 260 °C and 70 to 210 minutes. This study also found that the addition of lysine-rich casein or glucose to bread formulations increased production of the antioxidant compound (pronyl-L-lysine) by more than 200%. These studies support the role that the Maillard reaction may play in altering the antioxidant properties and contents of wheat-based food systems.

Several studies from the 1990s examined the influence of processing on antioxidative vitamin contents in wheat-based food systems. Rodgers and others (1993) evaluated the stability of  $\beta$ -carotene during baking and pre-baking processing steps for yellow cake, sugar cookie, and bagels. This study found pre-baking processing steps such as mixing, rolling, and proofing to cause no significant decreases in  $\beta$ -carotene. This study also found baking to cause significant reductions in the all-*trans*  $\beta$ -carotene isomer contents ranging from 74 to 85%. Ranhotra and others (1995) found  $\beta$ -carotene fortified bread and cracker baking to decrease total carotenoids from 4 to 23% with up to 20% of remaining carotenoids isomerized to 13- or 9-*cis* isomers. This study also found pre-baking steps for these products, including mixing and proofing, to cause minimal decreases in total carotenoids. Evaluating the effects of semolina pasta dough mixing on antioxidant contents, one study found this processing step to significantly decrease the contents of carotenoids (Icard-Verniere and Feillet, 1999). A recent study by Leenhardt and others (2006) found the most significant decreases (66%) in total carotenoid contents during bread making to occur during kneading with a high correlation of these losses to the lipoxygenase activity of wheat variety. This same study found less than 10% of losses in total carotenoids as a result of dough fermentation at 30 °C, and losses during baking of 36–45%. Together, these studies indicate that food processing in wheat-based food systems can alter its  $\beta$ -carotene contents and induce isomerization. The magnitude of these changes, however, is dependent on the food matrix and processing steps utilized.

Food processing induced changes in the antioxidative vitamin E contents of wheat food products have been investigated. Hakansson and Jagerstad (1990) evaluated the effects of mixing and drum drying on the vitamin E retention of wheat flours. Their study found the mixing of water with flour to induce significant losses of vitamin E likely from lipoxygenase activity, and continued processing with drum drying to degrade 90% of vitamin E present. Hakansson and Jagerstad (1992) evaluated the effect of bread-making steps on vitamin E losses and found fermentation to have no effect on vitamin E levels, while sour dough fermentation and dough mixing resulted in 20–60% decreases in vitamin E contents. Ranhotra and others (2000) found vitamin E fortified bread to retain 67% of the original vitamin E content after all processing steps including mixing, kneading, and baking. A recent study by Leenhardt and others found significant decreases in the tocopherol contents during bread making with 10–12% losses attributed to kneading and 15–20% losses attributed to baking (Lindenmeier and Hofmann, 2004). Further evaluating these changes, the study attributed the losses of vitamin E to direct oxidation and thermal destruction of vitamin E during processing (Lindenmeier and Hofmann, 2004). Together, these studies indicate that food processing in wheat-based food systems can alter its vitamin E contents. The magnitudes of these changes, however, are dependent on the food matrix and processing steps utilized.

## References

- Adam, A., Crespy, V., Levrat-Verny, M., Leenhardt, F., Leuillet, M., Demigne, C., and Remesy, C. (2002) The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. *Journal of Nutrition*, **132**: 1962–1968.
- Adom, K.K., Sorrells, M.E., and Liu, R.H. (2003) Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry*, **51**: 7825–7834.
- Adom, K.K., Sorrells, M.E., and Liu, R.H. (2005) Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *Journal of Agricultural and Food Chemistry*, **53**: 2297–2306.
- Alabaster, O., Tang, Z.C., Frost, A., and Shivapurkar, N. (1993) Potential synergism between wheat bran and psyllium: enhanced inhibition of colon cancer. *Cancer Letters*, **75**: 53–58.
- Alabaster, O., Tang, Z.C., Frost, A., and Shivapurkar, N. (1995) Effect of  $\beta$ -carotene and wheat bran fiber on colonic aberrant crypt and tumour formation in rats exposed to azoxymethane and high dietary fat. *Carcinogenesis*, **16**: 127–132.
- Anderson, J.W. (2003) Whole-grains protect against atherosclerotic cardiovascular disease. *Proceedings of the Nutritional Society*, **62**: 135–142.
- Anderson, J.W. (2004) Whole grains and coronary heart disease: the whole kernel of truth. *American Journal of Clinical Nutrition*, **80**: 1459–1466.
- Anson, N.M., Van Den Berg, R., Havenaar, R., Bast, A., and Haenen, G.R.M.M. (2008) Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry*, **56**: 5589–5594.
- Anson, N.M., Selinheimo, E., Havennar, R., Aura, A.-M., Mattila, I., Lehtinen, P., Bast, A., Poutanen, K., and Haenen, G.R.M.M. (2009) Bioprocessing of wheat bran improves in vitro bioaccessibility and colonic metabolism of phenolic compounds. *Journal of Agricultural and Food Chemistry*, **57**: 6148–6155.
- Barbolt, T.A. and Abraham, R. (1978) The effect of bran on dimethylhydrazine induced colon carcinogenesis in the rat. *Proceedings of the Society for Experimental Biology and Medicine*, **157**: 656–659.
- Barbolt, T.A. and Abraham, R. (1980) Dose response, sex difference and the effect of bran in dimethylhydrazine induced intestinal tumorigenesis in rats. *Toxicology and Applied Pharmacology*, **55**: 417–422.
- Bartolomé, B., Faulds, C.B., Tuohy, M., Hazlewood, G.P., Gilbert, H.J., and Williamson, G. (1995) Influence of different xylanases on the activity of ferulic acid esterases on wheat bran. *Biotechnology and Applied Biochemistry*, **22**: 65–73.
- Bourne, L.C., and Rice-Evans, C. (1998) Bioavailability of ferulic acid. *Biochemical and Biophysical Research Communications*, **253**: 222–227.
- Bourne, L., Paganga, G., Baxter, D., Hughes, P., and Rice-Evans, C. (2000) Absorption of ferulic acid from low-alcohol beer. *Free Radical Research*, **32**: 273–280.
- Bowden, F.P. and Moore, T. (1933) Absorption spectrum of the vitamin E fraction of wheat-grem oil. *Nature*, **132**: 204.
- Bressa, F., Tesson, N., Dallaroas, M., Sensidoni, A., and Tubaro, F. (1996) Antioxidant effect of Maillard reaction products – applications to a butter cookie of a competition kinetic analysis. *Journal of Agricultural and Food Chemistry*, **44**: 692–695.
- Carter, J.W., Madl, R., and Padula, F. (2006) Wheat antioxidants suppress intestinal tumor activity in Min mice. *Nutritional Research*, **26**: 33–38.
- Cheng, Z., Moore, J., and Yu, L. (2006a) A high-throughput relative DPPH radical scavenging capacity (RDSC) assay. *Journal of Agricultural and Food Chemistry*, **54**: 7429–7436.
- Cheng, Z., Zhou, H., Luther, M., Yin, J., and Yu, L. (2008) Effects of wheat antioxidants on oxygen diffusion-concentration products in liposomes and mRNA levels of HMG-CoA reductase and cholesterol 7 $\alpha$ -hydroxylase in primary rat hepatocytes. *Journal of Agricultural and Food Chemistry*, **56**: 5033–5042.
- Cheng, Z., Su, L., Moore, J., Zhou, K., Luther, M., Yin, J., and Yu, L. (2006b) Effects of post-harvest treatment and heat stress on availability of wheat antioxidants. *Journal of Agricultural and Food Chemistry*, **54**: 5632–5629.
- Crosby, G. (2005) Lignans in food and nutrition. *Food Technology*, **59**: 32–36.
- El-Basyouni, S.Z. and Towers, G.H.N. (1964) The phenolic acids in wheat. I. *Changes during growth and development*. *Canadian Journal of Biochemistry*, **42**: 203–210.
- Evans, H.M. and Burr, G.O. (1925) The anti-sterility vitamin fat soluble E. *PNAS*, **11**: 334–341.

- Faulds, C.B., Mandalari, G., LoCurto, R., Bisignano G., and Waldron, K.W. (2004) Arabinoxylan and mono- and dimeric ferulic acid release from brewer's grain and wheat bran by feruloyl esterases and glycosyl hydrolases from *Humicola insolens*. *Applied Microbiology and Biotechnology*, **64**: 644–650.
- Fausch, H., Kundig, W., and Neukom, H. (1963) Ferulic acid as a component of a glycoprotein from wheat flour. *Nature*, **199**: 287.
- Febles, C.I., Arias, A., Hardisson, A., Rodriguez-Alvarez, C., and Sierra, A. (2002) Phytic acid level in wheat flours. *Journal of Cereal Science*, **36**: 19–23.
- Fuchs, C.S., Giovannucci, E.L., and Colditz, G.A. (1999) Dietary fiber and the risk of colorectal cancer and adenoma in women. *New England Journal of Medicine*, **340**: 169–176.
- Fulcher, R.G., O'Brien, T.P., and Lee, J.W. (1972) Studies on the aleurone layer. I. Conventional and fluorescence microscopy of the cell wall with emphasis on phenol-carbohydrate complexes in wheat. *Australian Journal of Biological Science*, **25**: 23–34.
- Gallus, H.P.C. and Jennings, A.C. (1971) Phenolic compounds in wheat flour and dough. *Australian Journal of Biological Science*, **24**: 747–753.
- Garcia-Conesa, M.T., Plumb, G.W., Waldron, K.W., Ralph, J., and Williamson, G. (1997) Ferulic acid dehydrodimers from wheat bran: isolation, purification and antioxidant properties of 8-O-4-diferulic acid. *Redox Report*, **3**: 319–323.
- Gelinas, P. and McKinnon, C.M. (2006) Effect of wheat variety, farming site, and bread-baking on total phenolics. *International Journal of Food Science and Technology*, **41**: 329–332.
- Graf, E. and Easton, J.W. (1990) Antioxidant functions of phytic acid. *Free Radical Biology and Medicine*, **8**: 61–69.
- Hakansson, B. and Jagerstad, M. (1990) The effect of thermal inactivation of lipoxygenase on the stability of vitamin E in wheat. *Journal of Cereal Science*, **12**: 177–185.
- Hakansson, B. and Jagerstad, M. (1992) Breadmaking and storage of various wheat fractions affect vitamin E. *Journal of Food Science*, **57**: 1205–1209.
- Halliwell, B., Gutteridge, J.M.C., eds. (1989) The chemistry of oxygen radicals and other oxygen-derived species. In *Free Radicals in biology and medicine*, 3rd ed., Oxford University Press: New York, pp. 22–81.
- Humphries, J.M. and Khachik, F. (2003) Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of Agricultural and Food Chemistry*, **51**: 1322–1327.
- Icard-Verniere, C. and Feillet, P. (1999) Effects of mixing conditions on pasta dough development and biochemical changes. *Cereal Chemistry*, **76**: 558–565.
- Iqbal, S., Bhangerm, M.I., and Anwar, F. 2007. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. *LWT Food Science and Technology*, **40**: 361–367.
- Irmak, S., Jonnala, R.S., and MacRitchie, F. (2008). Effect of genetic variation on phenolic acid and policosanols contents of Pegaso wheat lines. *Journal of Cereal Science*, **48**: 20–26.
- Jenab, M. and Thompson, L.U. (1998) The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. *Carcinogenesis*, **19**: 1087–1092.
- Jensen, M.K., Koh-Banerjee, P., Hu, F.B., Franz, M., Sampson, L., Gronbaek, M., and Rimm, E.B. (2004) Intakes of whole-grains, bran, and germ and the risk of coronary heart disease in men. *American Journal of Clinical Nutrition*, **80**: 1492–1499.
- Jones, J.M. (2006) Grain-based foods and health. *Cereal Food World*, **51**: 108–113.
- Kim, K., Tsao, R., Yang, R., and Cui, S.W. (2006) Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effects of hydrolysis conditions. *Food Chemistry*, **95**: 466–473.
- Kern, S.M., Bennett, R.N., Mellon, F.A., Kroon, P.A., and Garcia-Conesa, M. (2003) Absorption of hydrocinamates in humans after high-bran cereal consumption. *Journal of Agricultural and Food Chemistry*, **51**: 6050–6055.
- Konishi, Y., Hitomi, Y., Yoshida, M., and Yoshioka, E. (2005) Pharmacokinetic study of caffeic and rosmarinic acids in rats after oral administration. *Journal of Agricultural and Food Chemistry*, **53**: 4740–4746.
- Konishi, Y., Hitomi, Y., and Yoshioka, E. (2004) Intestinal absorption of p-coumaric and gallic acids in rat after oral administration. *Journal of Agricultural and Food Chemistry*, **52**: 2527–2532.
- Konishi, Y. and Shimizu, M. (2003) Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Bioscience, Biotechnology and Biochemistry*, **67**: 856–862.
- Konishi, Y., Kobayashi, S., and Shimizu, M. (2003) Transepithelial transport of p-coumaric acid and gallic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Bioscience, Biotechnology and Biochemistry*, **67**: 2317–2324.

- Krings, U., Johansson, L., Zorn, H., and Berger, R.G. (2006) In-vitro DNA-protective activity of roasted wheat germ and fractions thereof. *Food Chemistry*, **97**: 712–718.
- Kroon, P.A., Clifford, M.N., Crozier, A., Day, A.J., Donovan, J.L., Manach, C., and Williamson, G. (2004) How should we assess the effects of exposure to dietary polyphenols in vitro? *American Journal of Clinical Nutrition*, **80**: 15–21.
- Kroon, P.A., Garcia-Conesa, M.T., Hazlewood, G.P., and Williamson, G. (1999) Release of ferulic acid dehydromers from plant cell walls by feruloyl esterases. *Journal of the Science of Food Agriculture*, **79**: 428–434.
- Leenhardt, F., Lyan, B., Rock, E., Boussard, A., Potus, J., Chanliaud, E., and Remesy, C. (2006) Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *Journal of Agricultural and Food Chemistry*, **54**: 1710–1715.
- Lequart, C., Nuzillard, J., Kurek, B., and Debeire, P. (1999) Hydrolysis of wheat bran and straw by and endoxylanase: production and structural characterization of cinnomoyl-oligosaccharides. *Carbohydrate Research*, **319**: 102–111.
- Li, W., Shan, F., Sun, S., Corke, H., and Beta, T. (2005) Free radical scavenging properties and phenolic content of Chinese black-grained wheat. *Journal of Agricultural and Food Chemistry*, **53**: 8533–8536.
- Lindenmeier, M. and Hofmann, T. (2004) Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *Journal of Agricultural and Food Chemistry*, **52**: 350–354.
- Liu, S.M., Stampfer, M.J., Hu, F.B., Giovannucci, E., Rimm, E., Manson, J.E., Hennekens, C.H., and Willett, W.C. (1999) Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *American Journal of Clinical Nutrition*, **70**: 412–419.
- Liyana-Pathirana, C.M. and Shahidi, F. (2006a) Antioxidant properties of commercial soft and hard wheats (*Triticum aestivum* L.) and their milling fractions. *Journal of the Science of Food and Agriculture*, **86**: 477–485.
- Liyana-Pathirana, C.M. and Shahidi, F. (2006b) Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural Food Chemistry*, **54**: 1256–1264.
- Liyana-Pathirana, C.M. and Shahidi, F. (2007a) Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chemistry*, **101**: 1151–1157.
- Liyana-Pathirana, C.M. and Shahidi, F. (2007b) The antioxidant properties of milling fractions from bread-wheat and durum. *Journal of Cereal Science*, **45**: 238–247.
- Liyana-Pathirana, C.M., Dexter, J., and Shahidi, F. (2006) Antioxidant properties of wheat as affected by pearling. *Journal of Agricultural and Food Chemistry*, **54**: 6177–6184.
- Mathew, S. and Abraham, T.E. (2004) Ferulic acid: an antioxidant found naturally in plant cell walls and feruloyl esterases involved in its release and their applications. *Critical Review of Biotechnology*, **24**: 59–83.
- Mattila, P., Pihlava, J.H., and Hellström, J. (2005) Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, **53**: 8290–8295.
- Miller, E., Rigelhof, F., Marquart, L., Prakash, A., and Kanter, M. (2000) Antioxidant content of whole grain breakfast cereals, fruits, and vegetables. *Journal of the American College of Nutrition*, **19**: 312S–319S.
- Moore, J., Hao, Z., Zhou, K., Luther, M., Costa, J., and Yu, L. (2005) Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *Journal of Agricultural and Food Chemistry*, **53**: 6649–6657.
- Moore, J., Liu, J-G., Zhou, K., and Yu, L. (2006a) Effects of Genotype and Environment on the Antioxidant Properties of Hard Winter Wheat Bran. *Journal of Agricultural and Food Chemistry*, **54**: 5313–5322.
- Moore, J., Yin, J., and Yu, L. (2006b) Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation. *Journal of Agricultural and Food Chemistry*, **54**: 617–626.
- Moore, J. and Yu, L. (2008a) Effects of extraction method and conditions on wheat antioxidant estimation. In: *Wheat antioxidants*, Yu, L., John Wiley & Sons, Inc.: Hoboken, NJ, pp. 100–118.
- Moore, J., Yu, L. (2008b) Methods for antioxidant capacity estimation of wheat and wheat-based food products. In: *Wheat antioxidants*, Yu, L., John Wiley & Sons, Inc.: Hoboken, NJ, pp. 118–172.
- Moore J., Cheng, Z., Hao, J., Su, L., and Yu, L. (2006c) Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *Journal of Agricultural and Food Chemistry*, **54**: 9032–9045.
- Moore J., Cheng, Z., Hao, J., Guo, G., Liu, J., Lin, C., and Yu, L. (2007) Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *Journal of Agricultural and Food Chemistry*, **55**: 10173–10182.



- Moore, J., Luther, M., Cheng, Z., and Yu, L. (2009) Effects of baking conditions, dough fermentation, and bran particle size on antioxidant properties of whole-wheat pizza crusts. *Journal of Agricultural and Food Chemistry*, **57**: 832–839.
- Mpofu, A., Sapirstein, H.D., and Beta, T. (2006) Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry*, **54**: 1265–1270.
- Nyström, L., Mäkinen, M., Lampi, A., and Piironen, V. (2005) Antioxidant activity of steryl ferulate extracts from rye and wheat bran. *Journal of Agricultural and Food Chemistry*, **53**: 2503–2510.
- Olcott, H.S. and Mattill, H.A. (1936) Antioxidants and the auto-oxidation of fats. *Journal of the American Chemistry Society*, **58**: 1627–1630.
- Panfili, G., Alessandra, F., and Irano, M. (2003) Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *Journal of Agricultural and Food Chemistry*, **51**: 3940–3944.
- Park, Y., Hunter, D.J., and Spiegelman, D. (2005) Dietary fiber intake and risk of colorectal cancer - A pooled analysis of prospective cohort studies. *Journal of the American Medical Association*, **294**: 2849–2857.
- Qu, H., Madl, R.L., Takemoto, D.J., Baybutt, R.C., and Wang, W. (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. *Journal of Nutrition*, **135**: 598–602.
- Ranhotra, G.S., Gelroth, J.A., Langemeier, J., and Rodgers, D.E. (1995) Stability and contribution of beta carotene added to whole wheat bread and crackers. *Cereal Chemistry*, **72**: 139–141.
- Ranhotra, G.S., Gelroth, J.A., and Okot-Kotber, B.M. (2000) Stability and dietary contribution of vitamin E added to bread. *Cereal Chemistry*, **77**: 159–162.
- Reddy, B.S. and Mori, H. (1981) Effect of dietary wheat bran and dehydrated citrus fiber on 3,2-dimethyl-4-aminobiphenyl induced intestinal carcinogenesis in F344 rats. *Carcinogenesis* **2**: 21–25.
- Reddy, B.S., Hirose, Y., Cohen, L.A., Simi, B., Cooma, I., and Rao, C.V. (2000) Preventative potential of wheat bran fractions against experimental colon carcinogenesis: implications for human colon cancer prevention. *Cancer Research* **60**: 4792–4797.
- Rhodes, D.I., Sadek, M., and Stone, B.A. (2002) Hydroxycinnamic acids in walls of wheat aleurone cells. *Journal of Cereal Science*, **36**: 67–81.
- Rodgers, D.E., Malouf, R.B., Langemeier, J., Gelroth, J.A., and Ranhotra, G.S. (1993) Stability and nutrient contribution of beta-carotene added to selected bakery products. *Cereal Chemistry*, **70**: 558–561.
- Santana-Rios, G. and Dashwood, R.H. (2005) Time to discontinue antigenotoxicity studies of dietary fiber? *Mutation Research: Reviews in Mutation Research*, **429**: 269–271.
- Slavin, J. (2004) Whole-grains and human health. *Nutrition Research Reviews*, **17**: 99–110.
- Slavin, J., Marquart, L., and Jacobs, D. (2000) Consumption of whole-grain foods and decreased risk of cancer: proposed mechanisms. *Cereal Foods World*, **45**: 54–58.
- Slover, H.T., Lehmann, J., and Robert, J.V. (1969) Nutrient composition of selected wheats and wheat products. III. Tocopherols. *Cereal Chemistry*, **46**: 635–651.
- Stahl, W., van den Berg, H., Arthur, J., Bast, A., Dainty, J., Faulks, R.M., Gärtner, C., Haenen, G., Hollman, P. *et al.* (2002) Bioavailability and metabolism. *Molecular Aspects of Medicine*, **23**: 39–100.
- Sørensen, H.R., Meyer, A.S., and Pedersen, S. (2003) Enzymatic hydrolysis of water-soluble wheat arabinoxylan. *Biotechnology and Bioengineering*, **81**: 726–731.
- Trowell, H. (1972) Ischemic heart disease and dietary fiber. *American Journal of Clinical Nutrition*, **25**: 926.
- Trowell, H. (1978) The development of the concept of dietary fiber in human nutrition. *American Journal of Clinical Nutrition*, **31**: S3.
- van Hung, P., Maeda, T., Miyatake, K., and Morita, N. (2009) Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. *Food Research International*, **42**: 185–190.
- Velioglu, Y.S., Mazza, G., Gao, L., and Oomah, B.D. (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, **46**: 4113–4117.
- Verma, B., Hucl, P., and Chibbar, R.N. (2009) Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, **116**: 947–954.
- Yu, L., Zhou, K., and Parry, J.W. (2005) Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *Lebensm.-Wiss. Technology*, **38**: 463–470.
- Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., and Qian, M. (2002a) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, **50**: 1619–1624.

- Yu, L., Haley, S., Perret, J., and Harris, M. (2002b) Antioxidant properties of hard winter wheat extracts. *Food Chemistry*, **78**: 457–461.
- Yu, L. and Zhou, K. (2004) Antioxidant properties of bran extracts from ‘Platte’ wheat grown at different locations. *Food Chemistry*, **90**: 311–16.
- Yu, L., Perret, J., Harris, M., Wilson, J., and Haley, S. (2003) Antioxidant properties of bran extracts from “Akron” wheat grown at different locations. *Journal of Agricultural and Food Chemistry*, **51**: 1566–1570.
- Yuan, X., Wang, J., and Yao, H. (2006) Production of feruloyl oligosaccharides from wheat bran insoluble dietary fibre by xylanases from *Bacillus subtilis*. *Food Chemistry*, **95**: 484–492.
- Zechmeister, L. and Cholnoky, L. (1940) Carotenoids of Hungarian wheat flour. *Journal of Biological Chemistry*, **135**: 31–36.
- Zhao, Z., Egashira, Y., and Sanada, H. (2003) Ferulic acid sugar esters are recovered in rat plasma and urine mainly as the sulfoglucuronide of ferulic acid. *Journal of Nutrition*, **133**: 1355–1361.
- Zhokhov, S.S., Jastrebova, J.A., Kenne, L., and Broberg A. (2009) Antioxidant hydroquinones substituted by B-1,6-linked oligosaccharides in wheat germ. *Journal of Natural Products*, **72**: 656–661.
- Zhou, K. and Yu, L. (2004a) Antioxidant properties of bran extracts from Trego wheat grown at different locations. *Journal of Agricultural and Food Chemistry*, **52**: 1112–1117.
- Zhou, K., Su, L., and Yu, L. (2004a) Phytochemicals and antioxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry*, **52**: 6108–6114.
- Zhou, K., Yin, J., and Yu, L. (2005) Phenolic acid, tocopherol and carotenoid compositions, and antioxidant functions of hard red winter wheat bran. *Journal of Agricultural and Food Chemistry*, **53**: 3916–3922.
- Zhou, K., Hao, J., Griffey, C., Chung, H., O’Keefe, S.F., Chen, J., and Hogan, S. (2007) Antioxidant properties of Fusarium head blight-resistant and -susceptible soft red winter wheat grains grown in Virginia. *Journal of Agricultural and Food Chemistry*, **55**: 3729–3736.
- Zhou, K., Laux, J.J., and Yu, L. (2004b). Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry*, **52**: 1118–1123.
- Zhou, K. and Liangli, Y. (2004b) Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT (Food Science and Technology)*, **37**: 717–721.
- Zielinski, H. and Kozłowska, H. (2000) Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry*, **48**: 2008–2016.
- Zile, M.H., Welsch, C.W., and Welsch, M.A. (1998) Effect of wheat bran fiber on the development of mammary tumors in female intact and ovariectomized rats treated with 7,12-dimethylbenz(a)anthracene and in mice with spontaneously developing mammary tumors. *International Journal of Cancer*, **75**: 439–443.

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# 10 Buckwheat: A novel pseudocereal

Min Wang, Xudan Guo, Yujie Ma, and Jinming Gao

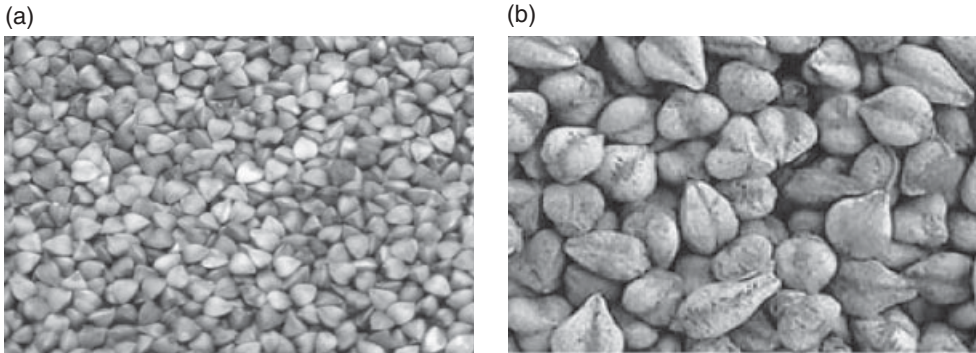
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## 10.1 Introduction of buckwheat

Buckwheat is a medical and edible dual-purpose dicotyledonous plant belonging to the *Fagopyrum* genera of the Polygonaceae family (Chai and Wang, 2002). It originated in the Himalayas and south west in the People's Republic of China (Chai and Wang, 2002). Generally, there are 15 species of buckwheat in the world, and only two of them are used for food preparation: common buckwheat and tartary buckwheat (Chai and Wang, 2002). Common buckwheat groats and tartary buckwheat seeds are shown in Figure 10.1. Buckwheat has a strong ecological adaptability so that it can grow well in almost all kinds of rough environments (Li and Zhang, 2001). Nowadays, buckwheat is ubiquitous almost everywhere but grows mainly in the northern hemisphere (Li and Zhang, 2001). Common buckwheat is more commonly grown, while tartary buckwheat is grown in mountainous regions (Krkošková and Mrázová, 2005).

According to the Food and Agriculture Organization of the United Nation (FAO), in the year of 2006, 22 countries in the world produced 2.53 million tons of buckwheat and 89.93% of the total buckwheat was produced by China, Russia, Ukraine, France and the United States, which were the five countries with the largest production yield (Chai and Wang, 2002). China has a buckwheat cultivating area of more than a million hm<sup>2</sup>, including 0.7 and 0.3 million hm<sup>2</sup> cultivating area for common buckwheat and tartary buckwheat, respectively (Chai and Wang, 2002). In addition, China is the only country that cultivates tartary buckwheat on a large scale. The world output of buckwheat in 2006 is listed in Table 10.1.

Buckwheat is rich in nutrients and has strong healing effects, thus it can serve as a potential material for the development and production of functional foods. Buckwheat has been made into various popular food products in many countries. The primary consumption form of buckwheat is seed, while its nutritious leaves can also be used in different kinds of food. In China, buckwheat is used in ethnic foods and local snacks, such as buckwheat noodle, pancake, cold jelly, 'cats' ears', and acetarious (Chai and Wang, 2002). Buckwheat tea and wine are also sold commercially (Lin *et al.*, 2005). The total consumption of common buckwheat is about 0.12 million tons in Japan, one of the largest markets of



**Figure 10.1** Seeds of buckwheat a) common buckwheat groats and b) tartary buckwheat seeds.

**Table 10.1** World buckwheat production (ton)

Countries	1997	2002	2003	2005
World	2,839,660	2,148,796	2,007,813	2,529,794
China	1,500,000	1,300,000	800,000	1,300,000
Russia	800,000	304,000	525,350	605,000
Ukraine	300,000	210,500	311,000	210,000
France	21,000	76,735	101,075	85,000
USA	34,000	65,000	65,000	65,000
Brazil	53,000	48,000	48,000	48,000
Poland	40,000	35,377	44,068	90,204
Kazakhstan	18,000	29,647	30,000	58,000
Japan	18,000	26,000	26,800	20,000
Belarus		14,200	13,000	15,000
Lithuania		13,000	14,700	15,000
Latvia		8,300	8,000	9,000
Canada	16,500	5,100	9,900	1,000
South Korea	49,000	4,000	3,800	3,000
Moldova		3,264	1,340	1,300
Bhutan	6,000	3,000	2,800	2,100
Hungary		1,200	1,200	850
Slovenia		785	1,100	200
South Africa		300	300	300
Estonia		188	200	200
Croatia		140	140	140
Georgia		60	40	100
Kyrgyzstan				400

Source: FAO, 2006.

buckwheat (Lin *et al.*, 2005). Most European countries consider buckwheat to be a basic food ingredient primarily in the preparation of porridges and soups (Krkošková and Mrázová, 2005). The functional properties of buckwheat also create an opportunity for developing new puffed snack products, as buckwheat grit cakes (Krkošková and Mrázová, 2005), which are popular in America.

## 10.2 Nutritional composition of buckwheat

### 10.2.1 The variety and content of nutritional components

Buckwheat is a good source of nutrients and is rich in starch, protein, unsaturated lipids, minerals, vitamins and dietary fibre.

Starch content in buckwheat seeds is 65–75% (Li and Zhang, 2001) and varies with the producing area and species (Zheng, 2009). Amylose accounts for 21.53% of total starch, with a higher content in tartary buckwheat than in common buckwheat (Zheng, 2009). The buckwheat starch granule is commonly in a polygonal shape with a small quantity of round and oval-shaped granules. The particle size is 2–14 µm. Some defects are found on the surface of both tartary and common buckwheat starch granules (Zheng, 2009). Scanned electron micrographs of four tartary buckwheat and four common buckwheat starches are shown in Figure 10.2.

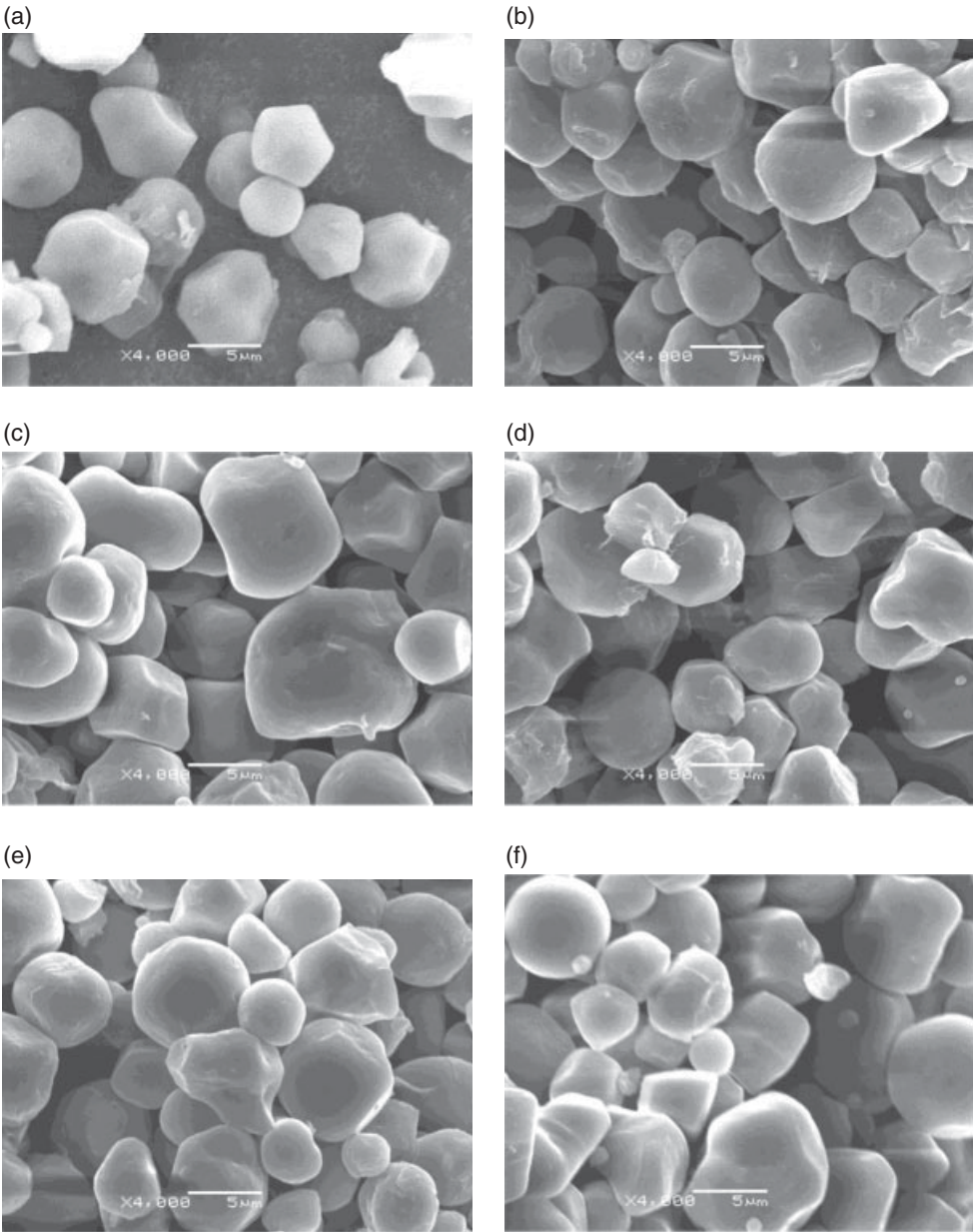
Protein content of buckwheat flour ranges from 8.51 to 18.87%, depending on the variety, which is only just less than that in oat, but significantly higher than that in rice, wheat, millet, sorghum and maize (Krkošková and Mrázová, 2005). The main difference regarding protein fractions between buckwheat flour and wheat flour is that buckwheat is rich in albumin and globulin, but very low in prolamin and glutelin (Krkošková and Mrázová, 2005). While buckwheat is a pseudo-cereal, buckwheat protein is closer to legume plant protein because of its high level of albumin, globulin and the low content of prolamin and glutelin (Du *et al.*, 2004).

Compared with other cereals, buckwheat proteins are well balanced and rich in essential amino acids. Comparison of the content of eight essential amino acids in buckwheat with the large bulks of grains is listed in Table 10.2. Buckwheat proteins are rich in lysine, which is the first limiting amino acid in other grain proteins, and arginine. In all buckwheat strains threonine and methionine are the first and the second limiting amino acids, respectively, but threonine and methionine are quite rich in other cereal proteins (Krkošková and Mrázová, 2005). Therefore, buckwheat proteins can have a strong supplemental effect with cereal proteins to improve the dietary amino acid balance and increase the protein biological value (BV) (Li and Zhang, 2001).

Buckwheat seeds contain 1–3% lipid, which is composed of nine fatty acids (Wang and Li, 2004). The fatty acid composition in common and tartary buckwheat is listed in Table 10.3. Unsaturated fatty acids are abundant in buckwheat, especially linoleic and oleic acids that make up approximately 80% of the total unsaturated fatty acids in buckwheat (Wang and Li, 2004a). Common buckwheat also contains a low amount of linolenic acid. The content of arachidic acid, eicosenoic acid, eicosadienoic acid and behenic acid in buckwheat is below 1% (Wang and Li, 2004a). The unsaturated fatty acids may lower serum cholesterol and inhibit the formation of arterial thrombus. They could also have a good preventive effect on cardiovascular diseases, such as atherosclerosis and myocardial infarction (Wang and Li, 2004a).

Buckwheat has a higher content of K, Mg, Cu, Zn, Ca, Fe, Mn and Cr than other cereals (Zhang and Hu, 2004). Some kinds of common and tartary buckweats in China's Sichuan Province contain as much as 0.63 and 0.74% calcium, respectively, which is 80 times that of rice (Wang and Li, 2004a). Tartary buckwheat also contains natural organic selenium, which is an essential trace element for humans and is absent in other cereal crops.

Buckwheat seeds show a good content of B vitamins and vitamin E. The B vitamins content is higher in tartary buckwheat than in common buckwheat (Bonafaccia *et al.*, 2003). Buckwheat may also serve as a major source of dietary rutin, which helps increase capillary



**Figure 10.2** Scanning electron micrographs of buckwheat starches.

- a) starch from Xinong 9909 Tartary buckwheat,  $\times 4,000$ ;
- b) starch from Qianku 1 Tartary buckwheat,  $\times 4,000$ ;
- c) starch from Dingbian red flower common buckwheat,  $\times 4,000$ ;
- d) starch from Inner Mongolia small grain common buckwheat,  $\times 4,000$ ;
- e) starch from Chaselimadao common buckwheat,  $\times 4,000$ ; and
- f) starch from Guizhou red flower common buckwheat,  $\times 4,000$ .

Source: Zheng, 2009. Reproduced with permission from the author.

**Table 10.2** Comparison the content of eight essential amino acids between buckwheat and other cereal grains

Parameter (%)	Common buckwheat seed	Tartay buckwheat seed	Wheat	Rice	Corn
Thr	0.2736	0.4173	0.328	0.288	0.347
Val	0.3805	0.5493	0.454	0.403	0.444
Met	0.1504	0.1834	0.151	0.141	0.161
Leu	0.4754	0.757	0.763	0.662	1.128
Lys	0.4214	0.6884	0.262	0.277	0.251
Trp	0.1094	0.1876	0.122	0.119	0.053
Ile	0.2735	0.4542	0.384	0.245	0.402
Phe	0.3864	0.5431	0.487	0.343	0.395

Source: Zhang and Hu, 2004. Data was obtained from Institute of Cereal Chemistry, Ministry of Commerce, The People's Republic of China, 1989. Reproduced with permission.

**Table 10.3** Oleaginousness and content of fatty acids in buckwheat seed oils (%)

Fatty acids	Tartary buckwheat oil	Common buckwheat oil
Palmitic acid	14.6	16.6
Stearic acid	2.2	1.6
Oleic acid	47.1	35.8
Linoleic acid	36.1	40.2

Source: Wang *et al.*, 2004b. Reproduced with permission.

permeability, maintain microvascular circulation and is commonly used as a supplementary drug in the treatment of hypertension and hemorrhagic disease caused by capillary degeneration (Wang and Li, 2004a). A comparison of minerals and vitamins between buckwheat and common grains is provided in Table 10.4.

Buckwheat seeds contain 3.4–5.2% dietary fibre; 20–30% of which is soluble dietary fibre (Yi *et al.*, 2002). Several surveys have demonstrated that the consumption of buckwheat fibre can decrease blood lipids, serum total cholesterol and blood glucose, and may improve glucose tolerance. Dietary fibre may bind minerals and proteins and reduce their absorption or digestibility, respectively, in the intestine (Krkošková and Mrázová, 2005). Indeed buckwheat protein appears to be less readily utilized than wheat protein, and dietary fibre may play a role (Krkošková and Mrázová, 2005). However, high intake of dietary fibre is not likely to be problematic when protein and minerals in the diet are adequate (Krkošková and Mrázová, 2005).

## 10.2.2 The distribution of average nutritional components in buckwheat seed

Buckwheat seed milling fractions contain various proportions of central endosperm, embryo and maternal tissues, and may differ in their chemical compositions (Krkošková and

**Table 10.4** Comparison minerals and vitamins between buckwheat and other cereal grains

	<b>Common buckwheat</b>	<b>Tartary buckwheat</b>	<b>Wheat flour</b>	<b>Rice</b>	<b>Corn</b>
K (%)	0.29	0.40	0.195	1.72	0.27
Na (%)	0.032	0.033	0.0018	0.0017	0.0023
Ca (%)	0.038	0.016	0.038	0.009	0.022
Mg (%)	0.14	0.22	0.051	0.063	0.060
Fe (%)	0.014	0.086	0.0042	0.024	0.0016
Cu (10 <sup>-6</sup> )	4.0	4.59	4.0	2.2	–
Mn (10 <sup>-6</sup> )	10.3	11.70	–	–	–
Zn (10 <sup>-6</sup> )	17	18.50	22.8	17.2	–
Se (10 <sup>-6</sup> )	0.431	–	–	–	–
Vitamin B <sub>1</sub> (10 <sup>-3</sup> )	0.08	0.18	0.46	0.11	0.31
Vitamin B <sub>2</sub> (10 <sup>-3</sup> )	0.12	0.50	0.06	0.02	0.10
Vitamin PP (10 <sup>-3</sup> )	2.7	2.55	2.5	1.4	2.0
Vitamin P (%)	0.095–0.21	3.05	0	0	0

Source: Zhang and Hu, 2004. Reproduced with permission.

**Table 10.5** Nutritional composition of buckwheat seed, groat, and flour

<b>Buckwheat product (g/100g)</b>	<b>Seed*</b>	<b>Groats</b>	<b>Dark flour</b>	<b>Light flour</b>	<b>White flour</b>
Protein	12.3	16.8	14.1	11.7	6.4
Carbohydrate	73.3	67.8	68.6	72.0	79.5
Fat	2.3	3.2	3.5	2.5	3.2
Fiber	10.9	0.6	8.3	1.6	0.5
Ash	2.1	2.2	1.8	1.8	0.9

Source: Li and Zhang, 2001. Flour data was obtained from commercial sources, and from Watt, B.K. and A.L. Merrill, 1963, *Composition of Foods*, USDA Handbook 8. Percentages are presented on a 12% moisture basis. Reproduced with permission of Taylor & Francis.

\*Buckwheat seed composition are based on 14% moisture content.

Mrázová, 2005). Table 10.5 lists the nutritional composition of buckwheat seed, groat and flour. Starch is concentrated in the central endosperm (Krkošková and Mrázová, 2005), while bran milling fractions contain the highest concentration of protein, lipid, soluble dietary fibre and minerals (Krkošková and Mrázová, 2005). Among the minerals in buckwheat seeds, P, K and Mg are most concentrated in the bran, particularly that from the hulls which are removed (Steadman *et al.*, 2001a). Conversely, Ca is more concentrated in the bran with hulls than in bran fractions from which hulls are removed (Steadman *et al.*, 2001a). Generally, the flour of the outer layers has a higher content of vitamin than the inner layers (Zheng, 2009).

### 10.3 Unique health components of buckwheat

Besides its high-quality protein, buckwheat is also rich in many unique components with health promoting properties against several chronic diseases. Among them, the most attractive ones are phenolic compounds, D-chiro-inositol, phytosterol, resistant starch, resistant protein, the thiamin – binding proteins found in buckwheat.



### 10.3.1 Phenolic compounds in buckwheat

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups, and are generally categorized as phenolic acid, flavonoids, stilbenes, coumarins and tannins (Liu *et al.*, 2007). It is well known that dietary antioxidants may prevent oxidative damage to physiologically important molecules by modulating the oxidative status of cells and consequently reducing the risk of aging-related disease and improving general human health (Yu *et al.*, 2002). Phenolic compounds are one of the most effective antioxidant constituents in many plant foods, including fruits, vegetables and grains (Velioglu *et al.*, 1998). In buckwheat, flavonoids and phenolic acids constitute the common phenolics present.

#### 10.3.1.1 Flavonoids

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are characterized by a benzo-*y*-pyrone structure. Flavonoids are ubiquitous in fruits and vegetables, however, the flavonoids in the buckwheat have received growing attention because of their contents, especially the concentration of rutin.

The flavonoid content and composition in buckwheat seeds vary by the species during different growing phases and may be altered by the growing circumstances. Briefly, the flavonoid content in *Fagopyrum tataricum* is generally higher than that in *Fagopyrum esculentum*. In *Fagopyrum tataricum* seeds, the flavonoid content is about 40 mg/g, while in *Fagopyrum esculentum* seeds is around 10 mg/g (Li and Zhang, 2001). In *Fagopyrum tataricum* flowers, leaves and stems, the flavonoid content can surpass 100 mg/g. Rutin is the major flavonoid present in buckwheat seeds. The content of rutin in tartary buckwheat seed is 9–300 times more than that in common buckwheat (Steadman *et al.*, 2001a). To date, 14 flavonoids have been isolated and identified from *Fagopyrum tataricum*, *Fagopyrum esculentum*, *Fagopyrum cymosum* seed, hull, root and sprout (Wang, 2005; Kim *et al.*, 2007). These compounds are summarized in Table 10.6 and their main flavonoids skeletons are shown in Figure 10.3.

Flavonoids extracted from tartary buckwheat bran could effectively reduce triacylglycerols and cholesterol in the serum and liver of hyperlipidemic rats. Meanwhile, they could also raise serum glutathione peroxidase activity and antiatheromatous plaque formation index and lower the atherogenic index of plasma, the artherogenic index and serum malondialdehyde of hyperlipidemic rats (Wang *et al.*, 2009a).

#### 10.3.1.2 Phenolic acids

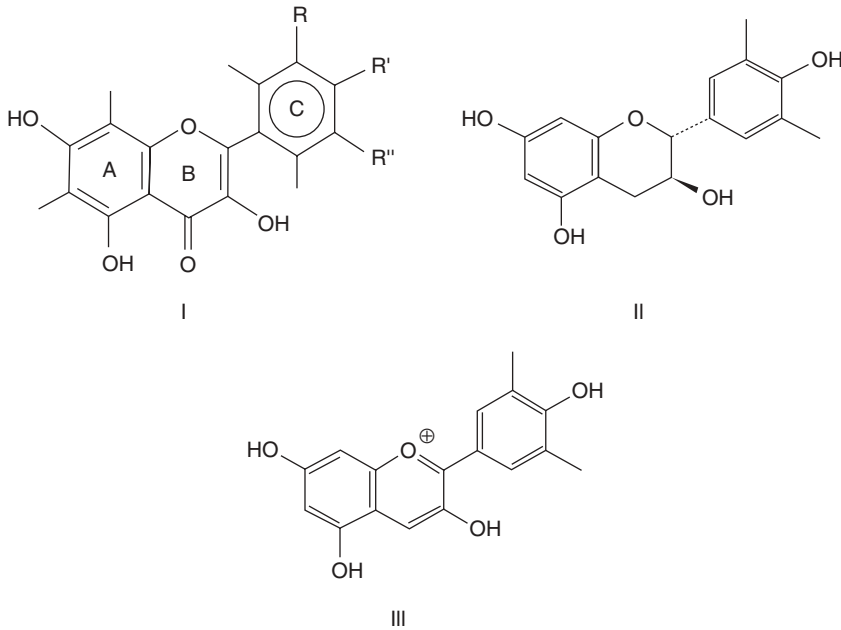
Phenolic acids are secondary metabolites that are commonly found in plant-derived foods. Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in plants (Herrmann, 1989).

Phenolic acids exist in different parts of buckwheat seeds. Several phenolic acids such as *p*-hydroxybenzoic, syringic, vanillic, gallic, protocatechuic, ferulic, *p*-coumaric and  $\delta$ -coumaric acids have been found in buckwheat (Xu *et al.*, 2002). Protocatechuic and *p*-hydroxybenzoic acids were identified as the major phenolic acids in tartary buckwheat seeds (Xu *et al.*, 2002). Oomah *et al.* (1996) found that buckwheat seed contained 12–16 g/kg of total phenolic acids. In addition, Xu *et al.* (2002) determined the phenolic acid content of different parts of tartary buckwheat seeds and demonstrated that the phenolic acid contents in

**Table 10.6** Flavonoids isolated from *Fagopyrum Gaerth*

<b>Number</b>	<b>Compounds</b>	<b>Skeleton</b>	<b>Substituent group</b>	<b>References</b>
1	rutin	I	5,7,3',4'-OH,3-O-glucoside - rhamnoside	Tian <i>et al.</i> , 2002 Dietrych-Szostak <i>et al.</i> , 1999
2	quercetin	I	3,5,7,3',4'-OH	Fabjan <i>et al.</i> , 2003 Tian <i>et al.</i> , 2002 Dietrych-Szostak <i>et al.</i> , 1999
3	kaempferol	I	3,5,7,4'-OH	Fabjan <i>et al.</i> , 2003 Tian <i>et al.</i> , 2002 Dietrych-Szostak <i>et al.</i> , 1999
4	morin	I	3,5,7,2',4'-OH	Tian <i>et al.</i> , 2002
5	hyperin	I	5,7,4',5'-OH,3-O-glucoside	Zhu <i>et al.</i> , 2003
6	vitexin	I	5,7,4'-OH, 8- glucoside	Dietrych-Szostak <i>et al.</i> , 1999
7	isovitexin	I	5,7,4'-OH, 6- glucoside	Dietrych-Szostak <i>et al.</i> , 1999
8	orientin	I	5,7,3',4'-OH, 8- glucoside	Dietrych-Szostak <i>et al.</i> , 1999
9	isoorientin	I	5,7,3',4'-OH, 6- glucoside	Dietrych-Szostak <i>et al.</i> , 1999
10	kaempferol-3-O- rutinoside	I	5,7,4'-OH, 3-O-rutinoside	Tian <i>et al.</i> , 2002
11	quercetin-3-O-rutinoside-7-O-galactoside	I	5,3',4'-OH, 3-O-rutinoside,7-O- galactoside	Zhu <i>et al.</i> , 2003
12	quercetin-3-O-dirhamnoside	I	5,7,3',4'-OH, 3-O-rhamnoside-rhamnoside	Zhu <i>et al.</i> , 2003
13	quercetin-3-O-rhamnoside-diglucoside	I	5,7,3',4'-OH, 3-O-rhamnoside-glucoside-glucoside	Zhu <i>et al.</i> , 2003
14	3',4',5,7-4-O-methylquercetin-3-O- $\alpha$ -L-rhamnopyranoside-(1-6)-O- $\beta$ -D-glucoopyranoside	I	3',4',5,7-4-O-methyl,3-O- $\alpha$ -L-rhamnopyranoside -O- $\beta$ -D- glucopyranoside	Zhu <i>et al.</i> , 2003
15	(-)epicatechin	II		Liu <i>et al.</i> , 1983
16	3-galloyl(-)epicatechin	II	3-galloyl	Liu <i>et al.</i> , 1983
17	procyanidinB-2	III		Liu <i>et al.</i> , 1983
18	procyanidinB-4	III		Liu <i>et al.</i> , 1983

19	3,3'-digalloyl-procyanidin B-2	III	3,3'-digalloyl	Peng <i>et al.</i> , 1996
20	(+)catechin	II		Peng <i>et al.</i> , 1996
21	(+)catechin-7-O- $\beta$ -D-glucopyranoside	II	7-O- $\beta$ -D-glucopyranoside	Watanabe <i>et al.</i> , 1998
22	(-)epicatechin 3-O-p- hydroxybenzoate	II	3-O-p-hydroxybenzoate	Watanabe <i>et al.</i> , 1998
23	(-)epicatechin-3-O-(3,4-di-O-methyl)gallate	II	3-O-(3,4-di-O-methyl)gallate	Watanabe <i>et al.</i> , 1998
24	cyanidin 3 - O - glucoside	III	5,7,3',4'-OH, 3-O-glucoside	Kim <i>et al.</i> , 2007
25	cyanidin 3 - O - rutinoside	III	5,7,3',4'-OH, 3-O-rutinoside	Kim <i>et al.</i> , 2007
26	cyanidin 3 - O - galactoside	III	5,7,3',4'-OH, 3-O-galactoside	Kim <i>et al.</i> , 2007
27	cyanidin 3 - O - galactopyranosyl - rhamnoside	III	5,7,3',4'-OH, 3-O-galactopyranosyl-rhamnoside	Kim <i>et al.</i> , 2007



**Figure 10.3** Main flavonoids skeleton of *Fagopyrum gaerth*. I) quercetin, II) catechin and III) anthocyanins.

Source: Wang, 2005.

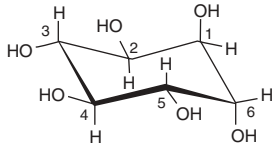
the tartary buckwheat bran, hull, outer flour and whole flour were 1745.33, 955.64, 162.65 and 94.61 mg/kg, respectively.

### 10.3.2 *D-chiro*-inositol and fagopyritols in buckwheat

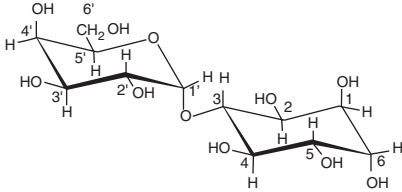
Buckwheat seeds accumulate *D-chiro*-inositol (DCI), which may lower blood sugar, and fagopyritols, which are the mono-galactosyl, di-galactosyl and tri-galactosyl derivatives of *D-chiro*-inositol, during seed development and maturation, instead of raffinose series oligosaccharides that accumulate in maturing seeds of a large number of food and feed seed crops (Horbowicz *et al.*, 1998). Six different fagopyritols have been found and divided into two series: fagopyritol A series including fagopyritol A1, fagopyritol A2, fagopyritol A3; and their positional isomer fagopyritol B series including fagopyritol B1, fagopyritol B2 and fagopyritol B3 (Steadman *et al.*, 2001b). The molecular structures of *D-chiro*-inositol and fagopyritols are shown in Figure 10.4.

Free *D-chiro*-inositol not only exists in buckwheat, but is also present in lupine, pigeon pea, soybean, chickpea and mungbean (Kawa *et al.*, 2003). Among these seeds, only mungbean seeds have a higher content of free *D-chiro*-inositol than buckwheat (Kawa *et al.*, 2003). In addition, fagopyritol B1 has been detected in the seeds of soybean, lupin, lentil, chickpea and jojoba bean (Steadman *et al.*, 2000). Fagopyritol B2 has also been identified in seed balls in sugar beet (Steadman *et al.*, 2000).

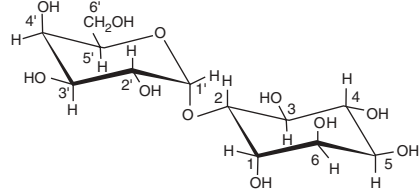
Fagopyritols are concentrated in aleurone and embryo cells of the seed, but not in the hull, seed coat or starchy endosperm according to Steadman *et al.* (2000). Therefore, milling fractions originating from achenes that contain fragments of hull have lower concentrations



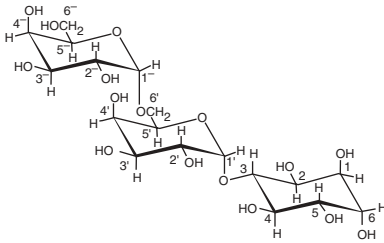
free D-chiro-inositol



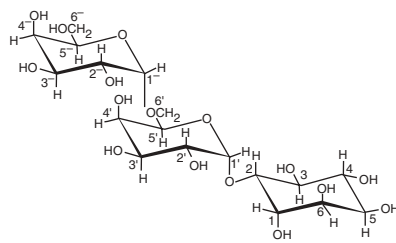
fagopyritol A1



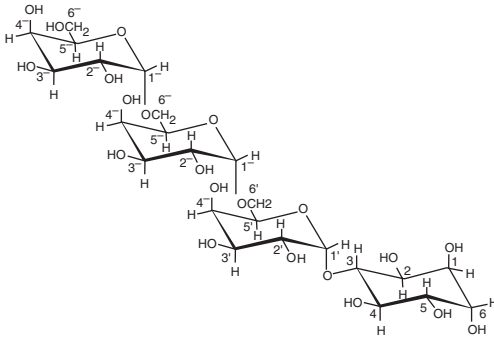
fagopyritol B1



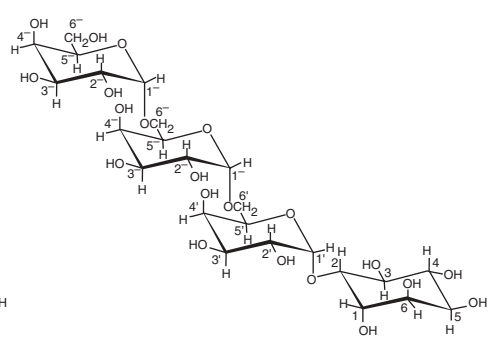
fagopyritol A2\*



fagopyritol B2\*



fagopyritol A3\*



fagopyritol B3\*

**Figure 10.4** Molecular structures of Free D-chiro-inositol and fagopyritols.

(Reproduced from Steadman *et al.*, 2001b; \*Obendorf *et al.*, 2000, copyright 2001 and 2000, with permission of Elsevier.)

of fagopyritols than dehulled milling fractions, due to dilution with tissues deficient in fagopyritols (Steadman *et al.*, 2000).

After observing the accumulation of free D-chiro-inositol and fagopyritols during seed development and maturation, Horbowicz *et al.* (1998) found that D-chiro-inositol was present from the earliest stages throughout embryo development and maturation. Fagopyritol B1 and fagopyritol A1 were first detected at 12 days after pollination (DAP) and accumulated

**Table 10.7** Changes in fasting blood-glucose (FBG) and postprandial blood sugar (PG) of diabetics after taking extract of DCI from tartary buckwheat sprout

Medical record Number	FBG (mmol/L)		PG (mmol/L)	
	Before taking	After taking for 15 days	Before taking	After taking for 15 days
1	9.5	8.6	14.6	11.2
2	8.0	7.0	11.6	10.9
3	7.9	9.0	11.0	11.0
4	8.1	7.0	10.8	9.6
5	9.7	7.8	10.4	9.1
6	9.8	8.8	16.7	11.5
7	6.7	7.2	13.2	11.4
8	7.8	6.7	12.7	10.9
9	9.9	7.7	15.0	8.3
10	9.3	8.8	9.3	8.0

Source: Cao *et al.*, 2006.

very rapidly between 12 and 16 DAP. Fagopyritol A2 and fagopyritol B2 were first detected at 16 DAP and accumulated rapidly to 20 DAP (Horbowicz *et al.*, 1998).

Fagopyritols are of considerable interest for the treatment of non-insulin dependent diabetes mellitus (NIDDM) and polycystic ovary syndrome (PCOS), both insulin response disorders (Ueda *et al.*, 2005). DCI have been identified as components of inositolphosphoglycan (IPG), whose role as putative insulin secondary messengers has been demonstrated in numerous studies (Kawa *et al.*, 2003). IPGs are released from glycosylphosphatidylinositols (GPIs) in cell membranes in response to insulin. Following GPI hydrolysis by phospholipases, IPGs are incorporated into the cell, where they can affect enzymes implicated in insulin action (Kawa *et al.*, 2003). Additionally, some fagopyritols have structural similarities with IPGs (Ogawa *et al.*, 1997).

Ostlund *et al.* (1993) have found that both the blood and urine of a healthy person contained certain amount of DCI, while the blood samples of patients with type 2 diabetes had an almost undetectable level of DCI. However, the urine DCI content of patients suffering from type 2 diabetes was several times higher than that of normal individuals. This suggests metabolic disorders lead to the fast loss of DCI, which causes signal transmission delay in insulin action.

The hypoglycemic effect of tartary buckwheat bran extract (TBBE) rich in DCI was examined using KK-*A<sup>y</sup>* mice and demonstrated that DCI-enriched TBBE played an important role in hypoglycemic control and was capable of ameliorating the impaired renal function in KK-*A<sup>y</sup>* mice (Yao *et al.*, 2008). This research also showed that extract of DCI from tartary buckwheat sprout could markedly ameliorate both fasting blood-glucose (FBG) and postprandial blood sugar (PG) of diabetics (Table 10.7). Another study showed that TBBE that was rich in DCI and poor in flavonoids was more effective than one with high content of flavonoids and low content of DCI in reduction of the diabetic index in the diabetic rats (Shan *et al.*, 2006).

Since humans and other monogastric animals do not have  $\alpha$ -D-galactosidase, the bioavailability of fagopyritols is very low (Cao *et al.*, 2006). Buckwheat seeds germinating in NaCl solution with the concentration of 0–30 mM for 7–22 hours, at 22–30 °C might have significantly lower levels of flavonoids and higher content of free DCI (Wen and

Xia, 2002). Budding buckwheat induced with  $\text{Cr}^{3+}$  also could boost the content of free DCI (Cao *et al.*, 2006). In addition, it has been reported that pressure and heat might disrupt galactosidic bonds and release free DCI (Yao *et al.*, 2008).

### 10.3.3 Phytosterols in buckwheat

Phytosterols (plant sterols) are similar to cholesterol in function (stabilization of phospholipid bilayers in cell membranes) and structure (steroid nucleus,  $3\beta$ -hydroxyl group, 5, 6-double bond). Whereas the cholesterol side chain is comprised of eight carbon atoms, most phytosterol side chains contain nine or ten carbon atoms (Kritchevsky and Chen, 2005). Phytosterols found in buckwheat seeds, although at a low level, also showed positive effects in lowering blood cholesterol level. Phytosterols have shown pharmaceutical effects in many chronic diseases. Plant sterols have antiviral effects and might improve the immunological status of host subjects (Abid Ali Khan *et al.*, 1991). Antitumour effects of phytosterols were also reported by Yasukawa *et al.* (1991), in the early 1990s. Heinemann *et al.* (1991) observed that the ingestion of phytosterols significantly inhibited the absorption of cholesterol *in vivo* and sitosterol showed different effects as that of sitostanol. Phytosterols, particularly  $\beta$ -sitosterol that cannot be absorbed in the human body, have similar structures to cholesterol and may have strong competitive inhibitory effects on cholesterol absorption *in vivo*.

Phytosterols are ubiquitous through the whole buckwheat, but their distribution and content vary significantly from different tissues and growing phases. Horbowicz and Obendorf (1992) reported their research results on the plant sterol composition and variation during growing embryo and endosperm tissues 6–20 days after pollination (DAP). The most abundant sterol in embryo and endosperm tissues is  $\beta$ -sitosterol, which accounts for 70% of the total sterols. Other sterols are campesterol, an unknown one, and trace amount of stigmasterol. In addition, tartary buckwheat oil contained three phytosterols, including  $\beta$ -sitosterol (54.37%), ergosta-5-en-3- $\beta$ -ol (5.03%) and 4,14-dimethylergosta-8,24(28)-dien-3- $\beta$ -ol (2.56%), while  $\beta$ -sitosterol (57.29%) was found only in common buckwheat oil (Wang, 2005).

### 10.3.4 Other components with special healing functions in buckwheat seeds

Buckwheat is rich in resistant starch, which is not absorbed in the small intestine but is fermented in the large intestine. Resistant starch is capable of reducing the risk of colorectal carcinoma and lowering plasma total cholesterol and triacylglycerol levels (Xu *et al.*, 2006). Research has also shown that resistant starch helps to maintain homeostasis of postprandial blood glucose and improve insulin sensitivity *in vivo* (Wang *et al.*, 2003; Zhang and Zhang, 2008). Therefore, resistant starch can be used as a nutraceutical product for diabetics.

Buckwheat protein has a balanced amino acid composition and a high biological value, but its digestion and absorption rates are much lower than that of wheat and legumes. Consequently, excessive consumption can also cause abdominal inflation. However, protein with low digestibility has certain physiological effects. A negative correlation existed between the digestibility of protein and its reducing effect on blood cholesterol (Li and Zhang, 2001). A number of studies have shown that buckwheat protein could increase bile excretion and reduce gallstone formation, and has a health effect similar to that of dietary fibre, thus preventing constipation, obesity and cancer (Zheng, 2009).

The low digestion and absorption rates of buckwheat protein are due to two reasons. Firstly, they are hampered by the endogenous anti-nutritional factors, such as protease inhibitors including tannins and dietary fibre. Secondly, they are affected by the discrepant sensitivity of different components of buckwheat protein to protease (Du *et al.*, 2004).

Like most Spermatophyta seeds, buckwheat seeds contain thiamin-binding proteins (TBP), which function for thiamin transportation and storage in the plant (Li and Zhang, 2001). Thiamin, or vitamin B1, is one of the water-soluble vitamins that play an important role in regulating metabolism. It is easily destroyed in food processing when encountering high temperatures or alkaline conditions (Gu and Guo, 2008). Patients who suffer from chronic gastrointestinal disease, gastrointestinal cancer and pancreatitis have barriers to absorption and storage of thiamin (Gu and Guo, 2008). The above two effects may lead to the lack of thiamin in the body. TBP can be exploited to improve the stability of thiamin during processing and enhance its bioavailability (Li and Zhang, 2001). It was reported that the TBP in buckwheat seeds was in the oligomeric form (Krkošková and Mrázová, 2005). In addition, on SDS-PAGE, buckwheat TBP migrates as a single band corresponding to molecular weight of 42 000 to 45 000 Da depending on whether reducing agents have been applied, and its isoelectric point (pI) is about 5.3 (Li and Zhang, 2001).

A number of peptides in buckwheat are known to lower blood pressure. A tripeptide, which is the decomposition product of buckwheat protein by protease, has a structure similar to rattlesnake toxins (Yi *et al.*, 2002). This substance has a strong inhibitory effect on angiotensin-transferase, and may reduce blood pressure. It was also found that buckwheat peptides have antioxidant activity (Yi *et al.*, 2002). Wang *et al.* (2009b) reported the composition and antioxidant activity of peptides generated by simulating the gastrointestinal digestion process after tartary buckwheat protein was digested *in vitro*. The experimental results revealed that peptides produced after ten hours of being subjected to simulating gastrointestinal digestion had the strongest antioxidant activity, which was significantly higher than that of vitamin C. After gel separation, the peptide with the strongest antioxidant activity was found to have a smaller molecular weight of 900 Da and was susceptible to absorption (Wang *et al.*, 2009b).

Fagopyrins in buckwheat seeds are in very low concentrations and are hard to isolate (Krkošková and Mrázová, 2005). There are different opinions about this group of compounds. They may be utilized in the treatment of type 2 diabetes (Krkošková and Mrázová, 2005). On the other hand, they may cause the subjects to suffer from photosensitization (Krkošková and Mrázová, 2005).

## 10.4 Allergens in buckwheat

While there are so many possible health benefits of consuming buckwheat and its products, some cases of allergenic reactions are reported upon consumption of buckwheat. Buckwheat allergy has been described in China, Japan and Korea and ranked fourth as a cause of immediate-type food allergy in Japan (Shek and Lee, 2006). The main symptoms involved are asthma, allergic rhinitis, urticaria and angioedema (Krkošková and Mrázová, 2005). The mechanism involved is type I mechanism involving an IgE-related reaction (Li and Zhang, 2001). Allergenic symptoms occur not only from consumption, but also after exposure to buckwheat dust. Allergy to buckwheat has been reported to be one cause of occupational asthma among workers in noodle shops in Japan (Li and Zhang, 2001). Lee



*et al.* (2001) reported three cases of buckwheat flour (BF) allergy in children using buckwheat chaff-stuffed pillows (BCP), who had been treated as nonatopic asthmatics after undergoing the routine allergy skin and serologic tests. The result showed that a small amount of BF attached to BCP can induce BF sensitization, and BCP should be considered a main cause of childhood nocturnal asthma in those asthmatics exposed to these pillows (Lee *et al.*, 2001).

The distribution of buckwheat allergic proteins is widespread with other storage proteins throughout the whole seed (Li and Zhang, 2001). A number of studies with regard to buckwheat allergens have been performed, and various buckwheat allergens have been reported. Yanagihara (1980) reported that the major allergenic proteins in buckwheat had molecular weights of 100, 50 and 17 kDa (Li and Zhang, 2001). However, Urisu *et al.* (1995) reported the presence of approximately 67–70, 26 and 24 kDa proteins that showed IgE-binding activity within the sera from more than 50% of buckwheat allergic patients. Kondo *et al.* (1993) found that BW24KD (a protein has a molecular weight of 24 kDa in buckwheat seeds) was allergically positive to RAST, prick test and immunoblotting methods (Shu *et al.*, 2005). According to Park *et al.* (2000), the allergens of 24, 19, 16 and 9 kDa are strong candidates to be major ones, and the 19-kDa allergen was relatively specific for BW-allergic patients. Moreover, it was pointed out that measurement of BW-specific IgE and the features of immunoblotting should be very useful tools in the diagnosis of BW allergy (Krkošková and Mrázová, 2005).

Although there are such a variety of buckwheat allergens, comparative studies of protein epitopes, which may not be large in number, make it possible to prevent and treat the allergies caused by buckwheat, or nurture hypoallergenic species. At the same time, eliminating the allergic buckwheat protein through processing or breeding efforts may be more economical ways, but more processing characteristics of these allergenic proteins are needed (Shu *et al.*, 2005).

## 10.5 Research trends of buckwheat nutritional and functional properties

It is clear that buckwheat is rich in nutritional and functional components. More research is required to develop better use of buckwheat for health promotion and reducing the risk of chronic diseases. The following section provides examples of three important future research topics for buckwheat.

### A. Investigating the mechanisms of action on functional components and their interactions

Many reports show that functional components in buckwheat can promote health and prevent disease, but the exact mechanisms responsible for those beneficial health effects have not been elucidated. Among the mechanisms, the synergistic and antagonistic action of functional components may also play an important role in healthcare functions. Investigation of these mechanisms and their interactions among functional components has a long way to go, although more and more researchers in the field have realised the importance of this basic research topic. In addition, the dietary reference intakes of buckwheat health components also need to be researched.

## B. Understanding the distributions of health compounds in buckwheat and the correlation between these components and growing conditions for a buckwheat variety

To achieve the maximum benefits from buckwheat, it is critical to understand the distributions of beneficial components in these buckwheat ingredients. This is also important for improving the safety and quality of consuming functional buckwheat based foods.

The variety and growing conditions may affect the profile and content of health components, so seeking the correlation between functional components and growing conditions is important for production of buckwheat high in health components. In other words, understanding of the distribution of bioactive components in buckwheat and optimizing the growing conditions of a selected buckwheat variety may lead to value-added production and consumption of buckwheat and buckwheat-based products. This will benefit both consumers and buckwheat producers.

## C. Retaining these health components during processing

The health promoting components of buckwheat may be lost or made unabsorbable due to processing. It would be necessary to examine processing conditions under which health promoting components are maximally retained.

## References

- Abid Ali Khan, M.M., Jain, D.C., Bhakuni, R.S., Zaim, M., and Thakur, R.S. (1991) Occurrence of some antiviral sterols in *Artemisia annua*, *Plant Science*, **75**: 161–165.
- Bonafaccia, G., Marocchini, M., and Kreft, I. (2003) Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry*, **80**: 9–15.
- Cao, W.M., Zhang, Y.Q., and Su, Y. (2006) Study on Extracting of the D-chiro-inositol in Fagopyrium Tartarian and Curative Effect of the Extraction on Diabetes. *Journal of Cereals and Oils in Chinese*, **1**: 22–24.
- Chai, Y. and Wang, F.S. (2002) Industry development report on Chinese minor cereal. Beijing: Publishing House of China Agricultural Science and Technology.
- Dietrych-Szostak, D. and Oleszek W. (1999) Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum Moench*) grain. *Journal of Agricultural and Food Chemistry*, **47**(10): 4384–4387.
- Du, S. K., Li, Z.X., and Yu X.Z. (2004) Research Progress on Buckwheat Protein. *Food Science in Chinese*, **25**(10): 409–414.
- Fabjan, N., Rode, J., Kosir, I.J., Wang, Z., Zhang, Z., and Kreft, I. (2003) Tartary buckwheat (*Fagopyrum tataricum Gaertn.*) as a source of dietary rutin and quercitrin. *Journal of Agricultural and Food Chemistry*, **51**: 6452–6455.
- Gu, C. and Guo, Y.P. (2008) Improve the recognition of the lack of vitamin B<sub>1</sub>. *Chinese Journal of Medicinal Guide*, **10**(6): 942–944.
- Heinemann, T., Kullak-Ublick, G.A., Pietruck, B., and Von Bergmann, K. (1991) Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol, *European Journal of Clinical Pharmacology*, **40**: S59–63.
- Herrmann, K. (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Critical Review of Food Science and Nutrition*, **28**: 315–347.
- Howbawicz, M. and Obendorf, R. L. (1992) Changes in sterols and fatty acids of buckwheat endosperm and embryo during seed development, *Journal of Agricultural and Food Chemistry*, **40**: 742–752.
- Horbowicz, M., Brenac, P., and Obendorf, R.L. (1998) Fagopyritol B1, O- $\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 2)-D-chiro-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. *Planta*, **205**: 1–11.

- Kawa, J.M., Taylor, C.G., and Przybylski, R. (2003) Buckwheat concentrate reduces serum glucose in streptozotocin-diabetic rats. *Journal of Agricultural and Food Chemistry*, **51**: 7287–7291.
- Kim, S.J., Maeda, T., Sarker, M.Z.I., Takigawa, S., Chie M.E., Yamauchi, H., Mukasa, Y., Saito K., Hashimoto, N., Noda, T., Saito, T., and Suzuki, T. (2007) Identification of anthocyanins in the sprouts of buckwheat. *Journal of Agricultural and Food Chemistry*, **55**: 6314–6318.
- Kondo, Y., Urisu, A., Wada, E., Tsuruta, M., and Yasaki, T. (1993) Allergen analysis of buckwheat by the immunoblotting method. *Allergy*, **42**: 142–148.
- Kritchevsky, D. and Chen, S.C. (2005) Phytosterol – health benefits and potential concerns: a review. *Nutrition Research*, **25**: 413–428.
- Krkošková B. and Mrázová Z. (2005) Prophylactic components of buckwheat. *Food Research International*, **38**: 561–568.
- Lee, S.Y., Lee, K.S., Hong, C.H., and Lee, K.Y. (2001) Three cases of childhood nocturnal asthma due to buckwheat allergy. *Allergy*, **56**: 763–766.
- Li, S. and Zhang, Q.H. (2001) Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition*, **41**(6): 451–464.
- Lin, R.F., Zhou, X.L., Ren, G.X., Bian, J.S., and Shan, F. (2005) Production and trading of buckwheat in China, nutrition and food. *Food Science in Chinese*, **26**(1): 259–263.
- Liu, R.H. Whole grain phytochemicals and health. (2007) *Journal of Cereal Science*, **46**: 209–217.
- Liu, Y.L., Fang, Q.N., and Zhang, Q.Q. (1983) Studies on the Chemical Constituents of Fagopyrum Cymosum (Trev.) Meisn. *Acta Pharmaceutica Sinica in Chinese*, **7**: 545–547.
- Obendorf, R.L., Steadman, K.J., Fuller, D.J., Horbowicz, M., and Lewis, B.A. (2000) Molecular structure of fagopyritol A1 (O- $\alpha$ -D-galactopyranosyl-(1  $\rightarrow$  3)-D- chiro- inositol) by NMR. *Carbohydrate Research*, **328**: 623–627.
- Ogawa, K., Watanabe, T., Ikeda, Y., Komdo, S. (1997) A new glycoside, 1D-2-O- $\alpha$ -D-galactopyranosyl-chiro-inositol from jobo beans. *Carbohydrate Research*, **302**: 219–221.
- Oomah, B.D., Campbell, C.G., and Giuseppe, M. (1996) Effects of cultivar and environment on phenolic acids in buckwheat. *Euphytica*, **90**: 73–77.
- Ostlund, R.E., Jr, McGill, J.B., Herskowitz, I. et al. (1993) D-chiro-inositol metabolism in diabetes mellitus. *Proceedings of the National Academy of Sciences*, **90**: 9988–9992.
- Park, J.W., Kang, D.B., Kim, C.W., Ko, S.H., Yum, H.Y., Kim, K.E., and Hong, C.S. (2000) Identification and characterization of the major allergens of buckwheat. *Allergy*, **55**: 1035–1041.
- Peng, Y., Sun, Z.M., and Xiao, P.G. (1996), The Research and Development of *Fagopyrum Cymosum* (Trev.) Meisn. *Chinese Traditional and Herbal Drugs*, **10**: 625–626.
- Shan F., Li W. D., Lin R.F., and Jin Z.X. (2006) Effects of tartary extracts on the blood glucose of diabetic rat. *Journal of Chinese Institute of Food Science and Technology*, **6**(1): 208–211.
- Shek, L.P. and Lee, B.W. (2006) Food allergy in Asia. *Current Opinion in Allergy and Clinical Immunology*, **6**: 197–201.
- Shu, S.G., Feng, B., and Wang, T. (2005) The Research on Protein of Buckwheat Seeds. *Seed in Chinese*, **24**(12): 42–49.
- Steadman, K.J., Burgoon, M.S., Schuster, R.L., Lewis, B.A., Edwardson, S.E., and Obendorf, R.L. (2000) Fagopyritols, D-chiro-inositol, and other soluble carbohydrates in buckwheat seed milling fractions. *Journal of Agricultural and Food Chemistry*, **48**: 2843–2847.
- Steadman, K.J., Burgoon, M.S., Lewis, B.A., Edwardson, S.E., and Obendorf, R.L. (2001a) Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *Journal of the Science of Food and Agriculture*, **81**: 1094–1100.
- Steadman, K.J., Fuller, D.J., and Obendorf, R.L. (2001b) Purification and molecular structure of two digalactosyl D-chiro-inositols and two trigalactosyl D-chiro- inositols from buckwheat seeds. *Carbohydrate Research*, **331**: 19–25.
- Tian, Q., Li, D., and Patil B.S. (2002) Identification and determination of flavonoids in buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) by high-performance liquid chromatography with electrospray ionisation mass spectrometry and photodiode array ultraviolet detection. *Phytochemical Analysis*, **13**(5): 251–6.
- Ueda T., Coseo M.P., Harrellb T.J., and Obendorfa R.L. (2005) A multifunctional galactinol synthase catalyzes the synthesis of fagopyritol A1 and fagopyritol B1 in buckwheat seed. *Plant Science*, **168**: 681–690.
- Urisu, S., Kondo, Y., Morita, Y., Yagi, E., Tsuruta, M., Yasaki, T., and Yamada, K. (1995) Isolation and characterization of a major allergen in buckwheat seeds. *Current Advance in Buckwheat Research*, 965–974.

- Velioglu, Y.S., Mazza, G., Gao, L., and Oomah, B. (1998) Antioxidant activity and total polyphenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, **46**: 4113–4117.
- Wang, Z., Yang, Y.X., Zhou, R.H., Vonk, R.J., and Jang, J. (2003) Metabolism of resistant starch and its regulative effect on plasma glucose. *Acta Nutrimenta Sinica in Chinese*, **25**(2): 190–195.
- Wang H.Y. and Li, Y. (2004a) Research status and applied prospect of buckwheat. *Food Science in Chinese*, **25**(10): 388–391.
- Wang, M., Wei, Y.M., and Gao, J.M. (2004b) Analysis of fatty acid and unsaponifiable matters from tartary buckwheat oil and buckwheat oil. *Acta Nutrimenta Sinica in Chinese*, **26**(1): 40–44.
- Wang M. (2005) Study on the composition and mechanisms of hypolipidemic property of tartary buckwheat. Dissertation for PhD at Northwest A and F University.
- Wang, M., Liu, R.J., Gao, J.M., Wei, Y.M., and John W.P. (2009a) Antioxidant activity of tartary buckwheat bran extracts and its effect on the lipid profile of hyperlipidemic rats. *Journal of Agricultural and Food Chemistry*, **57**: 5106–5112.
- Wang, X., Huang, Z.M., Wang, L., Hao, C.C., and Wang, M. (2009b) The antioxidation activity and composition of tartary buckwheat protein products produced by simulating gastrointestinal digestion. *Journal of Chinese Institute of Food Science and Technology*, **9**(6): 10–15.
- Watanabe M. (1998) Catechins as antioxidants from buckwheat (*Fagopyrum esculentum Moench*) groats. *Journal of Agricultural and Food Chemistry*, **46**: 839–845.
- Wen, L.P. and Xia, T. (2002) The method of transformation from D-*chiro*-inositol derivatives to free D-*chiro*-inositol in buckwheat seeds. Patent of China: CN1380276A.
- Xu B.C., Xiao G., and Ding X.L. (2002) Determination of Phenolic acids and proanthocyanidin in buckwheat (*Fagopyrum tataricum*). *Production and Scientific research experience in Chinese*, **12**(28): 32–37.
- Xu, G.F., Shi, M., Li, H., Zhou, X., Zhang, J., and Feng, D.J. (2006) Case-control study on the association between resistant starch intake and colorectal cancer. *Acta Nutrimenta Sinica in Chinese*, **28**(1): 11–14.
- Yanagihara, Y. (1980) Buckwheat hypersensitivity. *Kansensho*, **10**: 184–188.
- Yao, Y., Shan, F., Bian, J.S., Chen, F., Wang, M.F., and Ren, G.X. (2008) D-*chiro*-inositol-enriched tartary buckwheat bran extract lowers the blood glucose level in KK- $A^y$  mice. *Journal of Agricultural and Food Chemistry*, **56**: 10027–10031.
- Yasukawa, K., Takido, M., Matsumoto, T., Takeuchi, M., and Nakagawa, S. (1991) Sterol and triterpene derivatives from plants inhibit the effects of tumor promoter, and sterol and betulinic acid inhibit tumor formation in mouse skin two-stage carcinogenesis, *Oncology*, **48**: 72–76.
- Yi, L.G., Zhong, G., Liu, X., and Zeng, F.K. (2002) Research development of nutritional characteristics, physiological function and medicinal value of buckwheat. *Journal of Cereals and Oils in Chinese*, **5**: 32–34.
- Yu, L., Haley, S., Perret, J., Hamis, M., Wilson, J., and Qian, M. (2002) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, **50**: 1619–1624.
- Zhang M.L. and Hu, X.S. (2004) Research advance of buckwheat biological active substance and function. *Rain Fed Crops in Chinese*, **24**(1): 26–29.
- Zhang, W.Q. and Zhang, M.Y. (2008) Effects of resistant starch on insulin resistance in diabetes mellitus rats. *Acta Nutrimenta Sinica in Chinese*, **30**(3): 257–261.
- Zheng, J.J. (2009) Research on silty features and the processing applicability of gel food of different varieties of buckwheat. Dissertation for Master Degree at Northwest A and F University.
- Zhu, R., Gao, N.N., and Cheng, J.M. (2003) Chemical Composition and Pharmacological Function of Tartary Buckwheat. *Chinese Wild Plant Resources*, **22**(2): 7–9.

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# 11 Nutraceutical and health properties of psyllium

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## 11.1 Introduction

Psyllium refers to a group of mucilaginous materials prepared from the seeds and the seed husks of the *Plantago* genus plants including, but not limited to, *P. ovata*, *P. psyllium*, and *P. indica*. Psyllium is produced in certain sub-tropical regions including parts of Asia, North Africa, and Mediterranean regions of Europe. Psyllium contains a densely substituted acidic arabinoxylan (Yu *et al.*, 2009). The xylan backbone has both ( $\beta$ 1 $\rightarrow$ 4) and ( $\beta$ 1 $\rightarrow$ 3) linkages, with some single xylopyranosyl branches at C-2 and some trisaccharide side chains at C-3 positions. The trisaccharide side chains were identified as L-Araf- $\alpha$ -(1 $\rightarrow$ 3)-D-Xylp- $\beta$ -(1 $\rightarrow$ 3)-L-Araf according to NMR and methylation studies. Other monosaccharides reported in psyllium include D-galactose, D-galacturonic acid, D-rhamnose, 4-*O*-methyl-D-glucuronic acid and 2-*O*-(2-D-galactopyranosyluronic acid)-L-rhamnose (Yu *et al.*, 2009).

Psyllium has been utilized as a health component in food and dietary supplements, as well as in cosmetic and pharmaceutical products such as lotions and drug delivery systems (Yu *et al.*, 2009). Psyllium has been used for potential treatment of constipation, diarrhea, and weight gain, and is found to reduce the risk of cardiovascular diseases, irritable bowel syndrome, anti-inflammation, and colon cancer (Yu *et al.*, 2009; Wörnberg *et al.*, 2009). Psyllium may also be used as a functional ingredient in foods or other consumer products, such as a deflocculant in paper and textile manufacturing and a binder in meat products (Yu *et al.*, 2009). In addition, derivatives of psyllium polysaccharides have been studied as oral delivery systems for bioactive components (Yu *et al.*, 2009; Singh and Chauhan, 2009; Singh *et al.*, 2009). This chapter summarizes the health properties of psyllium, the potential biochemical mechanisms involved in its biological actions, effects of structural modification on bioactivities, and the possible adverse effects of psyllium intake.

## 11.2 Health beneficial effects of psyllium

### 11.2.1 Hypolipidemic effect

Dietary fibers have been well recognized for their potential in reducing plasma total and LDL cholesterol concentrations and the risk of cardiovascular diseases (Wärnberg *et al.*, 2009). In 1998, the US FDA approved a health claim for soluble fibers from certain foods and reduction of the risk of coronary heart disease. Products that contain 1.7 g soluble fiber from psyllium seed husk per reference amount customarily consumed are able to carry a health claim “may reduce the risk of heart disease” (21 CFR 101.81). Some countries of the European Union have also had similar health claims related to psyllium and cardiovascular disease (Wärnberg *et al.*, 2009). The US National Cholesterol Education Program (NCEP) (NCEP ATP III) encourages the intake of viscous (soluble) fiber (psyllium) at 10–25 g/day to reduce LDL cholesterol.

Psyllium has been evaluated in different animal models and human clinic trials for its effectiveness in reducing plasma cholesterol concentration and the possible mechanisms involved in this beneficial activity (Yu *et al.*, 2009; Chan and Heng, 2008). Tables 11.1 and 11.2 summarize the previous studies on hypocholesterolemic effects of psyllium using animal models. Some of these previous studies have been reviewed by Yu and others (Yu *et al.*, 2009). Briefly, several hamster feeding studies indicated that psyllium significantly reduced plasma cholesterol comparable to the effect of other dietary fibers including pectin and guar gum. The primary mechanisms involved in the cholesterol lowering effects of psyllium have been the enhanced fecal bile acid excretion and hepatic bile acid syntheses in hamster models. In addition, psyllium could reduce triacylglycerols to an extent comparable to that of cholestyramine. Additive hypocholesterolemic effect of psyllium and cholestyramine has also been observed in hamsters. Not only psyllium, but two enzymatically modified psyllium preparations also exhibited elevated hypocholesterolemic and bile acid excretion in these studies. Other modified psyllium preparations such as acid-treated, sulfated, and hydroxypropylated psyllium preparations showed bile acid-binding capacity *in vitro* (Cheng *et al.*, 2009; Liu *et al.*, 2010a, 2010b). Since binding of bile acids *in vitro* can be an indicator of enhanced excretion of bile acids or reduced total plasma and LDL cholesterols, the strong binding capacity of the bile acid by these psyllium derivatives is suggestive of good potential in treating hypercholesterolemia.

Consistent with the findings from the hamster feeding studies, the hypocholesterolemic effects of psyllium were also observed in rat and mouse models. Anderson and others (Anderson *et al.*, 1994) found that rats fed psyllium had the lowest serum and liver cholesterol concentrations compared with those fed nine other different dietary fibers under the same experimental conditions. Terpstra *et al.* (2000) found that psyllium significantly reduced plasma and liver cholesterol concentrations in female rats fed a cholesterol enriched diet. The mechanisms were proposed as increased excretion of fecal sterols and bile acids, along with altered regulation of cholesterol 7 $\alpha$ -hydroxylase, ileal apical sodium-dependent bile acid transporter (ASBT), and 3-hydroxyl-3-methylglutaryl CoA reductase (HMGR) (Yu *et al.*, 2009). In 2008, Chan and Heng investigated the effects of diet containing psyllium on the expression levels of hepatic genes and plasma lipid levels in mice. The psyllium diet was able to reduce plasma total cholesterol and triglyceride concentrations, and to down regulate genes involved in lipogenesis and up regulate the gene for biosyntheses of cholesterol and bile acids. Changes in genes related to bile acid synthesis such as Cyp7A1, Cyp39A1 and Ldlr support that the cholesterol-lowering effects were related to the increased conversion

**Table 11.1** Effect of dietary psyllium on plasma/serum and liver cholesterol concentrations in hamsters and rats<sup>1</sup>

Number per group	Days on diet	Animal models	Cholesterol in diet (%)	Psyllium in diet (%)	Fat in diet (%)	Plasma/Serum cholesterol (mmol/L)		Decrease (%)	Liver cholesterol (µmol/g)		Decrease (%)	References		
						Cellulose	Psyllium		Cellulose	Psyllium				
8	21	Hamsters	0.2	5	10	8.84	6.52	26	42.9	15.8	63	Yu <i>et al.</i> , 2009		
10	28		2	20	NR	6.81	2.83	58	ND	ND	-			
12	35		0.4	5	5	9.72	6.69	31	107.3	114.0	-6			
10	35		0.12	8	20	5.54	2.96	47	92.8	10.5	89			
16	28		0.1	7.5	10	10.30	3.15	69	42.9	6.7	84			
8/10	17		0	7.5	4.6	3.20	2.11	34	6.5	5.2	19			
8	18		0.1	7.5	10	8.61	4.11	52	20.0	6.2	69			
14	56		0.1	3	20	5.72	4.21	26	25.6	15.0	41			
11	21		Rats	1.2	6.8	6	4.4	2.9	34	110	52		53	Yu <i>et al.</i> , 2009
10	28			0	5	NR	2.25	2.17	4	7.50	7.24		3	
6	28			0.25	5	NR	2.15	2.07	4	38.0	20.2		47	
4	21			0	10	10	2.19	1.96	11	7.37	5.69		23	
4	21			0.3	10	10	2.37	2.45	-3	12.90	7.68		40	
15	21			0.3	5	NR	3.26	2.66	18	33.6	17.3		49	
15	21	0.3		10	NR	3.26	2.62	20	33.6	14.7	56			
10	21	0		3.33	NR	ND	ND	-	10.3	8.29	20			
10	21	0		6.67	NR	ND	ND	-	10.3	8.29	20			
10	21	0		10	NR	ND	ND	-	10.3	8.15	21			
14	56	1.2		3	20	16.47	8.92	46	90.31	60.49	33			

<sup>1</sup> Statistical significance was  $P < 0.05$  in all studies. ND = not determined; NR = not reported.

**Table 11.2** Effect of dietary psyllium on plasma/serum and liver cholesterol concentrations in guinea pigs, mice, rabbits and monkeys<sup>1</sup>

Number per group	Days on diet	Animal models	Cholesterol in diet (%)	Psyllium in diet (%)	Fat in diet (%)	Plasma/Serum cholesterol (mmol/L)		Decrease (%)	Liver cholesterol (µmol/g)		Decrease (%)	References
						Cellulose	Psyllium		Cellulose	Psyllium		
6	28	Guinea	0.04	7.5	15	1.99	1.63	18	6.85	3.59	48	Yu <i>et al.</i> , 2009
6	28	pigs	0.25	7.5	15	6.98	4.42	37	19.40	10.68	45	
24	28		0.25	7.5	15.1	3.6	2.3	36	ND	ND	-	
6	21	Mice	0	10	4	2.02	1.49	26	ND	ND	-	Chan and Heng, 2008
6	70		0	10	4	2.14	1.39	35	ND	ND	-	
6	30	Monkeys	0.29	10	19.5	6.65	4.06	39	ND	ND	-	

<sup>1</sup> Statistical significance was  $P < 0.05$  in all studies. ND = not determined; NR = not reported.



of cholesterol to bile acids (Chan and Heng, 2008). In addition, the hypocholesterolemic effects of psyllium were also evaluated using other animal models such as guinea pigs and monkeys (Table 11.2), and the mechanisms behind might be related to fecal bile acid excretion, hepatic acyl coenzyme: cholesterol acyltransferase (ACAT) activity, hepatic membrane apoB/E receptors, and the regulation of lecithin cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), cholesterol 7 $\alpha$ -hydroxylase, and 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase (Yu *et al.*, 2009).

The cholesterol lowering effect has also been evaluated in several human studies (Table 11.3). In a study involving 28 hypercholesterolemic subjects, psyllium was found to significantly lower the total plasma cholesterol (7.2%) and LDL cholesterol (13.5%) after three months of treatment (8.06 g daily) (Segawa *et al.*, 1998). A similar study conducted by Anderson and his co-workers (Yu *et al.*, 2009) showed greater cholesterol (14.8%) and LDL-cholesterol (20.2%) lowering effect of psyllium in a ten-week study. Jenkins and others (2002) found that psyllium, along with beta-glucan, was able to significantly lower serum cholesterol, LDL-cholesterol, total triglycerides, and lipoprotein B in 68 hyperlipidemic subjects. Their studies also concluded that part of the beneficial effect on lipid and lipoprotein ratios was due to lowering the glycemic index of the diet. Sprecher and others compared the hypocholesterolemic effect of psyllium between a high-fat diet and a low-fat diet. Subjects on high-fat diet tended to have a slightly more decrease in serum total and LDL cholesterol levels, suggesting that psyllium might physically disrupt the intraluminal formation of micelles (Sprecher *et al.*, 1993). Diet containing psyllium was found to reduce total plasma cholesterol (19.7%), LDL cholesterol (23.7%), triglycerides (27.2%), and the ratio of LDL-cholesterol to HDL-cholesterol (24.1%) in patients with non-insulin dependent diabetes mellitus and hyperlipidaemia (Gupta *et al.*, 1994). Moreover, Sola and others studied 28 men with cardiovascular disease, and found psyllium, compared with insoluble fibers, decreased the ratio of apo B to A-I, plasma triacylglycerol, and ratio of LDL-cholesterol to HDL-cholesterol (Sola *et al.*, 2007). Psyllium might also reduce cholesterol concentration in healthy human subjects. The hypocholesterolemic effect was also found in an intervention study of 24 healthy men taking 22.2 g psyllium cereal per day for 18 weeks (Stoy *et al.*, 1993). Interestingly, the hypolipidemic effect of psyllium was found to be different among men, pre-menopausal and post-menopausal women (Vega-Lopez *et al.*, 2001, 2002, 2003; Ganji and Kuo, 2008). Although no significant difference was observed for cholesterol levels, psyllium induced a considerable decrease on total triglycerides (TG) in men, an increase in post-menopausal women, and no change in pre-menopausal women. Vega-Lopez *et al.* (2001, 2002, 2003) suggested that the effects of psyllium on TG may be partially due to the influence of hormonal status on lipoprotein gene expression. Decreased insulin resistance might be related to the TG lowering effect of psyllium in men. The hypertriglyceridemic activity in post-menopausal women may be due to an enhanced VLDL production. Postprandial effects of psyllium on lipids were examined by several research groups (Khossousi *et al.*, 2008; Sartore *et al.*, 2009). The hypocholesterolemic effect of psyllium was observed in both short- and long-term treatments. Psyllium-enriched cereal significantly reduced serum cholesterol, LDL-cholesterol, as well as HDL-cholesterol in a two-week study, and its long-term effect was supported by 24-week and 26-week studies (Yu *et al.*, 2009). A meta-analysis by Wei *et al.* further confirmed that psyllium could produce dose- and time-dependent serum cholesterol and LDL-cholesterol lowering effect (Wei *et al.*, 2009). The combination of psyllium with other bioactive compounds such as monounsaturated fatty acid, sterols, and statin might result in an additive hypocholesterolemic

**Table 11.3** Effect of dietary psyllium on plasma and liver cholesterol concentrations in humans

Number of subjects	Status of subjects	Type of diet	Psyllium (g/day)	Time	% Change			Reference	Note
					LDL-C	TC	TG		
28	Hypercholesterolemia	NR	8.06	3 mo	-13.5	-7.2	+15.8	Segawa <i>et al.</i> , 1998	
26	mild-to-moderate hypercholesterolemia	normal	10.2	10 wk	-20.2	-14.8	-12.7	Yu <i>et al.</i> , 2009	
68	Hyperlipidemia	NCEP Step 2	7.2	1 mo	-4.1	-2.2	+6.4	Jenkins <i>et al.</i> , 2002	
24	NIDDM w/hyperlipidemia	normal	7	90 d	-23.7	-19.7	-27.2	Yu <i>et al.</i> , 2009	
286	mild-to-moderate hypercholesterolemia	NCEP Step 1	3.4	24 wk	-1.2	-	+13.2	Yu <i>et al.</i> , 2009	
		NCEP Step 1	6.8	24 wk	-1.2	-0.3	+13.1		
		NCEP Step 1	10.2	24 wk	-1.9	-0.8	-		
18	modestly hypercholesterolemia	NCEP Step 2	7.3	2 wk	-11.3	-8.3	-	Yu <i>et al.</i> , 2009	compared to placebo
248	Hypercholesterolemia	NCEP Step 1	10.2	26 wk	-2.9	-2.1	+3.2	Yu <i>et al.</i> , 2009	
135	Hypercholesterolemia	hi-fat	10.2	8 wk	-7.2	-5.8	-	Yu <i>et al.</i> , 2009	
		lo-fat	10.2	8 wk	-6.4	-4.2	-		
20	moderate hypercholesterolemia	normal	15.3	40 d	-8.2	-5.3	-	Everson <i>et al.</i> , 1992	
28	CVD	low-saturated-fat diet	10.5	8 wk	-6.9	-3.8	-2.8	Yu <i>et al.</i> , 2009	compared to placebo

NR, not reported; LDL-C, LDL-cholesterol; TC, total cholesterol; TG, triglycerides; NIDDM, Non-insulin-dependent diabetes mellitus; CVD, cardiovascular disease; NCEP, National Cholesterol Education Program.

effect (Jenkins *et al.*, 1997; Shrestha *et al.*, 2006; Moreyra *et al.*, 2005; Agrawal *et al.*, 2007). In summary, these data indicate the potential of psyllium in reducing total serum and LDL cholesterol and total serum triglycerides, as well as increasing HDL cholesterol.

Binding of bile acids in the small intestine to prevent their reabsorption, similar to cholestyramine, is considered a possible mechanism for the cholesterol-lowering activity of psyllium, although the precise mechanisms are still under investigation (Yu *et al.*, 2009; Vega-Lopez *et al.*, 2003). Another proposed mechanism is that psyllium physically disrupts the intraluminal formation of micelles, which causes decreased cholesterol absorption and reabsorption of bile acids, and then results in depletion of hepatic cholesterol (Yu *et al.*, 2009). In addition, the short chain fatty acids produced by fermentation of psyllium fiber in the colon, may play a role in suppressing hepatic synthesis of fatty acids (Chen *et al.*, 1984). Other proposed mechanisms include a possible influence of psyllium on hormonal (insulin and glucagon) status, lipid metabolism, and effect on intestinal motility. It should be pointed out that these mechanisms are not necessarily mutually exclusive.

### 11.2.2 Hypoglycaemic effect

Psyllium has been shown to improve postprandial glycemic index and insulin sensitivity in a number of studies (Yu *et al.*, 2009). For instance, a recent study showed that the hot-water extractable components of *Plantago ovata* at 0.5 g/kg body weight/day significantly inhibited the rise of blood glucose level induced by oral intake of glucose or sucrose in rats, with no alteration in fasting blood glucose level and insulin status. Psyllium intake reduced sucrose absorption in the gastrointestinal tract, suppressed intestinal glucose absorption during the 30 minutes of perfusion, and increased gastrointestinal motility, but exhibited no effect on disaccharidase activity, indicating psyllium might reduce hyperglycaemia by suppressing intestinal glucose absorption. In an earlier study, psyllium was shown to enhance blood glucose disposal or improve insulin sensitivity by significantly up regulating skeletal muscle plasma membrane GLUT-4 protein expression without phosphatidylinositol 3-kinase activation in stroke-prone spontaneously hypertensive rats (Yu *et al.*, 2009). GLUT-4 protein is a glucose transporter. Insulin enhances glucose uptake into skeletal muscle by transporting GLUT-4 from the intracellular membrane to the plasma membrane through phosphatidylinositol 3-kinase activation.

Psyllium has been reported for its potential to reduce postprandial glucose and insulin concentrations in diabetic, hypercholesterolemia, hypertensive, and overweight patients (Cicero *et al.*, 2007; Khossousi *et al.*, 2008; Sartore *et al.*, 2009; Yu *et al.*, 2009). Table 11.4 summarizes the hyperglycaemia effects of psyllium in humans from these studies. Early in 1991, a study involving 18 non-insulin-dependent diabetic patients using a crossover design showed that psyllium ingestion right before meals reduced postprandial glucose elevation up to 14% at breakfast and 20% at dinner relative to placebo, whereas the second-meal effects after lunch had a 31% reduction of postprandial glucose elevation compared to placebo (Yu *et al.*, 2009). The proposed mechanisms for the hyperglycaemic effect included suppressing the diffusion of glucose to the small intestinal epithelium for absorption, delaying gastric empty time, and reducing carbohydrate digestibility by retarding their access to digestive enzymes. All the three mechanisms are related to the strong gel forming capacity of psyllium and its ability to significantly increase fluid viscosity in the gastric and intestinal systems. This finding was supported by several recent human studies showing that dietary psyllium intake reduced postprandial serum glucose and insulin concentrations in hypercholesterolemic

**Table 11.4** Hypoglycaemia effects of psyllium in humans<sup>1</sup>

Number of subjects	Status of subjects	Type of diet	Psyllium	Serum/Plasma glucose concentration decrease (%)		Serum/Plasma insulin concentration decrease (%)		References
				Cmax	AUC	Cmax*	AUC*	
18	Non-insulin-dependent patients	Standard diet	6.8 g/meal	14.1	12.75	11.81	16.59	Yu <i>et al.</i> , 2009
10	Hypercholesterolemia	High/low fibre diet	5.1/3.4 g/meal	20.83/18.75	NR	NR	NR	
34	Type 2 diabetes and mild-to-moderate hypercholesterolemia	Diabetes exchange diet	5.1 g/meal 10.2 g/day	19.2 11.0	NR	NR	NR	
20	Type 2 diabetes	Controlled diet	3.5 g/meal	10	12.2	5.5	5	
45	Type 2 diabetes mellitus	High/low glycaemic load breakfast	6.6 g/meal	NR	1.4	NR	8.0	

<sup>1</sup> Statistical significance was  $P < 0.05$  in all studies. Cmax, maximum serum/plasma glucose concentration; AUC, area under glucose curve; Cmax\*, maximum serum/plasma insulin concentration; AUC\*, area under insulin curve.

men and in type 2 diabetic human subjects (Yu *et al.*, 2009). Anderson and others (1994) reported that psyllium at a level of 5.1 g/day and twice a week for two weeks reduced post-lunch postprandial glucose concentrations in men with type 2 diabetes by 11 and 19%, respectively. In 2005, a study showed that eight-week treatment with 5.1 g psyllium twice a day half an hour before breakfast and dinner significantly reduced fasting plasma glucose and controlled glucose fluctuations by reducing glycosylated hemoglobin (HbA1c) in type 2 diabetic patients (Yu *et al.*, 2009). A recent study confirmed that two-month treatment with psyllium significantly decreased fasting plasma glucose and HbA1c levels as well as triglycerides and blood pressure in type 2 diabetic patients (Sartore *et al.*, 2009). It needs to be noted that conflicting results were obtained for the possible residual effect of psyllium after the second meal in type 2 diabetic patients. In addition, these previous studies suggest that addition of psyllium to conventional diet is safe, and may not adversely affect the bioavailability of dietary mineral and vitamin (Yu *et al.*, 2009).

### 11.2.3 Effects in gastrointestinal system

Psyllium is well known as an outstanding laxative and its laxative activity has been reviewed by Yu and others (2009). Early in 1998, a multi-state, double-blind, parallel-design study involving 170 subjects proved that psyllium was superior to docusate sodium as a stool softener and laxative for treatment of chronic idiopathic constipation. The results also suggested that psyllium might increase stool water content, total stool output, and bowel movement in two weeks of treatment at a level of 5.1 g/d. (Bi-daily) (McRorie *et al.*, 1998). The beneficial effect might be associated with the water-holding and water up-taking ability of psyllium. The laxative effect of psyllium was also observed in another study using human subjects. It was found out that a gel-forming, unfermented component of psyllium in the colon might contribute a bulkier and more moist, slick stool. In addition, psyllium was also reported to increase the excretion of short chain fatty acids in stools, while not significantly affecting the fecal bacterial mass (Marteau *et al.*, 1994). Changes in colon pH could be another explanation of the laxative effect of psyllium.

Interestingly, psyllium has been shown to have paradoxical properties of both improving constipation and causing ameliorating diarrhea (Yu *et al.*, 2009; Bliss *et al.*, 2001). Diarrhea is a symptom of having loose or liquid stools at a high frequency. Psyllium husk was found to delay gastric emptying and to reduce the speed of colon transit by increasing meal viscosity and delaying the formation of gaseous products due to colon fermentation, respectively (Yu *et al.*, 2009).

In addition, several research studies have examined the potential use of psyllium for treatment of irritable bowel syndrome (IBS) (Jalihal and Kurian, 1990; Longstreth *et al.*, 1981; Bijkerk *et al.*, 2009). IBS is a common functional gastrointestinal disorder related to recurrent episodes of abdominal pain or discomfort bowel habit change. The results from these previous studies suggested that psyllium might be used in treatment of IBS.

### 11.2.4 Cancer prevention

The potential application of psyllium in the prevention of cancer, particularly colon and breast cancers, has been reviewed by Yu and others (2009). Morita and others (1999) reported that psyllium at a level of 15 g/kg diet was able to shift the fermentation site of high-amylose corn starch toward the distal colon and increase the fecal butyrate content in rats. Distal colon is the common site of colon cancer. Butyrate is an important short chain fatty acid for

colon cancer prevention since it may dose-dependently suppress cancer cell proliferation, promote differentiation marker expression, and lead to reversion of cells from a neoplastic to a nonneoplastic phenotype (Yu *et al.*, 2009). Psyllium might also alter colonic sphingomyelin metabolism and apoptosis, which may have implications for colon tumourigenesis and inflammation in mice (Cheng *et al.*, 2004). In addition, psyllium extract and pure  $\beta$ -sitosterol isolated from psyllium extract restored gap junctional intercellular communication in Ha-ras transfected rat liver cells. Up regulation of gap junctional intercellular communication is a possible mechanism for several anticarcinogenic compounds such as retinoids and carotenoids (Yu *et al.*, 2009). It should be pointed out that conflicting evidence also exists about the preventive effect of psyllium on colon cancer. Ma and others reviewed the existing data and concluded that psyllium and other soluble fibers such as pectin might not reduce the risk of colon cancer (Ma *et al.*, 1996).

Five different combinations of psyllium and wheat bran, at ratios of wheat bran to psyllium at 12 and 0%, 8 and 2%, 6 and 3%, 4 and 4%, and 0 and 6% in the diet, were evaluated and compared for their potential in reducing the risk of chemically induced mammary tumourigenesis using F344 rats (Yu *et al.*, 2009). After 19 weeks of treatment, rats on the 4%:4% (wheat bran/psyllium) diet had the lowest mammary tumourigenesis, and rats on the diet with other ratios or psyllium alone diets had intermediate tumourigenesis. Rats on the higher psyllium diet had lower fecal estrogen excretion, although no difference was detected in their circulating estrogens or urinary estrogen excretions among rats on different diets. In addition, psyllium intake suppressed bacterial  $\beta$ -D glucuronidase activity in rats (Cohen *et al.*, 1996). The relationship between bacterial  $\beta$ -D glucuronidase activity and mammary tumor development was not clear, and other phytochemicals such as phytates, isoflavonoids, and proteinase inhibitors may contribute to the overall anticarcinogenesis activity. Future research is warranted to investigate whether a mixture of insoluble and soluble fiber offers a better approach to reduce the risk of mammary cancer.

### 11.2.5 Anti-inflammation effect

A human study was conducted to investigate the potential effect of psyllium on inflammatory markers including C-reactive protein (CRP) which is known to be related to dietary fiber intake (King *et al.*, 2008). CRP is an independent predictor for the risk of cardiovascular disease, and has been associated with the risk of many other health problems such as diabetes and hypertension. In this study, no significant differences in inflammation markers including CRP, fibrinogen and IL-6 were observed between the control and psyllium treatment groups. It needs to be pointed out that the human subjects in this study were overweight or obese adults. The observation was supported by a recent human study reported by North and others (2009), who concluded that increased fiber intake reduced CRP levels, but psyllium supplementation did not. Additional studies are required to investigate the effect of psyllium on CRP in healthy human subjects.

## 11.3 Potential in controlled delivery of bioactives

The cross-linked polysaccharide derivatives of *Plantago psyllium* mucilage and *Plantago ovate* husks have been investigated for their potential in oral delivery of bioactives, since as a gel-forming edible polysaccharide there are less safety concerns. The former is considered

**Table 11.5** Psyllium-derived delivery systems for bioactives

	<b>Cross-linking approach*</b>	<b>Bioactive</b>	<b>Delivery characteristics</b>	<b>References</b>
<i>P. psyllium</i>	MAAm and N,N-MBAAm/radiation	Rifampicin	Non-Fickian diffusion, potential for colon delivery	Singh <i>et al.</i> , 2009
<i>P. psyllium</i>	N,N-MBAAm/APS	Insulin	Non-Fickian diffusion	Singh and Chauhan, 2009
<i>P. psyllium</i>	MAAm and N,N-MBAAm/APS	Tetracycline-HCl	Colon delivery	Singh <i>et al.</i> , 2008
<i>P. psyllium</i>	Acrylic acid/radiation	5-fluoruracil	Colon delivery	Singh <i>et al.</i> , 2008
<i>P. ovate</i>	Succinic acid/tartaric acid	Diltiazem-HCl	N/A	Gohel <i>et al.</i> , 2000, 2003

\*MAAm - Methacrylamide, N,N-MBAAm - N,N'-methylenebisacrylamide, APS stands for ammonium persulphate, a chemical inducer of polymerization; N/A means "not available".

excellent in terms of consumer acceptability (Singh *et al.*, 2009; Singh and Chauhan, 2009; Singh *et al.*, 2008a, 2008b, 2008c). The cross-link networks of polysaccharides may be induced using a chemical polymerization agent or by radiation initiated polymerization (Singh *et al.*, 2009; Singh and Chauhan, 2009).

Table 11.5 summarizes the representative previous studies in this area. Acrylic acid and its derivatives, including methacrylamide (MAAm) and N,N'-methylenebisacrylamide (MBAAm), have been used as the cross-linking agents to create novel cross-linked psyllium derivatives (Singh *et al.*, 2009; Singh and Chauhan, 2009; Singh *et al.*, 2008a, 2008b, 2008c). The radiation cross-linked polymerization of psyllium and MAAm or N,N-MBAAm was shown to potentially act as a colon specific delivery system for rifampicin, an antibiotic drug (Singh *et al.*, 2009). The release of rifampicin from the hydrogel system was proposed in a non-Fickian diffusion model. In 2008, radiation cross-linked psyllium and polyacrylic acid based hydrogels were found to be able to deliver 5-fluoruracil to colon in the controlled and sustained manner (Singh *et al.*, 2008a). In addition, ammonium persulphate (APS) has been used as a chemical inducer for polymerization of psyllium and MAAm or N,N'-MBAAm to produce hydrogels (Singh *et al.*, 2008b, 2008c). These hydrogels have been shown to be a delivery vehicle in the colon of tetracycline hydrochloric acid. In addition, polycarboxylic acids-treated psyllium derivatives have been demonstrated to modulate the release of diltiazem-HCl tablets (Gohel *et al.*, 2000, 2003). These studies warrant additional research that directly compares the psyllium-based oral hydrogels and the well accepted colon-targeted delivery systems using more bioactives including drugs with diversified chemical and physical properties.

## 11.4 Possible adverse effects

Adverse effects of psyllium intake have been reported, although psyllium is generally safe for human consumption. These include reduction of caloric availability, appetite suppression, bloating and flatulence, possible abdominal pain, potential anaphylactic symptoms, and alteration of nutrient and drug absorption (Roe *et al.*, 1988; Yu *et al.*, 2009). In 1988, a human study involving 12 healthy women aged 22–39 years showed that psyllium intake

could reduce the apparent absorption of a pharmaceutical dose of riboflavin whereas wheat bran had no effect (Roe *et al.*, 1988). It was suggested that the direct binding capacity of psyllium was a critical factor for the reduced absorption of riboflavin.

Effects of psyllium intake on calcium absorption in several human studies have been reviewed (Yu *et al.*, 2009). In 1995, Heaney and Weaver showed that intake of a commercial psyllium preparation at typical therapeutic levels had little practical effect on the availability of co-ingested calcium in a human study (Heaney and Weaver, 1995). An *in vitro* study by Luccia and Kunkel also showed no binding of exogenous calcium to psyllium (Luccia and Kunkel, 2002a). However, an animal study using weanling Wistar rats showed that psyllium reduced calcium bioavailability and induced negative changes in bone composition (Luccia and Kunkel, 2002b). The effect of psyllium on iron bioavailability was also investigated and it was found that psyllium might inhibit iron absorption in dogs and that the inhibition may be due to the direct binding of iron under the intestinal conditions (Fly *et al.*, 1996; Fernandez and Phillips, 1982a, 1982b). In contrast, a chick feeding study concluded that bioavailability of iron was not affected by psyllium intake (Fly *et al.*, 1996). The viscosity and fermentability of psyllium were considered important factors related to the effect of psyllium on mineral absorption. A reduction in viscosity and fermentability might result in less inhibition on calcium, magnesium, and zinc absorption.

A recent study evaluated the prevalence of *P. ovate* seed allergy among 58 healthcare workers who had daily exposure to psyllium and compared it to that of another group of 63 healthcare workers who had no exposure (Bernedo *et al.*, 2008). The results showed that the exposure group had a rate of 13.8 and 8.6% for sensitization and clinical allergy to psyllium, respectively, whereas no sensitization was observed in the non-exposed group. The allergic symptoms of psyllium ingestion were reviewed by Lantner and others (Lantner *et al.*, 1990).

Psyllium may increase gas production and slow down intestinal gas transition, which is associated with pronounced gaseous symptoms such as uncomfortable abdominal distension, abdominal pain, flatulence, and bloating. In addition, psyllium intake has been reported to significantly suppress caloric intake over an 11-day period (Yu *et al.*, 2009).

In summary, many animal and human studies have demonstrated that psyllium consumption may reduce plasma and liver cholesterols. Psyllium also has shown postprandial glucose and insulin lowering effects in humans, probably related to its gel forming capacity and its high viscosity in GI system. In addition, psyllium has strong laxative effect and may potentially be used for treatment of IBS. More research is required to confirm the cancer prevention and anti-inflammatory effect of psyllium. It has been accepted that psyllium is generally safe for consumption, although it has been reported to potentially reduce the absorption of vitamins and minerals. Recent studies suggested that enzyme and chemical modified psyllium preparations may have good potential in treating hypercholesterolemia while reducing viscosity and gel-forming properties.

## References

- Anderson, J.W., Jones, A.E., and Riddellmason, S. (1994) 10 different dietary-fibers have significantly different effects on serum and liver lipids of cholesterol-fed rats. *Journal of Nutrition*, **124**: 78–83.
- Anderson, J., Allgood, L.D., Turner, J., Oeltgen, P.R., and Daggy, B.P. (1999) Effects of psyllium on glucose and serum lipid responses in men with type 2 diabetes and hypercholesterolemia. *American Journal of Clinical Nutrition*, **70**: 466–473.



- Agrawal, A.R., Tandon, M., and Sharma, P.L. (2007) Effect of combining viscous fibre with lovastatin on serum lipids in normal human subjects. *International Journal of Clinical Practice*, **61**: 1812–1818.
- Arjmandi, B.H., Sohn, E., Juma, S., Murthy, S.R., and Daggy, B.P. (1997) Native and partially hydrolyzed psyllium have comparable effects on cholesterol metabolism in rats. *Journal of Nutrition*, **127**: 463–469.
- Bernedo, N., Garcia, M., Gastaminza, G., Fernandez, E., Bartolome, B., Algorta, J., and Munoz, D. (2008) Allergy to laxative compound (Plantago ovata seed) among health care professionals. *Journal of Investigational Allergology and Clinical Immunology*, **18**: 181–189.
- Bijkerk, C.J., de Wit, N.J., Muris, J.W.M., Whorwell, P.J., Knottnerus, J.A., and Hoes, A.W. (2009) Soluble or insoluble fibre in irritable bowel syndrome in primary care? Randomised placebo controlled trial. *British Medical Journal*, **339**: b3956.
- Bliss, D.Z., Jung, H.J., Savik, K., Lowry, A., LeMoine, M., Jensen, L., Werner, C., and Schaffer, K. (2001) Supplementation with dietary fiber improves fecal incontinence. *Nursing Research*, **50**: 203–213.
- Chan, M.Y. and Heng, C.K. (2008) Sequential effects of a high-fiber diet with psyllium husks on the expression levels of hepatic genes and plasma lipids. *Nutrition*, **24**: 57–66.
- Chen, W.J.L., Anderson, J.W., and Jennings, D. (1984) Propionate May Mediate the Hypocholesterolemic Effects of Certain Soluble Plant Fibers in Cholesterol-Fed Rats. *Proceedings of the Society for Experimental Biology and Medicine*, **175**: 215–218.
- Cheng, Y.J., Ohlsson, L., and Duan, R.D. (2004) Psyllium and fat in diets differentially affect the activities and expressions of colonic sphingomyelinases and caspase in mice. *British Journal of Nutrition*, **91**: 715–723.
- Cheng, Z., Blackford, J., Wang, Q., and Yu L. (2009) Acid treatment to improve psyllium functionality. *Journal of Functional Foods*, **1**: 44–49.
- Cicero, A.F.G., Derosa, G., Manca, M., Bove, M., Borghi, C., and Gaddi, A.V. (2007) Different effect of psyllium and guar dietary supplementation on blood pressure control in hypertensive overweight patients: A six-month, randomized clinical trial. *Clinical and Experimental Hypertension*, **29**: 383–394.
- Cohen, L.A., Zhao, Z.L., Zang, E.A., Wynn, T.T., Simi, B., and Rivenson, A. (1996) Wheat bran and psyllium diets: Effects on N-methylnitrosourea-induced mammary tumorigenesis in F344 rats. *Journal of the National Cancer Institute*, **88**: 899–907.
- Everson, G.T., Daggy, B.P., McKinley, C., and Story, J.A. (1992) Effects of psyllium hydrophilic mucilloid on LDL-cholesterol and bile acid synthesis in hypercholesterolemic men. *Journal of Lipid Research*, **33**: 1183–1192.
- Fernandez, R. and Phillips, S.F. (1982a) Components of Fiber Bind Iron In Vitro. *American Journal of Clinical Nutrition*, **35**: 100–106.
- Fernandez, R. and Phillips, S.F. (1982b) Components of Fiber Impair Iron-Absorption in the Dog. *American Journal of Clinical Nutrition*, **35**: 107–112.
- Fly, A.D., CzarnieckiMaulden, G.L., Fahey, G.C., and Titgemeyer, E.C. (1996) Hemicellulose does not affect iron bioavailability in chicks. *Journal of Nutrition*, **126**: 308–316.
- Ganji, V. and Kuo, J. (2008) Serum lipid responses to psyllium fiber: differences between pre- and post-menopausal, hypercholesterolemic women. *Nutrition Journal*, **7**: 22.
- Gohel, M.C., Amin, A.F., Chhabaria, M.T., Panchal, M.K., and Lalwani, A.N. (2000) Modulation of drug release rate of diltiazem-HCl from hydrogel matrices of succinic acid-treated ispaghula husk. *Pharmaceutical Development and Technology*, **5**: 375–381.
- Gohel, M.C., Patel, M.M., and Amin, A.F. (2003) Development of modified release diltiazem HCl tablets using composite index to identify optimal formulation. *Drug Development and Industrial Pharmacy*, **29**: 565–574.
- Gupta, R.R., Agrawal, C.G., Singh, G.P., and Ghatak, A. (1994) Lipid-lowering efficacy of psyllium hydrophilic mucilloid in non insulin dependent diabetes mellitus with hyperlipidaemia. *India Journal of Medicine Research*, **100**: 237–241.
- Heaney, R.P. and Weaver, C.M. (1995) Effect of psyllium on absorption of co-ingested calcium. *Journal of the American Geriatrics Society*, **43**: 261–263.
- Jalihal, A. and Kurian, G. (1990) Ispaghula therapy in irritable-bowel-syndrome – improvement in overall well-being is related to reduction in bowel dissatisfaction. *Journal of Gastroenterology and Hepatology*, **5**: 507–513.
- Jenkins, D.J.A., Kendall, C.W.C., Vuksan, V., Vidgen, E., Parker, T., Faulkner, D., Mehling, C.C., Garsetti, M., Testolin, G., Cunnane, S.C., Ryan, M.A., and Corey, P.N. (2002) Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid

- risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *American Journal of Clinical Nutrition*, **75**: 834–839.
- Jenkins, D.J.A., Wolever, T.M.S., Vidgen, E., Kendall, C.W.C., Ransom, T.P.P., Mehling, C.C., Mueller, S., Cunnane, S.C., Oconnell, N.C., Setchell, K.D.R., Lau, H., Teitel, J.M., Garvey, M.B., Fulgoni, V., Connelly, P.W., Patten, R., and Corey, P.N. (1997) Effect of psyllium in hypercholesterolemia at two monounsaturated fatty acid intakes. *American Journal of Clinical Nutrition*, **65**: 1524–1533.
- Khossousi, A., Binns, C.W., Dhaliwal, S.S., and Pal, S. (2008) The acute effects of psyllium on postprandial lipaemia and thermogenesis in overweight and obese men. *British Journal of Nutrition*, **99**: 1068–1075.
- King, D.E., Mainous, A.G., Egan, B.M., Woolson, R.F., and Geesey, M.E. (2008) Effect of psyllium fiber supplementation on c-reactive protein: The trial to reduce inflammatory markers (TRIM). *Annals of Family Medicine*, **6**: 100–106.
- Lantner, R.R., Espiritu, B.R., Zumerchik, P., and Tobin, M.C. (1990) Anaphylaxis following ingestion of a psyllium-containing cereal. *Journal of the American Medical Association*, **264**: 2534–2536.
- Liu, W., Xie, Z.H., Zhang, B.C., Wang, Q., Yao, W.B., Gao, X.D., and Yu, L.L. (2010a) Effects of hydroxypropylation on the functional properties of psyllium. *Journal of Agricultural and Food Chemistry*, **58**: 1615–1621.
- Liu, W., Zhang, B., Wang, Q., Xie, Z.H., Yao, W.B., Gao, X.D., and Yu, L.L. (2010b) Effects of sulfation on the physicochemical and functional properties of psyllium. *Journal of Agricultural and Food Chemistry*, **58**: 172–179.
- Longstreth, G.F., Fox, D.D., Youkeles, L., Forsythe, A.B., and Wolochow, D.A. (1981) Psyllium therapy in the irritable bowel syndrome – a double-blind trial. *Annals of Internal Medicine*, **95**: 53–56.
- Luccia, B.H.D. and Kunkel, A.E. (2002a) Psyllium reduces relative calcium bioavailability and induces negative changes in bone composition in weanling Wistar rats. *Nutrition Research*, **22**: 1027–1040.
- Luccia, B.H.D. and Kunkel, M.E. (2002b) In vitro availability of calcium from sources of cellulose, methylcellulose, and psyllium. *Food Chemistry*, **77**: 139–146.
- Ma, Q.Y., Hoper, M., Halliday, I., and Rowlands, B.J. (1996) Diet and experimental colorectal cancer. *Nutrition Research*, **16**: 413–426.
- Marteau, P., Flourie, B., Cherbut, C., Correze, J.L., Pellier, P., Seylaz, J., and Rambaud, J.C. (1994) Digestibility and bulking effect of ispaghula husks in healthy humans. *Gut*, **35**: 1747–1752.
- McCall, M.R., Mehta, T., Leathers, C.W., and Foster, D.M. (1992) Psyllium husk .1. Effect on plasma-lipoproteins, cholesterol-metabolism, and atherosclerosis in african-green monkeys. *American Journal of Clinical Nutrition*, **56**: 376–384.
- McRorie, J.W., Daggy, B.P., Morel, J.G., Diersing, P.S., Miner, P.B., and Robinson, M. (1998) Psyllium is superior to docusate sodium for treatment of chronic constipation. *Alimentary Pharmacology and Therapeutics*, **12**: 491–497.
- Moreyra, A.E., Wilson, A.C., and Koraym, A. (2005) Effect of combining psyllium fiber with siravastatin in lowering cholesterol. *Archives of Internal Medicine*, **165**: 1161–1166.
- Morita, T., Kasaoka, S., Hase, K., and Kiriya, S. (1999) Psyllium shifts the fermentation site of high-amylose cornstarch toward the distal colon and increases fecal butyrate concentration in rats. *The Journal of Nutrition*, **129**(11): 2081–2087.
- North, C.J., Venter, C.S., and Jerling, J.C. (2009) The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. *European Journal of Clinical Nutrition*, **63**(8): 921–933.
- Roe, D.A., Kalkwarf, H., and Stevens, J. (1988) Effect of fiber supplements on the apparent absorption of pharmacological doses of riboflavin. *Journal of the American Dietetic Association*, **88**: 211–213.
- Sartore, G., Reitano, R., Barison, A., Magnanini, P., Cosma, C., Burlina, S., Manzato, E., Fedele, D., and Lapolla, A. (2009) The effects of psyllium on lipoproteins in type II diabetic patients. *European Journal of Clinical Nutrition*, **63**: 1269–1271.
- Segawa, K., Kataoka, T., and Fukuo, Y. (1998) Cholesterol-lowering effects of psyllium seed associated with urea metabolism. *Biological and Pharmaceutical Bulletin*, **21**: 184–187.
- Shrestha, S., Volek, J.S., Udani, J., Wood, R.J., Greene, C.M., Aggarwal, D., Contois, J.H., Kavoussi, B., and Fernandez, M.L. (2006) A combination therapy including psyllium and plant sterols lowers LDL cholesterol by modifying lipoprotein metabolism in hypercholesterolemic individuals. *Journal of Nutrition*, **136**: 2492–2497.
- Singh, B. and Chauhan, N. (2009) Modification of psyllium polysaccharides for use in oral insulin delivery. *Food Hydrocolloids*, **23**: 928–935.

- Singh, B., Chauhan, N., and Kumar, S. (2008a) Radiation crosslinked psyllium and polyacrylic acid based hydrogels for use in colon specific drug delivery. *Carbohydrate Polymers*, **73**: 446–455.
- Singh, B., Chauhan, N., Kumar, S., and Bala, R. (2008b) Psyllium and copolymers of 2-hydroxyethylmethacrylate and acrylamide-based novel devices for the use in colon specific antibiotic drug delivery. *International Journal of Pharmaceutics*, **352**: 74–80.
- Singh, B., Bala, R., and Chauhan, N. (2008c) In vitro release dynamics of model drugs from psyllium and acrylic acid based hydrogels for the use in colon specific drug delivery. *Journal of Material Science: Material Medicine*, **19**: 2771–2780.
- Singh, B., Sharma, V., Kumar, A., and Kumar, S. (2009) Radiation crosslinked polymerization of methacrylamide and psyllium to develop antibiotic drug delivery device. *International Journal of Biological Macromolecules*, **45**: 338–347.
- Sola, R., Godas, G., Ribalta, J., Vallve, J.C., Girona, J., Anguera, A., Ostos, M., Recalde, D., Salazar, J., Caslake, M., Martin-Lujan, F., Salas-Salvado, J., and Masana, L. (2007) Effects of soluble fiber (*Plantago ovata* huak) on plasma lipids, lipoproteins, and apolipoproteins in men with ischemic heart disease. *American Journal of Clinical Nutrition*, **85**: 1157–1163.
- Sprecher, D.L., Harris, B.V., Goldberg, A.C., Anderson, E.C., Bayuk, L.M., Russell, B.S., Crone, D.S., Quinn, C., Bateman, J., Kuzmak, B.R., and Allgood, L.D. (1993) Efficacy of psyllium in reducing serum cholesterol levels in hypercholesterolemic patients on high- or low-fat diets. *Annals of Internal Medicine*, **119**(1): 545–554.
- Stoy, D.B., LaRosa, J.C., Brewer, B.K., Mackey, M., and Meusing, R.A. (1993) Cholesterol-lowering effects of ready-to-eat cereal containing psyllium. *Journal of the American Dietetic Association*, **93**: 910–912.
- Terpstra, A.H.M., Lapre, J.A., de Vries, H.T., and Beynen, A.C. (2000) Hypocholesterolemic effect of dietary psyllium in female rats. *Annals of Nutrition and Metabolism*, **44**: 223–238.
- Vega-Lopez, S., Vidal-Quintanar, R.L., and Fernandez, M.L. (2001) Sex and hormonal status influence plasma lipid responses to psyllium. *American Journal of Clinical Nutrition*, **74**: 435–441.
- Vega-Lopez, S., Conde-Knape, K., Vidal-Quintanar, R.L., Shachter, N.S., and Fernandez, M.L. (2002) Sex and hormonal status influence the effects of psyllium on lipoprotein remodeling and composition. *Metabolism-Clinical and Experimental*, **51**: 500–507.
- Vega-Lopez, S., Freake, H.C., and Fernandez, M.L. (2003) Sex and hormonal status modulate the effects of psyllium on plasma lipids and monocyte gene expression in humans. *Journal of Nutrition*, **133**: 67–70.
- Wärnberg, J., Marcos, A., Bueno, G., and Moreno, L.A. (2009) Functional benefits of psyllium fiber supplementation. *Current Topics in Nutraceutical Research*, **7**: 55–63.
- Wei, Z.H., Wang, H., Chen, X.Y., Wang, B.S., Rong, Z.X., Wang, B.S., Su, B.H., and Chen, H.Z. (2009) Time- and dose-dependent effect of psyllium on serum lipids in mild-to-moderate hypercholesterolemia: a meta-analysis of controlled clinical trials. *European Journal of Clinical Nutrition*, **63**: 821–827.
- Yu, L.L., Lutterodt, H., and Cheng, Z. (2009) Beneficial health properties of psyllium and approaches to improve its functionalities. *Advances in Food and Nutrition Research*, **55**: 193–220.

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# 12 Nutraceutical and health properties of sorghum and millet

Genyi Zhang and Bruce Hamaker

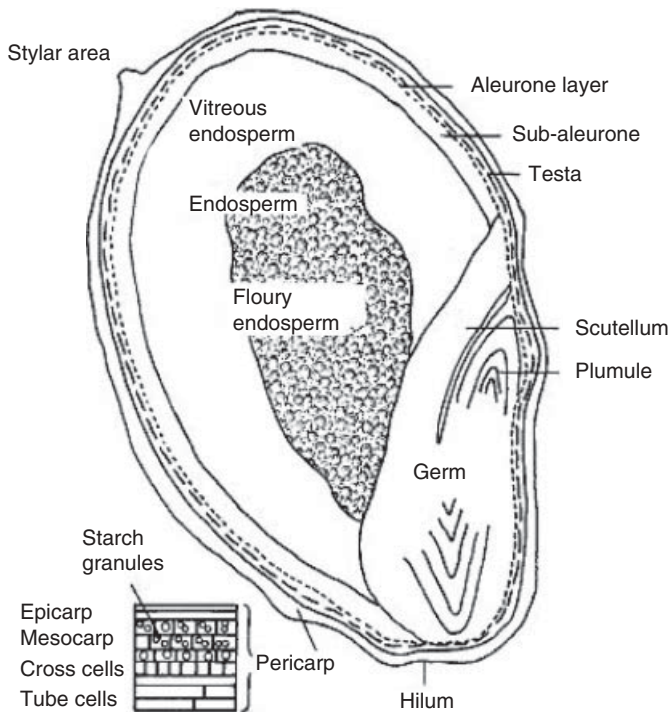
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## 12.1 Introduction

Sorghum and millet taxonomically belong to the grass family Poaceae, and are the primary cereal crops cultivated in the semi-arid tropics of Asia and Africa as the staple food grain. They provide energy, protein, vitamins, and minerals for more than 300 million people with their distinct and unique characteristic of being drought-resistant compared with other cereal grains. Sorghum [*Sorghum bicolor* L. Moench] is currently the major cultivated species whereas pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet, and finger millet are the widely-cultivated millet species. Globally, sorghum and millet are the fifth and sixth, respectively, important crops after maize, wheat, rice, and barley in annual world production. Although sorghum and millet were considered as “coarse grain” or “inferior grains,” they may become more important for the world food supply as the climate changes and as a result of global warming.

The basic kernel structure is similar in sorghum and millet with principal anatomical components of pericarp, germ, or embryo and endosperm, but there exists a great diversity in their color, shape, and size. The bran is layered by pericarp, testa, aleurone, and sub-aleurone layers (Figure 12.1) using sorghum kernel as an example. Among millets, the kernel structure can be divided as utricles and caryopses. Finger, proso, and foxtail millets are utricles in which the seed is loosely covered by the pericarp while pearl millet belongs to caryopses in which the pericarp is fused to the seed. Starch granules and protein bodies are located in the endosperm, and lipids are stored in the germ whereas the bran, also called seed coat, is the location for a variety of phytochemicals and pigments giving different colors to grains. Although the traditional research on sorghum has been mainly concentrated on its protein digestibility and quality (Duodu *et al.*, 2003), the increased interest in phytochemicals with versatile health functions has made sorghum a unique cereal crop with the highest phytochemical content among cereal crops on a per weight basis. Millet, on the other hand, known as a high-quality crop with many beneficial effects to human health, also has a considerable content of phytochemicals.

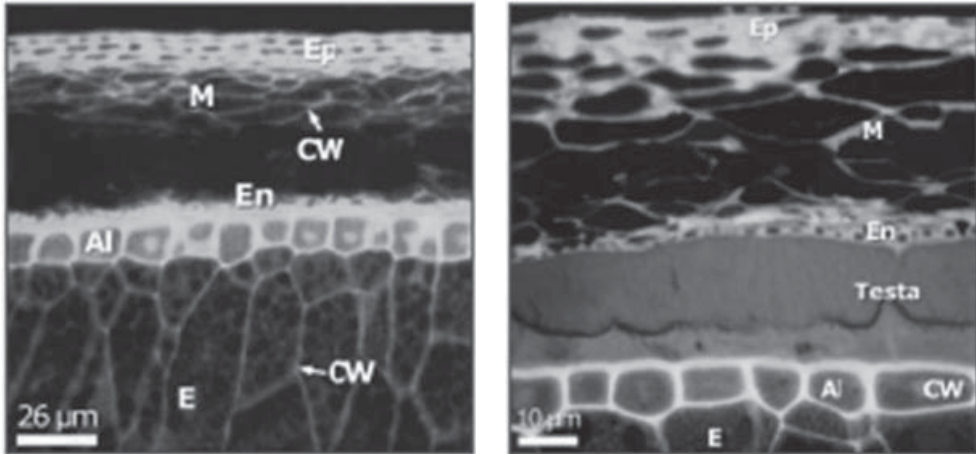
Sorghum phytochemicals, mainly located in the bran layers of testa and pericarp of the grain, can be classified as simple phenols, phenolic acids (ferulic, *p*-coumaric, procatechuic,



**Figure 12.1** Schematic representation of the cross-section of sorghum grains with component parts. Source: Adapted from Earp *et al.*, copyright 2004, with permission of Elsevier.

vanillic, caffeic, *p*-hydroxybenzoic, gallic, and cinnamic acids), tannins, phytosterols, policosanols, carotenoids, lignans, and flavonoids including flavanols, flavanones, flavones, isoflavones, and anthocyanins (Krueger *et al.*, 2003). Sorghum has been categorized into different types according to the content of its characteristic tannins. Type I sorghum is almost tannins-free, tannins in Type II are present in the pigmented testa, and tannins in Type III sorghum are present in pigmented testa and pericarp (Hahn *et al.*, 1984; Hahn and Rooney, 1986). This classification emphasizes the relationship between the tannin content and testa layer in sorghum grain that is genetically controlled by the *B1B2* genes, and the tannin-free sorghums (with recessive *B1B2* genes) do not have this layer (Figure 12.2). Another broad classification incorporating both the appearance of sorghum kernel and the phenolic compound profiles has also been proposed. According to this classification, sorghum grain with a white kernel (tannin-free) has the lowest content of phytochemicals; the brown sorghum with a pigmented testa has the highest content of tannins; red sorghum with a red pericarp has significant phenols; black sorghum with a black pericarp has a high level of anthocyanins; whereas the yellow sorghum yields carotene and xanthophylls (Awika and Rooney, 2004). Apparently, the broader range of phytochemicals in sorghum grains (Table 12.1) is not only controlled by its genetic makeup but also affected by the growing environment.

Phenolic compounds, mainly derivatives of flavonoids and tannins, are the major phytochemicals in millet, and similar rules as in sorghum are followed such as the location of phytochemicals, the effect of genetic makeup, and growth environment on the content of specific phytochemicals. However, limited information about millet phytochemicals is available



**Figure 12.2** Fluorescence photomicrograph of cross-sections of a non-tannin (left) and a tannin sorghum kernel (right).

Source: Adapted from Earp *et al.*, copyright 2004, with permission of Elsevier.

Ep - epicarp; M - mesocarp; CW - cell wall; En - endocarp; Al - aleurone; E - endosperm cell.

**Table 12.1** The phenolic content and antioxidant activity ( $\mu\text{mol TE/g}$ ) of different categories of sorghum

Sorghum type	Phenolic content (mg gallic acid equivalent/g)	Oxygen radical absorbance capacity (ORAC)	2,2'-Azinobis (3-ethyl-benzothiaziline-6-sulphonic acid) activity (ABTS)
White sorghum	0.8	22	6
Red sorghum	6.6	140	53
Black sorghum	6.4	220	57
Tannin sorghum	19.8	870	226

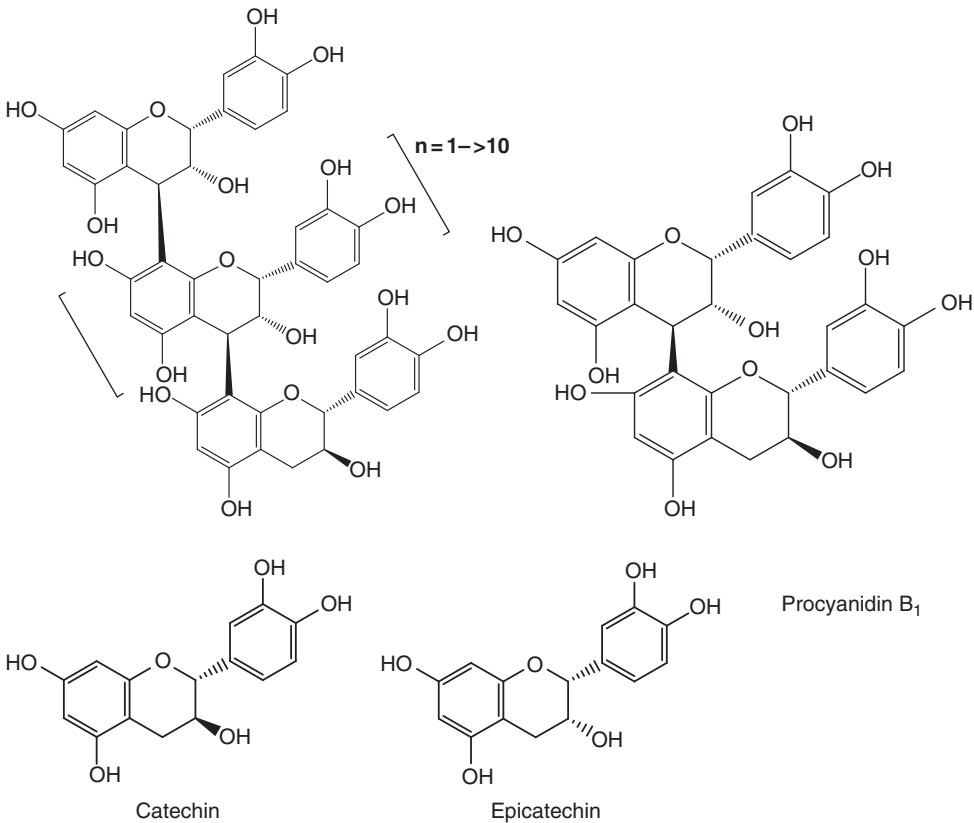
Source: Reproduced from Awika and Rooney, copyright 2004, with permission of Elsevier.

to give a detailed illustration. In this chapter, the fractions of phytochemicals and their benefits to human health will be reviewed to facilitate a deep understanding of phytochemicals from sorghum and millet, and their unique chemical structures and health benefits.

## 12.2 Phytochemicals in sorghum and millet grains and fractions

### 12.2.1 Tannins

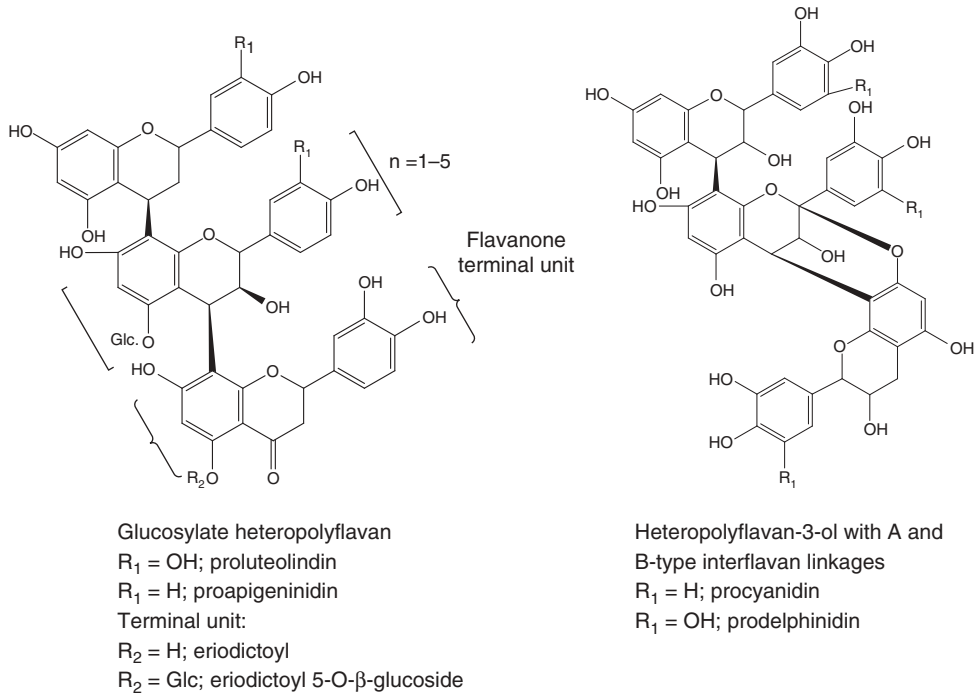
Tannin is a term used historically in the leather industry (tanning animal hides into leather) describing the large polyphenolic compounds that can bind and precipitate proteins with their sufficient hydroxyl and other functional groups, which sets it apart from other phenolics that could not precipitate proteins (e.g. simple phenolic acids). Tannin is divided into



**Figure 12.3** Chemical structure of proanthocyanidins and the repeating monomer (epicatechin) and termination unit (catechin) in sorghum. Procyanidin B<sub>1</sub> is the most abundant dimers in sorghum.

hydrolyzable tannins and condensed tannins. Sorghum is unique among cereals with its high content of condensed tannins that are oligomeric and polymeric flavanoids with a degree of polymerization (DP) of 2–50 of flavan, flavan-3-ol, and flavone monomers (Awika *et al.*, 2003a). The type of interflavan bonds (A and B type) and hydroxylation and glycosylation patterns greatly increase the heterogeneity of sorghum tannins (Reed *et al.*, 2005).

Proanthocyanidins (oligomer of polyflavan-3-ol) is one important group of phenolic tannins in sorghum (Figure 12.3). It is a polymeric product of flavan-3-ol (e.g. epicatechin, catechin) with different DPs. Gupta and Haslam (1978) identified (–)-epicatechin as the repeating units and (+)-catechin as the chain termination monomer in sorghum tannin. This was later confirmed by Gu *et al.* (2002) who showed that the most chain termination unit is (+)-catechin (88%) and the rest is (–)-epicatechin. Procyanidin B<sub>1</sub>, composed of catechin and epicatechin, is the abundant dimer in sorghum tannins. A great heterogeneity of proanthocyanidins was also found in sorghum due to different substitutions of functional groups at different locations in the repeating units and termination monomers (Figure 12.4). Prodelphinidins was reported by Brandon *et al.* (1982); dimmers and trimers of heteropolyflavans with glycosylated luteolinidin as a repeating monomer and eriodictoyl or its glucoside as chain terminator was reported by Gujer *et al.* (1986); mixture of DP2–DP7 of polyflavan of prodelphinidin and proapigeninidin with eriodictoyl or eriodictoyl-O-β-



**Figure 12.4** Heterogeneity of tannins (polyflavans) in sorghum.

glucoside as the terminal unit was found by Krueger *et al.* (2003). Great heterogeneity in the polyflavan-3-ol (procyanidin) polymers in terms of interflavan linkages (A or B type) and the presence of gallicatechin/epigallocatechin hydroxylation patterns in sorghum tannin polymers were also observed (Krueger *et al.*, 2003). Apparently, the structural complexity of sorghum tannin molecules makes it very difficult to be isolated and characterized, and novel approaches are necessary to gain a deep understanding of the structure-function relationship of sorghum tannins.

The content of sorghum tannins, which is mainly determined by the *BIB2* genes, is generally very high in tannin-containing cultivars (Table 12.2) although the tannin accumulation pattern varies during seed development. Additionally, the growing environment also affects the content possibly through altering enzyme activity under different temperature and water stress conditions. Hoshino and Duncan (1982) found that higher tannin content was obtained from the late planted sorghum seeds due to a higher temperature. It was also reported that water stress caused a decrease in tannin content and the degree of effect might be altered by the status of seed maturity. The DP of tannins is another important factor concerning the structure of tannin molecules. Mature seeds usually have a greater DP for tannins, which is associated with a decreased astringency. Food processing such as extrusion on the other hand could lead to a decreased DP of procyanidins (Awika *et al.*, 2003a). Both the content and the DP of tannin molecules need to be investigated for their potential physiological activities before they are used as functional ingredients.

Among millets, finger millet was reported to contain high amounts of tannins (Ramachandra *et al.*, 1977) ranging from 0.04 to 3.47% (catechin equivalents). In studies reported by Udayasekhara and Deosthale (1988), white varieties of finger millet had no detectable



**Table 12.2** Tannin content of sorghum grain and other edible crops

Commodity	mg/g (DW)
Tannin sorghum	10.0–68.0
Tannin-free sorghum	0.5–3.8
Finger millet	3.6–13.1
Buckwheat groats	1.7
Pinto beans	1.5–3.3
Faba beans	0.7
lentils	3.2–10.4
Cow pea	1.8–2.9

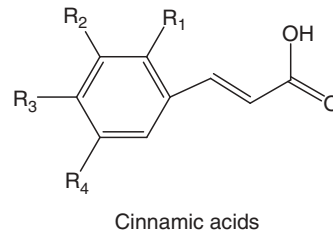
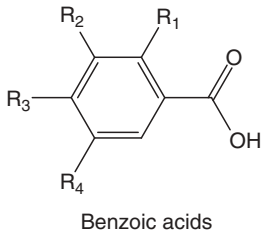
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tannin, while the tannin content of brown varieties ranged from 351 to 2392 mg/100 g. The pigmented testa, similar to that of sorghum, is the location of tannins, and dark brown and red millets usually have a high content of tannins. Pearl millet was also reported to contain tannins, but there is no detail about its content and distributions. For other types of millet, there are few reports about the presence of tannins in their kernel available (Dykes *et al.*, 2006).

### 12.2.2 Phenolic acids and flavonoids

Phenolic acids are mostly present in the bran of the sorghum and millet grains, and most of the phenolic acids are derivatives of hydroxybenzoic or hydroxycinnamic acids (Figure 12.5). Similar to other cereals, the phenolic acids also exist in either free form or bound with other macromolecules through ester bonds (Chadrasekara and Shahidi, 2010). Ferulic acid is predominantly bound phenolic acid. Other identified major phenolic acids in sorghum are syringic, protocatechuic, caffeic, *p*-coumaric, and sinapic acids (Hahn *et al.*, 1984; Waniska *et al.*, 1989) while diadzene, gallic, coumaric, syringic, and vanillic acids were identified as major phenolic acids in finger millet (Viswanath *et al.*, 2009). These phenolic acids are synthesized through the shikimate pathway (Herrmann and Weaver, 1999). The phenolic acid composition and existing forms also vary greatly in both millets and sorghum.

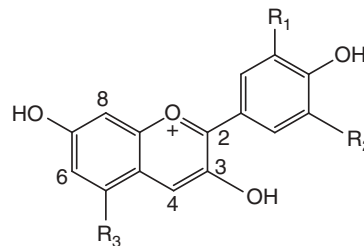
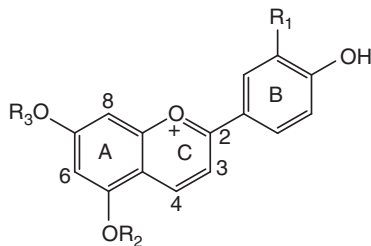
Some sorghum cultivars with a red/purple color have high levels of anthocyanins and their content may be comparable to that found in fruits and vegetables. The primary anthocyanins given the color of sorghum include yellow apigeninidin, apigeninidin-5-glucoside, orange luteolinidin, luteolinidin-5-glucoside, 7-O-methylapigeninidin, fisetinidin, cyanidin, and pelargonidin (Figure 12.6). The most common anthocyanins in sorghum belong to 3-deoxyanthocyanidin family, which is different from anthocyanidins present in fruits and vegetables. The 3-deoxyanthocyanidins have a high acid stability. Sorghum is the only known food source of 3-deoxyanthocyanidins that is stable to heat and light. The black sorghums generally have high levels (4.0–9.8 mg/g) of anthocyanins and may be a useful source of anthocyanidins for different purposes. Other flavonoids isolated from sorghum include flavan-4-ols, flavones apigenin and luteolin, flavanones of eriodictyoland and naringenin, and some flavonols such as kaempferol 3-rutinoside-7-glucuronide (Dykes and Rooney, 2007).



Gallic acid:  $R_1 = H, R_2 = R_3 = R_4 = OH$   
 Gentisic acid:  $R_1 = R_4 = OH, R_2 = R_3 = H$   
 Salicylic acid:  $R_1 = OH, R_2 = R_3 = R_4 = H$   
 p-hydroxybenzoic acid:  $R_1 = R_2 = R_4 = H, R_3 = OH$   
 Syringic:  $R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH$   
 Protocatechuic:  $R_1 = R_4 = H, R_2 = R_3 = OH$

Caffeic acid:  $R_1 = R_4 = H, R_2 = R_3 = OH$   
 Ferulic acid:  $R_1 = R_4 = H, R_2 = OCH_3, R_3 = OH$   
 o-coumaric acid:  $R_1 = OH, R_2 = R_3 = R_4 = H$   
 p-coumaric acid:  $R_1 = R_2 = R_4 = H, R_3 = OH$   
 Sinapic:  $R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH$

**Figure 12.5** Sorghum phenolic acids.



Apigeninidin:  $R_1 = H, R_2 = H, R_3 = H$   
 Apigeninidin-5-glucoside:  $R_1 = H, R_2 = Glc, R_3 = H$   
 Luteolinidin:  $R_1 = OH, R_2 = H, R_3 = H$   
 Luteolinidin-5-glucoside:  $R_1 = OH, R_2 = Glc, R_3 = H$   
 7-O-methylapigeninidin:  $R_1 = H, R_2 = H, R_3 = CH_3$

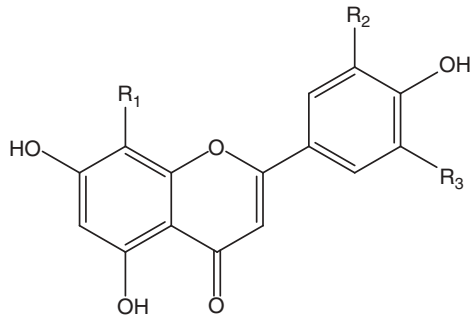
Fisetinidin:  $R_3 = H, R_1 = R_2 = OH$   
 Cyanidin:  $R_1 = R_3 = OH, R_2 = H$   
 Pelargonidin:  $R_1 = R_2 = H, R_3 = OH$   
 Peonidin:  $R_1 = OCH_3, R_2 = H, R_3 = OH$   
 Malvidin:  $R_1 = OCH_3, R_2 = OCH_3, R_3 = OH$   
 Delphinidin:  $R_1 = R_2 = R_3 = OH$   
 Petunidin:  $R_1 = OCH_3, R_2 = R_3 = OH$

**Figure 12.6** The 3-deoxyanthocyanidin in sorghum compared to anthocyanidins found in fruits and vegetables (right side).

Flavones are the only flavonoids reported in millets (Figure 12.7). Eight flavones in the leaves of finger millet were identified by Hilu *et al.* (1978): orientin, isorientin, vitexin, isovitexin saponarin, violanthin, lucenin-1, and triclin. Glucosylvitexin, glucosylorientin, and vitexin in pearl millet were detected by Reichert (1979) in a ratio of 29:11:4. Apigenin and luteolin in fonio with a content of 150 and 350 mg/kg, respectively, were reported by Sartelet *et al.* (1996).

### 12.2.3 Carotenoids

Carotenoids are lipophilic pigments naturally found in higher plants with diverse structures and generally present in carotenes and their hydroxylated derivatives such as xanthophylls (containing oxygen atoms). The color of carotenoids is determined by their structures and can



Apigenin: R<sub>1</sub> = H; R<sub>2</sub> = H; R<sub>3</sub> = H

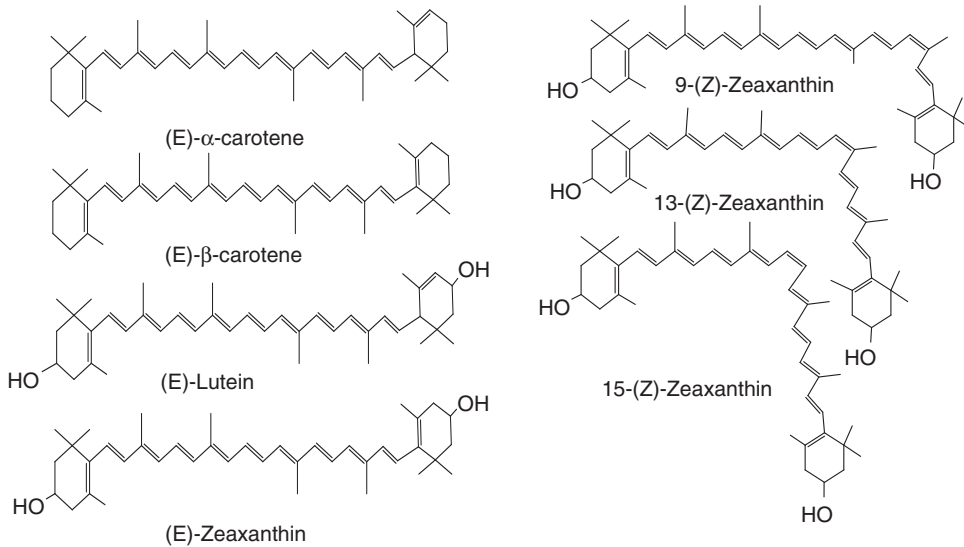
Luteolin: R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = H

Orientin: R<sub>1</sub> = Glc; R<sub>2</sub> = OH; R<sub>3</sub> = H

Tricin: R<sub>1</sub> = H; R<sub>2</sub> = OCH<sub>3</sub>; R<sub>3</sub> = OCH<sub>3</sub>

Vitexin: R<sub>1</sub> = Glc; R<sub>2</sub> = H; R<sub>3</sub> = H

**Figure 12.7** Structure of major millet flavones.



**Figure 12.8** Major carotenoids found in sorghum.

range from pale yellow through bright orange to deep red. Sorghum carotenoids, similar to those in maize, are yellow-orange pigments mainly existing in the endosperm. (E)- $\alpha$ -carotene, (E)- $\beta$ -carotene, (E)-lutein, and isomers of (E)-zeaxanthin, including 9-(Z)-zeaxanthin, 13-(Z)-zeaxanthin, and 15-(Z)-zeaxanthin are the primary carotenoids found in sorghum (Figure 12.8). The content of carotenoids in sorghum is less than that in corn, and only sorghums with yellow endosperm contain appreciable carotenoids at a level of  $\sim 10$  mg/kg of flour, and common sorghum grains may have a carotenoid content of 1.5–5.6 mg/kg (Blessin *et al.*, 1962). A recent study by Kean *et al.* (2007) evaluating eight sorghum cultivars showed

that total carotenoids in grains increased between 10 and 30 d after blooming, but decreased significantly from 30 to 50 d. In matured sorghum kernels, total carotenoid content ranged from 2.62 to 15.02  $\mu\text{g}$  per 1000 kernels, which was significantly lower than average values in maize. They also found that zeaxanthin was the most abundant carotenoid in sorghum. Carotenoid content is a quantitative trait of cereal grains. The genetic basis of carotenoid levels investigated by Fernandez *et al.* (2008) in sorghum showed several quantitative trait locus (QTLs) for each compound as well as for color and total carotenoids, which are correlated to each other as color QTL is co-localized with carotenoid QTL. For  $\beta$ -carotene (provitamin A), five QTLs were localized on chromosomes 1, 2, and 10, and the QTL on chromosome 2 was associated with a new phytoene synthase gene (*Psy3*) that is stable across environments with positive additive effects.

Lutein and zeaxanthin are the predominant carotenoids in white and red millet, with lutein being the major carotenoid in white millet while zeaxanthin is the major carotenoid in red millet (McGraw *et al.*, 2001). Wilkinson *et al.* (1968) reported carotene content between 339 and 427 mg/kg in pearl millet. A recent study (Asharani *et al.*, 2010) on millet antioxidant showed a content of 78–316  $\mu\text{g}/100\text{g}$  of carotenoid in finger millet, and a content of 126–191 and 249–518  $\mu\text{g}/100\text{g}$  of carotenoid in foxtail and proso millets, respectively.

#### 12.2.4 Other phytochemicals

Phytosterols and policosanols are two lipophilic phytochemicals present in sorghum bran, and their cholesterol-lowering effects make them important nutraceuticals related to the health of the cardiovascular system.

These compounds exist as part of the bran oil waxes, and are the structural component of cell membrane. The typical phytosterols in sorghum (in free forms) include sitosterols, campesterol, stigmasterol, sitosteryl trans-ferulate, sitosteryl glucoside, and sitosteryl oleate. Sitosterol is structurally very close to cholesterol, and it is the common structural component of other phytosterols. The most abundant policosanols in sorghum are octacosanol (C28) and triacontanol (C30).

Quantitative data about sorghum phytosterols and policosanols are limited in the literature. After hexane extraction, Singh *et al.* (2003) reported that the total phytosterols was around 0.5 mg/g for the sorghum kernels compared to that of 0.9 mg/g for corn. For policosanols, sorghum generally had a higher content of 0.2% of the grain, and comprised 19–46% of the sorghum wax (Avato *et al.*, 1990). In a recent study (Leguizamon *et al.*, 2009), the contents of sorghum phytosterol and poliscosanols were investigated using different extraction methods. The total content of phytosterol before alkaline hydrolysis was 12.51 mg/100 g, and it changed to 24.85 or 53.59 mg/100 g after alkaline treatment or acid and alkaline treatment, respectively. For poliscoanol, C28 was the most abundant one with 45% of the total policosanols. Additionally, these compounds may be concentrated in the bran fractions such as the by-product of wet-milling or fermentation.

### 12.3 Antioxidant properties of sorghum and millet grain and components

Redox reaction is a chemical reaction related to electron transfers between chemicals in which the one that obtains electrons is called oxidant, and the other, giving away electrons,

**Table 12.3** The antioxidant activities ( $\mu\text{mol TE/g}$  sample) of sorghum and sorghum products

Sample	ORAC	ABTS	DPPH	Phenols (mg GAE/g)
White grain	22	6	6	1
White extrudate	26	7	6	1
White bran	64	28	21	5
Red grain	140	53	28	5
Red bran	710	230	71	20
Black 2001 grain	219	57	41	6
Bk 2001 extrudate	94	37	32	5
Bk 2001 bran	1008	250	184	26
Hi tannin grain	454	108	118	13
Hi tannin extrudate	286	90	74	6
Hi tannin bran	2400	512	495	55

Source: Adapted from Awika *et al.*, 2003b. Copyright 2003, with permission of the American Chemical Society.

is called reductant. An antioxidant is a substance capable of slowing or preventing the oxidation of other molecules through scavenging free radicals generated in oxidation, donating hydrogen or electrons to oxidants (act as a reductant) by being oxidized. As phytochemicals, particularly phenolic compounds, which are capable of donating hydrogen or scavenging free radicals, are the major antioxidant molecules in cereals. The antioxidant capability is determined by both the quantity of the antioxidants and the specific phytochemicals, which vary because of genetic makeup and environmental conditions. Additionally, different *in vitro* measurement methods also give different quantitative values reflecting the complexity of antioxidants and possibly synergistic effects among different phytochemicals.

In sorghum, polyflavanols (tannins), anthocyanins, phenolic acids, carotenoids, and other antioxidant compounds are responsible for the antioxidant properties of sorghum grain. Awika *et al.* (2003a) reported that phenol contents of the sorghums is highly correlated with their antioxidant activity measured by the method of hydrogen atom transfer (HAT) based oxygen radical absorbance capacity (ORAC), and electron transfer (ET) based 2,2 $\phi$ -azino bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity assays. Significant varietal and grain component differences were observed as the tannins in sorghums and their bran have the highest antioxidant capacity (Table 12.3). A later study by Dykes *et al.* (2005) confirmed the association between phenol content and antioxidant activities, and further showed that genes for plant color, pericarp thickness, presence of a pigmented testa, and spreader genes are related to phenol content and antioxidant activity.

Tannins, as polymeric phenolic compounds, show stronger antioxidant activity than simple phenols although they have similar redox potentials (Table 12.4). Tannins in sorghums have strong antioxidant activity and the high tannin content is likely the major contributing factor for the overall antioxidant property of the grain. For simple phenols, the extended conjugation, number and arrangement of phenolic substituents, and molecular weight are associated with their antioxidant capacity whereas degree of polymerization (DP) of tannins is correlated with their antioxidant capacity, and a short distance among many aromatic rings and hydroxyl groups are more important for scavenging free radicals (Ariga and Hamano, 1990). Furthermore, tannins have little pro-oxidant activity compared to some simple phenols (Hagerman *et al.*, 1998). Procyanidin o-quinone can form oligomeric compound

**Table 12.4** The radical quench ability ( $\mu\text{mol TE/g}$ ) of polyphenols measured by the Metmyoglobin Method

Phenolics	TEAC	50% inhibition (mM)	Trolox ratio
catechin	2.61	0.86	2.64
Methyl gallate	2.60	0.87	2.61
PC (tannin)	>>3	0.08	28.4
Poly GG (hydrolyzed tannin)	>>3	0.15	15.1
Trolox	1.0	2.27	1.0

Source: Adapted from Dykes and Rooney, 2007. Reproduced with permission.

\*TEAC: Trolox equivalent antioxidant capacity.

through different coupling reactions that retain the number of hydroxyl groups resulting in a little or no pro-oxidant activity, but the simple flavanoid o-quinones could act as pro-oxidants by forming reactive oxygen species through redox cycling (Bors *et al.*, 2000). The high antioxidant activity and little pro-oxidant capability might suggest the role of tannin-rich sorghums in reducing the risk of human diseases. Tannin-rich sorghums may, therefore, serve as potential dietary sources of antioxidant nutraceuticals.

Phenolic acid is another category of compounds with antioxidant property in sorghum with the widest varieties among cereals, although the content is not the highest (Dykes and Rooney, 2007). The antioxidant activity of phenolic acids depends on their chemical structures including the number of hydroxyl groups and the electron-withdrawing property of carboxylate groups. For hydroxybenzoic acid derivatives, the monohydroxy benzoic acids show no antioxidant activity in the ortho and para position. The antioxidant activity of dihydroxybenzoic acids depends on the relative position of the hydroxyl groups in the ring, and the proximity of the  $-\text{CO}_2\text{H}$  group to the orthodiphenolic substituents, which apparently influences the availability of the hydrogen with the m-position being the most effective in 3,5-dihydroxybenzoic acid (resorcylic acid). Incorporation of an additional  $-\text{CH}_2-$  group between the phenyl ring and the carboxylic acid group in the hydroxyphenyl acetic acids decreases the electron withdrawing capacity of the carboxylate group and could almost double the antioxidant capacity. For hydroxycinnamic acid derivatives, insertion of an ethylenic group between a phenyl ring carrying a *p*-hydroxyl group and the carboxylate group, as in *p*-coumaric acid, has a highly favorable effect on the reducing properties of the OH group.

The high antioxidant capacity of black sorghums and their bran were correlated with their anthocyanin contents (Awika and Rooney, 2005). As described above, the 3-deoxyanthocyanin is the major form present in sorghum, and the antioxidant activity of the 3-deoxyanthocyanidins is similar to that of anthocyanins (Awika *et al.*, 2004). The flavylium cationic at low pH is the basic structure for anthocyanins, and different substituents on ring B alter their antioxidant properties. Depending on the pH, anthocyanins may exist in a variety of protonated, deprotonated, hydrated, and isomeric forms, which influence their antioxidant activity. The antioxidant activity of anthocyanin is fulfilled through hydrogen atom donation, metal chelation, and protein binding. A completely conjugated structure of anthocyanins could stabilize the radical product through electron delocalization; hydroxylation and methoxylation on the B ring certainly affect their antioxidant activity due to the enhanced stability of the radical structure. The *O*-diphenol substitution offers a higher stability to the *O*-semiquinone radical formed upon hydrogen donation compared to the stability of simple aryloxy radicals.

**Table 12.5** Comparison of antioxidant activity (ORAC) between sorghum and common fruit and vegetables

Materials	ORAC ( $\mu\text{mol TE/g}$ )	Materials	ORAC ( $\mu\text{mol TE/g}$ )
Tannin sorghum (grain)	868	Blueberry, lowbush	842
Tannin sorghum (bran)	3124	Strawberry	402
Black sorghum (grain)	219	Plum	495
Black sorghum (bran)	1008	Watermelon	18
Red sorghum (grain)	140	Apple, red delicious	295
Red sorghum (bran)	710	Orange, navel	137
White sorghum (grain)	22	Broccoli	173
White sorghum (bran)	64	Carrot	108
		Onion, red	93
		Sweet pepper	105
		Radishes	217
		Potatoes, russet	63

Source: Adapted from Wu *et al.*, 2005 and Awika *et al.*, 2003b\*.

\*Values are reported on a dry material weight basis. ORAC stands for oxygen radical absorbance capacity and TE stands for Trolox Equivalent.

In addition, the glycosylation generally decreases the antioxidant activity of the molecules (Jaldappagari *et al.*, 2008).

Compared to sorghum grains, there is only limited information on the antioxidant properties of millet. Sripriya *et al.* (1996) reported that the brown or red finger millet had higher antioxidant activity (94%) than the white variety (4%) using DPPH radical scavenging activity assay, and finger millet is a potent source of antioxidants higher than that of wheat, rice, and other millets. Recent studies by Chandrasekara and Shahidi (2010) showed that both soluble and bound fractions of millet grains are rich sources of phenolic compounds with antioxidant, metal chelating, and reducing power. Sorghum (not including white sorghum) has the highest antioxidant activity among cereals (Sun and Ho, 2008) while millet has an intermediate antioxidant activity, which is correlated to phenolic content. Ragae *et al.* (2006) showed a total phenol content of 1387 and 4128  $\mu\text{g/g}$  (gallic acid equivalents) for millet and sorghum, and the corresponding antioxidant activity of 195.8 and 23.83  $\mu\text{mol/g}$  in DPPH radical scavenging capacity in 10 minutes. In comparison, sorghum approaches or exceeds the antioxidant activity of common fruits and vegetables (Table 12.5) (Wu and Prior, 2005, Awika *et al.*, 2003b) indicating sorghum could be a potential cereal resource for phenolic compounds.

## 12.4 Potential beneficial effects of sorghum and millet consumption in human health

Sorghums and/or millet have shown potential to reduce the risks of cancer, obesity, diabetes, cardiovascular disease, and hyperlipidemia. These beneficial effects may be related to their phytochemical components and through multi-mechanisms such as reduction of oxidative stress or interactions with cell receptors that alter cell proliferation and apoptosis. This section will provide a brief summary of the potential health effects of sorghums and millets and possible biological mechanisms involved.

### 12.4.1 Cancer

The cell cycle is regulated by complex genes, some suppressing the division and some promoting it. Cancer results from unrestricted cell division due to the imbalance of these two types of regulatory genes, which is often caused by inactivation of genes suppressing the cell division such as p53. Specifically, carcinogenesis is a multistep process consisting of tumor initiation, promotion, and progression. Initiation is the conversion of a normal cell to a cancer cell due to gene mutation caused by carcinogens, the promotion is the proliferation of the tumor cell, and the progression is the further development of the tumor cells that cause metastasis. Limitless potential of growth and replication, self-sufficiency in growth signals, evading apoptosis, insensitivity to anti-growth signals, sustained angiogenesis, and tissue invasion and metastasis are the hallmarks of tumor cells. Many epidemiological studies have shown an inverse relationship between cancer incidence and consumption of vegetables and fruits, which are common sources of dietary phytochemicals. Thus, the mechanism of the anticancer effects of phytochemicals has become an important research area.

The anticancer function of phytochemicals with their high reactivity is mediated through multiple pathways during different stages of cancer development. Phytochemicals like ellagic acid, indole-3-carbinol, sulphoraphane, and flavonoids with a double bond between positions 2 and 3 in the C-ring can act at the initiation stage by activating intracellular detoxification and antioxidant enzymes (mainly phase II enzymes) to eliminate the carcinogens through biotransformation. After tumor initiation, phytochemicals like  $\beta$ -carotene, curcumin, and epigallocatechin gallate can act by slowing down the proliferation of the tumor cells through inactivating growth related signal transduction pathways such as ERK pathway or activating apoptosis related pathways such as JNK and p38 MAPK (Briviba *et al.*, 2002) to prevent the cell growth or to promote cell apoptosis. Recently, an oxidative breakage of DNA due to polyphenols, as a mechanism of anticancer, has been found in which the antioxidant polyphenols can form a ternary complex composed of DNA and  $\text{Cu}^{2+}$  in which the  $\text{Cu}^{2+}$  is reduced to  $\text{Cu}^+$  by polyphenols, and its reoxidation generates various ROS to break DNA to stop cell growth (Hadi *et al.*, 2007).

The reduced risk of cancer by sorghum consumption can be found as early as 1981 when van Rensburg reported that a low incidence of esophageal cancer in the world was correlated with sorghum consumption. Later, Chen *et al.* (1993) also reported similar findings in China. Grimmer *et al.* (1992) reported the anti-mutagenicity of sorghum polyphenol extracts when they were coincubated with mutants of *S. typhimurium* and other mutagens of sodium azide, daunomycin, and 2-aminofluorenet, and the high MW procyanidins (tannins, MW = 10,000–50,000 Da) fraction had the highest activity compared to low MW tannins. The mechanism of tannins' anticancer effect is probably related to cytochrome P-450, a protein that is capable of converting promutagens to mutagenic derivative as reported by Parbhoo *et al.* (1995). Shih *et al.* (2007) reported that the 3-deoxyanthoxyanins aglycons, apigeninidin, and luteolinidin, were more cytotoxic to human cancer cells than their anthocyanidin analogues. Recently, the anti-gastrointestinal cancer effect of 3-deoxyanthoxyanins (3-DXA) was investigated by Yang *et al.* (2009), and they found that 3-DXA can inhibit proliferation of the HT-29 human colon cancer cells and induce phase II enzymes of NAD(P)H:quinone oxidoreductase (NQO) that can protect cells from carcinogens and oxidant toxicity. Black sorghum extract is more



effective than white sorghum due to different compositions of 3-DXA in black sorghum extract. Specifically, methoxylation at position 7 or 5 of these 3-DXA molecules, particularly apigeninidin and luteolinidin, showed strong NQO induction activity, and methoxylation on the A-ring is as likely a prerequisite for their activity as enzyme inducer that was further evidenced by a synthetic double methylation at positions 5 and 7. This observation might be explained by an increased bioavailability as the lipophilicity of 3-DXA was increased when the -OH groups are converted to -OCH<sub>3</sub> groups leading to higher cellular absorption. It was also noted that flavone apigenin found in sorghum (Dykes *et al.*, 2009) showed strong activities to induce apoptosis of the HL-60 human leukemia tumor cells in an order of apigenin > quercetin > myricetin > kaempferol (Wang *et al.*, 1999). Apigenin also inhibited pancreatic tumor cell growth by inhibiting DNA synthesis, reducing the levels of cyclin A and B, phosphorylated forms of cdc2 and cdc25, which all are required for G2 to M stage transition indicating a G2/M phase arrest mechanism for inhibiting cell growth (Ujiki *et al.*, 2006). Recently, Awika *et al.* (2009) showed that the antiproliferative activity of sorghum extract in cancer cells was correlated with its phenol content, and tannin-containing sorghum extract was the most potent inhibitor of cell proliferation. However, the exact mechanism still needs investigation to better understand the structure–function relationship of sorghum flavonoids.

The effects of polyphenols on tumor cells may be mediated through telomerase repression to decrease the number of cell replications (Wang *et al.*, 2009), inhibiting Akt/mTOR signaling to suppress proliferation of tumor cells (Johnson *et al.*, 2009), ROS-related mitochondrial pathway for tumor cell apoptosis (Chen *et al.*, 2008), and direct modification of enzyme activity (Fang *et al.*, 2005). These are examples of anticancer mechanisms of commonly known polyphenols, and multiple anticancer mechanisms indicate that new methodologies, particularly the technologies developed in the -omics research (e.g. genomics, nutrigenomics, proteomics), are needed to connect the unique physicochemical properties of sorghum phytochemicals with gene expression/regulation and the cellular activity such as microarray to simultaneously analyze expression of multiple genes, proteomics to analyze the changes of key proteins and enzymes so that a deep understanding of the mechanism will be achieved. Another strategy is to examine the effect of various sorghum phytochemicals on the key molecular targets that have been identified from cancer genetics and cancer cell biology studies, such as topoisomerase II that is identified as a target for cancer treatment. From the perspective of phytochemicals, various phytochemicals may synergistically combat cancers such as the finding of Jo *et al.* (2005) that the flavonoid family of proanthocyanidin and anthocyanin from grape cell culture extract working together has the greatest effect. Thus, research on the interaction of different sorghum phytochemicals in anticancer also needs to be strengthened. Additionally, the effect of food processing on the bioactivities of these phytochemicals, and the bioavailability studies at cellular level (like ATP-binding cassette (ABC) transporters and cellular membrane property) are also needed to improve the efficiency of anticancer effects of sorghum phytochemicals. Furthermore, clinical trials need to be carried out in order to investigate the *in vivo* efficacy of phytochemicals as both tumor cells and normal cells are present simultaneously. The last intriguing point is the effect of phytochemicals on normal cells and tumor cells, which needs to be understood as it is well known that the fundamental difference between cancerous and normal cells is the unrestricted growth of the tumor cell. To what extent phytochemicals protect the normal cellular function and disrupt the tumor cell growth is likely important for understanding some ambiguous results from clinical trials related to cancer-preventive mechanisms.

### 12.4.2 Obesity

Obesity is becoming a prevalent problem in many countries worldwide, and is a risk factor for diabetes and cardiovascular diseases. Although there are predispositions to obesity in some populations due to their genetic backgrounds, the overconsumption of high energy foods and lack of physical activity are the major causes of obesity. Reducing body weight and preventing obesity have become the ultimate tasks for healthcare, research, and related industries, and reducing energy intake through diet is one essential route to prevention of obesity.

Sorghum and millet have long been known as low quality food materials due to their high content of tannins that were considered to be antinutritive materials that can form insoluble complexes with protein and carbohydrate, which reduce the digestibility of these nutrients leading to a lower nutritive value (Duodu *et al.*, 2003; Naczk and Shahidi, 1997). The mechanism of tannin binding protein is through hydrogen bonding and hydrophobic interaction, in which proline-rich proteins have high affinity for tannins. Additionally, phenolics also bind digestive enzymes such as amylases, trypsin, chymotrypsin, and lipases (Al-Mamary *et al.*, 2001) and brush-board anchored enzymes causing a decrease of enzyme activity and nutrient transportation leading to a reduction of nutrient absorption by the gastrointestinal tract. Chemically, tannins with high molecular weight (a high DP) are more effective in reducing nutrient bioavailability (Sarni-Manchado *et al.*, 1999). Many animal studies on the effect of tannins on body weight gain have suggested that there is a threshold to body weight gain reduction, and a high tannin content (2.7–3.5% CE) is needed to be effective. All these studies showed that there is strong potential to reduce or control body weight by consuming high tannin sorghum and millet. However, new technologies need to be developed to mask the astringent taste and to simultaneously maintain the nutrition value of diets caused by tannins with their inherent property of binding proteins and minerals that may lead to a decreased absorption of these nutrients.

Carbohydrate is the most abundant material in cereal food products, and reducing the bioavailability of carbohydrate using high tannin sorghum bran or its extract might be another possibility for applications in cereal-based food products. However, since tannins also affect absorption of some minerals (Afsana *et al.*, 2004), a systematic study is needed to reduce the energy availability and also maintain sufficient levels of other important nutrients. For providing sorghum itself, thick porridge is a common food in Africa with a satiety-maintaining property, which is related to carbohydrate that has a relatively lower digestibility compared to other cereals (Zhang and Hamaker, 1998). The combination of sorghum protein, starch, and tannins might slow the digestion of protein and starch so as to sustain the fullness of stomach.

### 12.4.3 Cardiovascular diseases

Cardiovascular disease (CVD) is a class of ailment that mainly involves heart and blood vessels related to atherosclerosis in which artery wall is thickened due to a build-up of fatty materials. Free radicals, particularly oxygen free radical species (ROS), and oxidized low-density lipoprotein molecules (LDL) are related to CVD. Thus, prevention of CVD needs to consider lowering levels of LDL cholesterol, triacylglycerols, and homocysteine, and increasing plasma antioxidant activity by higher dietary intake of antioxidants. The beneficial

effect of whole grain food in decreasing the CVD mortality from epidemiological data (Anderson, 2003) indicates that phytochemicals might play an important role in CVD prevention.

Reducing plasma LDL cholesterol concentration is a primary strategy for reducing CVD risk. The study of Carr *et al.* (2005) showed that lipid extract of sorghum grain significantly decreased the cholesterol absorption and plasma non-HDL cholesterol level in a dose-dependent manner using male hamsters as the animal model, and the decreased level of plasma non-HDL cholesterol was 18, 36, and 69% when 0.5, 1.0, and 5.0% lipid extract (by weight to diet), respectively, was used in the diet for four weeks compared to control diet. Liver cholesteryl ester concentration was also significantly reduced. The lipid extract from grain sorghum was analyzed to be sterols and policosanols. Phytosterols have a similar structure to cholesterol, so they might displace cholesterol in the intestine micelles and reduce the absorption of cholesterol (Ostlund, 2004, 2007). Policosanols are long chain alcohol, and can be oxidized to fatty acids to influence the cholesterol metabolism (Varady *et al.*, 2003) by reducing hepatic cholesterol biosynthesis while enhancing LDL clearance. Thus, sorghum lipid extract not only decreased the absorption of cholesterol, but also reduced the *in vivo* cholesterol synthesis. A combination of these two mechanisms should be important to a net reduction of non-HDL cholesterol, which is beneficial to CVD risk reduction.

The antioxidant compounds from sorghum and millet, including carotenoids and flavonoids, may influence the risk of CVD by preventing LDL oxidation and formation of foam cells.  $\alpha$ -Carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and other carotenoids showed beneficial effects on CVD (Voutilainen *et al.*, 2006). However, Osganian *et al.* (2003) reported that only a high intake of  $\alpha$ -carotene and  $\beta$ -carotene showed a significant inverse relationship with the risk for coronary artery disease (CAD), but no relationship was found for intake of other carotenoids such as lutein/zeaxanthin, lycopene, or  $\beta$ -cryptoxanthin after a 12-year follow-up human population study.

#### 12.4.4 Human melanoma

Melanoma is a malignant melanocytes tumor predominantly found in the skin with a high mortality rate, and ultraviolet (UV) radiation from sunlight exposure is one of the major etiologic agents in human melanoma (Yang *et al.*, 2009). UV radiation could cause DNA mutation, and a mutation of the tumor suppressor gene *PTEN* that is a key regulator for carcinogenesis is directly linked to melanoma in humans. There are around 60,000 new cases of invasive melanoma diagnosed in the US each year, especially for the people living in areas with higher UV levels. According to a WHO report, about 48 000 melanoma related deaths occurs worldwide each year. Currently, the most effective cure is early surgery to remove the tumor. The production of melanin in the melanocytes needs a tyrosinase (EC 1.14.18.1) enzyme that can oxidize tyrosine and certain diphenolic intermediates to quinones, which polymerize to become melanin. In a study on the effect of sorghum tannins on the activity of tyrosinase and melanogenesis (Gomez-Cordoves *et al.*, 2001), sorghum tannins increased melanogenic activity, but decreased the melanocyte colony formation at a high concentration (>40 mg/L) showing that sorghum tannins may be used as a therapeutic agent to treat melanoma. Actually, a high degree of polymerization of tannins can enhance their inhibitory activity on cell proliferation and induce apoptosis (Lizarraga *et al.*, 2008).

In another study on procyanidin from pine bark (Tourino *et al.*, 2005), procyanidin with different DPs showed mild inhibition of human melanoma cells with the monomer being the least efficient, also supporting the greater effectiveness of tannins with a high degree of polymerization. With the antioxidant activity and cell proliferation inhibition property, procyanidin is likely an excellent chemopreventive agent for skin cancer.

Although there are limited studies on the mechanism of preventing skin cancer by phytochemicals from sorghum and millet, research on the effect of green tea polyphenol (GTP) on melanoma showed that GTP can prevent photocarcinogenesis through IL-12-dependent DNA repair, activation of cytotoxic T cells and inhibition of angiogenesis in tumors (Mantena *et al.*, 2005). Since (–)-epigallocatechin-3-gallate (EGCG) is the main active component in GTP and the structural unit of proanthocyanidins that is enriched in sorghum polyphenols, it is reasonable to speculate that the sorghum polyphenols might work in a similar manner to prevent melanoma.

#### **12.4.5 Sorghum and millet bran consumption and risk of colon cancer**

The colon is the last part of the human gastrointestinal tract, starting from the cecum of the small intestine to anus, and its microbiota, which has billions of bacteria. The colon's significant components are intimately associated with human health, particularly colon cancer. Any food material that is not utilized in the small intestine may become the substrate for bacteria fermentation. For sorghum and millet bran, the indigestible component including hemicelluloses, cellulose, and some polyphenols become available for colon bacteria. Regarding the component of hemicelluloses and cellulose as dietary fiber, there have been extensive studies on its fermentation pattern and related colon health (Rose *et al.*, 2008), and the unabsorbed phytochemicals will be the focus of this section with a discussion on their metabolites by bacteria and the effect on colon cancer.

Sorghum and millet consumption through traditional African diets is associated with a low rate of colon cancer incidence in Africans, and the phytochemicals might be one of the factors contributing to this prevention, particularly the unabsorbed phytochemicals and their degradation products in the colon. Gu *et al.* (2007) studied sorghum tannins and observed that the total procyanidins and polymers disappeared progressively – and significant degradation occurred in the cecum and colon – and the predominant metabolites of procyanidins by gut microflora were phenolic acids that can be observed from urinary excretion and serum phenolic acid profiles, but depolymerization of procyanidins was not apparent throughout the GI tract. After sorghum bran consumption, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid, and 4-hydroxyphenylacetic acid dominated in the serum, and catechin and 3'-O-methylcatechin were dominant in the urine. Thus, the anticancer effect of phytochemicals of sorghum bran may be mediated by their direct effect on tumor cells or by the phenolic acids produced by gut microflora.

The anticancer effect of polyphenol metabolites from gut microflora has been demonstrated by the decrease of the reduced level of malonyldialdehyde (MDA) and related compounds as well as oxidative DNA damage (measured as 8-oxo-2-deoxyguanosine levels) in distal colon mucosa (Larrosa *et al.*, 2009). Similarly, Coates *et al.* (2007) showed that the “colon-available” raspberry polyphenols had significant protective effects against DNA damage induced by hydrogen peroxide in HT29 colon cancer cells and decreased the population of

HT29 cells in the G<sub>1</sub> phase of the cell cycle. For undigested polyphenols, Kim *et al.* (2006) showed that wine polyphenols had an apoptotic effect on the human colon cancer SNU-C4 cells by reducing the expression of *Bcl-2* and increasing the expression of *Bax* and *Caspase-3*. Treatment of Caco2 cell using pepsin- predigested chokeberry juice inhibited cell proliferation and viability, and caused changes of many genes related to DNA processing and transcription, cell signaling, apoptosis, cell cytoskeleton, RNA, protein translation, and transporting (Bermudez-Soto *et al.*, 2007). Thus, polyphenols from colonic fermentation or originally present could inhibit colon tumor cell growth. For sorghum polyphenols, 3-deoxyanthoxyanin (DXA) (Yang *et al.*, 2009) has been shown to inhibit the proliferation of colon HT-29 cells. However, studies of sorghum polyphenols on colon cancer are still limited in the literature, and even less for millet polyphenols.

It is well known that whole cereal grain consumption has been correlated with reduced risks of colon cancer. However, more studies of sorghum or millet consumption for colon cancer prevention are needed. Additionally, there have been extensive studies on the anticancer effect of dietary fibers, which is another factor that needs to be considered in fighting colon cancer, and to what extent fiber and phytochemicals contribute to the reduction of risk of colon cancer is not well understood. Dietary fibers and phytochemicals may synergistically contribute to colon cancer prevention, and the mechanism is likely an important research area related to food, gut microflora, and colon health.

## 12.5 Perspectives

Food innovation for human health is achieving a global consensus, and the important role of phytochemicals for human health has been established. Cereals, as the staple food, are a critical dietary component for human health, particularly the whole grain-based foods, whose health benefits have been widely accepted with evidence from numerous epidemiological studies. Sorghum and millet, as the major crops in semi-arid areas, are often considered as nutritionally inferior cereals. However, with the continuously increasing human population and the changes of the global climate, sorghum and millet with their unique drought-resistance and high level of phytochemicals will become important for combating growing health problems such as obesity, diabetes, cardiovascular diseases, and cancer. Although the high content of phytochemicals in sorghum is a fact, further investigations into these phytochemicals and their relation to human health, and an unraveling of the cellular and molecular mechanisms, are recommended. As for millet, although there have been good analyses of its phytochemicals and antioxidant property, more research into the effect of its consumption on human health is still needed. In addition, creating specialty sorghum and millet with particular phytochemicals is another important aspect for research. Finally, novel food processing techniques are required to promote sorghum and millet consumption.

## References

- Afsana, K., Shiga K., Ishizuka S., and Hara H. (2004) Reducing effect of ingesting tannic acid on the absorption of iron, but not zinc, copper and manganese by rats. *Bioscience, Biotechnology and Biochemistry*, **68**: 584–592.

- Al-Mamary, M., Al-habori M., Al-Aghbari A., and Al-Obeidi, A. (2001) In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research*, **21**: 1393–1401.
- Anderson, J.W. (2003) Whole grain protects against atherosclerotic cardiovascular disease, *Proceedings of the Nutrition Society*, **62**: 135–142.
- Ariga, T. and Hamano, M. (1990) Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxy radicals. *Agricultural and Biological Chemistry*, **54**: 2499–2504.
- Asharani V.T., Jayadeep A., and Malleshi, N.G. (2010) Natural antioxidants in edible flour of selected mill millets. *International Journal of Food Production*, **13**: 41–50.
- Avato, P., Bianchi, G., and Murelli, C. (1990) Aliphatic and cyclic lipid components of Sorghum plant organs. *Phytochemistry*, **29**: 1073–1078.
- Awika, J.M., Dykes, L., Gu, L., Rooney, L.W., and Prior, R.L. (2003a) Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry*, **51**: 5516–5521.
- Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., and Cisneros-Zevallo, L. (2003b) Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, **51**: 6657–6662.
- Awika, J.M. and Rooney L.W. (2004) Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, **65**: 1199–1221.
- Awika, J.M., Rooney, L.W., and Waniska, R.D. (2004) Properties of 3-deoxyanthocyanins from sorghum. *Journal of Agricultural and Food Chemistry*, **52**: 4388–4394.
- Awika, J.M., Yang, L., Browning, J.D., and Faraj, A. (2009) Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. *LWT (Food Science and Technology)*, **42**: 1041–1046.
- Bermudez-Soto, M.J., Larrosa, M., Garcia-Cantalejo, J., Espin, J.C., Tomas-Barberan, F.A., and Garcia-Conesa, M.T. (2007) Transcriptional changes in human Caco-2 colon cancer cells following exposure to a recurrent non-toxic dose of polyphenol-rich chokeberry juice. *Genes and Nutrition*, **2**: 111–113.
- Blessin, C.W., Dimler, R.J., and Webster, O.J. (1962) Carotenoids of corn and sorghum. II. Carotenoid loss in yellow-endosperm sorghum grain during weathering. *Cereal Chemistry*, **39**: 389–392.
- Bors, W., Michel, C., and Stettmaier, K. (2000) Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. *Archives of Biochemistry and Biophysics*, **374**: 347–355.
- Brandon, M.J., Foo, L.Y., Porter, L.J., and Meredith, P. (1982) Proanthocyanidins of barley and sorghum composition as a function of maturity of barley ears. *Phytochemistry*, **21**: 2953–2957.
- Briviba, K., Pan L., and Rechkammer G. (2002) Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. *Journal of Nutrition*, **132**: 2814–2818.
- Carr, T.P., Weller C.L., Schlegel, V.L., Cuppett, S.L., Guderian, D.M., and Johnson, K.R. (2005) Grain sorghum lipid extract reduces cholesterol absorption and plasma non-HDL cholesterol concentration in Hamsters. *Journal of Nutrition*, **135**: 2236–2240.
- Chandrasekara, A. and Shahidi F. (2010) Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry*, **58**: 6706–6714.
- Chen, F., Cole P., Mi Z.B., and Xing L.Y. (1993) Corn and wheat-flour consumption and mortality from esophageal cancer in Shanxi, China. *International Journal of Cancer*, **53**: 902–906.
- Chen, W., Zhao Z., Li L., Wu B., Chen S.F., Zhou H., Wang Y., and Li Y.Q. (2008) Hispolon induces apoptosis in human gastric cancer cells through a ROS-mediated mitochondrial pathway. *Free Radical Biology and Medicine*, **45**: 60–72.
- Coates, E.M., Popa, G., Gill, C.R., McCann, M.J., McDougal, G.J., Stewart, D., and Rowla, I. (2007) Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. *Journal of Carcinogenesis*, **6**: 4.
- Duodu, K.G., Taylor, J.R.N., Belton, P.S., and Hamaker, B.S. (2003) Factors affecting sorghum protein digestibility. *Journal of Cereal Science*, **38**: 117–131.
- Dykes, L., Rooney, L.W., Waniska, R.D., and Rooney, W.L. (2005) Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural Food Chemistry*, **53**: 6813–6818.
- Dykes L. and Rooney L.W. (2007) Phenolic compounds in cereal grains and their health benefits. *Cereal Foods Worlds*, **52**: 105–111.
- Dykes, L., Seitz, L.M., Rooney, W.L., and Rooney, L.W. (2009) Flavonoid composition of red sorghum genotypes. *Food Chemistry*, **116**: 313–317.

- Fang, J., Lu, J., and Holmgren, A. (2005) Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. *Journal of Biological Chemistry*, **280**: 25284–25290.
- Fernandez, M.G.S., Hamblin, M.T., Li, L., Rooney, W.L., Tuinstra, M.R., and Kresovich, S. (2008) Quantitative trait loci analysis of endosperm color and carotenoid content in sorghum grains. *Crop Science*, **48**: 1732–1743.
- Gomez-Cordoves, C., Bartolome, B., Vieira, W., and Virador, V.M. (2001) Effects of wine phenolics and sorghum tannins on tyrosinase activity and growth of melanoma cells. *Journal of Agricultural Food Chemistry*, **49**: 1620–1624.
- Grimmer, H.R., Parbhoo, V., and McGarth, R.M. (1992) Antimutagenicity of polyphenol-rich fractions from *Sorghum bicolor* grain. *Journal of Agricultural Food Chemistry*, **59**: 251–256.
- Gu, L., Kelm, M., Hammerstone, J.F., Beecher, G., Cunningham, D., Vannozzi, S., and Prior, L., (2002) Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *Journal of Agricultural Food Chemistry*, **50**: 4852–4860.
- Gu, L., House, S.E., Rooney, L., and Prior, R.L. (2007) Sorghum bran in the diet dose dependently increased the excretion of catechins and microbial-derived phenolic acids in female rats. *Journal of Agricultural Food Chemistry*, **55**: 5326–5334.
- Gujer, R., Magnolato, D., and Self, R. (1986) Glucosylated flavonoids and other phenolic compounds from sorghum. *Phytochemistry*, **25**: 1431–1436.
- Gupta, R.K. and Haslam, E. (1978) Plant proanthocyanidins. 5. Sorghum polyphenols. *Journal of the Chemical Society, Perkin Transactions*, **14**: 892–896.
- Hadi, S.M., Bhat, S.H., Azmi, A.S., Hanif, S., Shanmim, U., and Ullah, M.F. (2007) Oxidative breakage of cellular DNA by plant polyphenols: a putative mechanism for anticancer properties. *Seminars in Cancer Biology*, **17**: 370–376.
- Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., and Riechel, T.K. (1998) High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural Food Chemistry*, **46**: 1887–1892.
- Hahn, D.H. and Rooney L.W. (1986) Effect of genotype on tannins and phenols of sorghum. *Cereal Chemistry*, **63**: 4–8.
- Hahn, D.H., Rooney L.W., and Earp C.F. (1984) Tannins and phenols of sorghum. *Cereal Foods World*, **29**: 776–779.
- Hahn, D.H., Faubion, J.M., and Rooney, L.W. (1983) Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chemistry*, **60**: 255–259 .
- Herrmann, K.M. and Weaver, L.M. (1999) The shikimate pathway. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**: 473–503.
- Hilu, K.W., De Wet, J.M.J., and Seigler, D. (1978) Flavonoid patterns and systematics in Eleusine. *Biochemical Systematics and Ecology*, **6**: 247–249.
- Hoshino, T., and Duncan, R.R. (1982) Sorghum tannin content during maturity under different environmental conditions. *Japanese Journal of Crop Science*, **51**: 178–184.
- Jaldappagar, S., Motohashi, N., Gangeenahalli, M.P., and Naismith, J.H. (2008) Bioactive mechanism of interaction between anthocyanins and macromolecules like DNA and proteins. *Top Heterocyclic Chemistry*, **15**: 49–65.
- Jo, J.-Y., de Mejia, E.G., and Lila, M.A. (2005) Effects of grape cell culture extracts on human topoisomerase ii catalytic activity and characterization of active fractions. *Journal of Agricultural Food Chemistry*, **53**: 2489–2498.
- Johnson, S.M., Gulhati, P., Arrieta, I., Wang, X., Uchida, T., Gao, T., and Evers, B.M. (2009) Curcumin inhibits proliferation of colorectal carcinoma by modulating Akt/mTOR signaling. *Anticancer Research*, **29**: 3185–90.
- Kean, E.G., Ejeta, G., Hamaker, B.R., and Ferruzzi, M.G. (2007) Characterization of carotenoid pigments in mature and developing kernels of selected yellow-endosperm sorghum varieties. *Journal of Agricultural Food Chemistry*, **55**: 2619–2626.
- Kim, M.J., Kim, Y.J., Park, H.J., Chung, J.H., Leem, K.H., and Kim, H.K. (2006) Apoptotic effect of red wine polyphenols on human colon cancer SNU-C4 cells. *Food and Chemical Toxicology*, **44**: 898–902.
- Krueger, C.G., Vestling, M.A., and Reed, J.D. (2003) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum (*Sorghum bicolor* L.) Moench. *Journal of Agricultural Food Chemistry*, **51**: 538–543.

- Larrosa, M., Luceri, C., Vivoli, E., Pagliuca, C., Lodovici, M., Moneti, G., and Dolara, P. (2009) Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Molecular Nutrition and Food Research*, **53**: 1044–1054.
- Leguizamón, C., Weller, C.L., Schlegel, V.L., and Carr, T.P. (2009) Plant sterols and policosanols characterization of hexane extracts from grain sorghum, corn and their DDGS. *Journal of the American Oil Chemists' Society*, **86**: 707–716.
- Lizarraga, D., Lozano, C., Tourino, S., Centelles, J.J., Torres, J.L., and Cascante, M. (2008) Polymerization and galloylation: two important aspects for antiproliferative properties of procyanidin-rich natural extracts. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **7**: 3343–3347.
- Manach, C., Williamson, G., Morand, C., Scalbert, A., and Remesy, C. (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, **81**(Suppl.): 230S–242S.
- Mantena, S.K., Meeran, S.M., Elmetts, C.A., and Katiyar, S.K. (2005) Orally administered green tea polyphenols prevent ultraviolet radiation-induced skin cancer in mice through activation of cytotoxic T cells and inhibition of angiogenesis in tumors. *Journal of Nutrition*, **135**: 2817–2877.
- McGraw, K.J., Hill, G.E., Stradi, R., and Parker, R.S. (2001) The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiological and Biochemical Zoology*, **74**: 843–852.
- Naczki, M. and Shahidi, F. (1997) Nutritional implications of canola condensed tannins, *Antinutrients and Chemicals in Food: ACS Symposium Series*, **662**: 186–208.
- Osganian, S.K., Stampfer, M.J., Rimm, E., Spiegelman, D., Manson, J.A.E., and Willett, W.C. (2003) Dietary carotenoids and risk of coronary artery disease in women. *American Journal of Clinical Nutrition*, **77**: 1390–9.
- Ostlund, R.E. Jr. (2004) Phytosterols and cholesterol metabolism. *Current Opinion in Lipidology*, **15**: 37–41.
- Ostlund, R.E. Jr. (2007) Phytosterols, cholesterol absorption and healthy diets. *Lipids*, **42**: 41–45.
- Parbhoo, V., Grimmer, H.R., Cameron-Clarke, A., and McGarth, R.M. (1995) Induction of cytochrome P-450 in rat liver by a polyphenol-rich extract from a bird-resistant sorghum grain. *Journal of the Science of Food and Agriculture*, **69**: 247–252.
- Ragaee, S., Abdel-Ala, E.M., and Noaman, M. (2006) Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, **98**: 32–38.
- Ramachandra, G., Virupaksha, T.K., and Shadaksharaswamy, M. (1977) Relationship between tannin levels and in vitro protein digestibility in finger millet (*Eleusine caracana* Gaertn.). *Journal of Agricultural Food Chemistry*, **25**: 1101–1104.
- Reed, J.D., Krueger, C.G., and Vesting, M.M. (2005) MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry*, **66**: 2248–2263.
- Reichert, R.D. (1979) The pH-sensitive pigments in pearl millet. *Cereal Chemistry*, **56**: 291–294.
- Rice-Evens, C.A., Miller, N.J., and Paganga, G. (1996) Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **20**: 933–956.
- Rose, D.J., DeMeo, M.T., Keshavarzian, A., and Hamaker, B.R. (2008) Influence of dietary fiber on inflammatory bowel disease and colon cancer: importance of fermentation pattern. *Nutritional Review* **65**: 51–62.
- Sarni-Manchado, P., Cheyrier, V., and Moutounet, M. (1999) Interactions of grape seed tannins with salivary proteins. *Journal of Agricultural Food Chemistry*, **47**: 42–47.
- Sartelet, H., Serghat, S., Lobstein, A., Ingenbleek, Y., Petitfrere, E., Aguié-Aguie, G., Martiny, L., and Haye, B. (1996) Flavonoids extracted from Fonio millet (*Digitaria exilis*) reveal potent antithyroid properties. *Nutrition* **12**: 100–106.
- Shih, C.H., Siu, S.O., Ng, R., Wong, E., Chiu, L.C.M., Chi, I.K., and Lo, K. (2007) Quantitative analysis of anticancer 3-deoxyanthocyanidins in infected sorghum seedlings. *Journal of Agricultural Food Chemistry*, **55**: 254–259.
- Singh, V., Moreau, R.A., and Hicks, K.B. (2003) Yield and phytosterol composition of oil extracted from grain sorghum and its wet-milled fractions. *Cereal Chemistry*, **80**: 126–129.
- Sun, T. and Ho Chi-Tang. (2008) Total phenolic content and antioxidant activity of cereals. Chapter 13, pp. 143–150. In *Functional Food and Health*. ACS symposium Series Vol. **993**.
- Slabbert, N. (1992) Complexation of condensed tannins with metal ions. In *Plant Polyphenols: Synthesis, Properties, Significance*, Hemingway, R.W., Laks, P.E., (eds). Plenum Press: New York, pp. 421–436.



- Tourino S., Selga, A., Jimenez, A., Julia, L., Lozano, C., Lizarraga, D., Cascante, M., and Torres, J.L. (2005) Procyanidin fractions from pine (*Pinus pinaster*) bark: radical scavenging power in solution, antioxidant activity in emulsion, and antiproliferative effect in melanoma cells. *Journal of Agricultural Food Chemistry*, **53**: 4728–4735.
- Udayasekhara Rao, P. and Deosthale, Y.G. (1988) In vitro availability of iron and zinc in white and coloured ragi (*Eleusine coracana*): role of tannin and phytate. *Plant Foods for Human Nutrition*, **38**: 35–41.
- Ujjiki, M.B., Ding, X-Z., Salabat, M.R., Bentrem, D.J., Golkar, L., Milam, B., Talamonti, M.S., Bell, Jr R.H., Iwanamura, T., and Adrian, T.E. (2006) Apigenin inhibits pancreatic cancer cell proliferation through G2/M cell cycle arrest. *Molecular Cancer*, **5**: 76.
- van Rensburg, S.J. (1981) Epidemiological and dietary evidence for a specific nutritional predisposition to esophageal cancer. *Journal of the National Cancer Institute*, **67**: 243–251.
- Varady, K.A., Wang, Y., and Jones, P.J.H. (2003) Role of policosanols in the prevention and treatment of cardiovascular disease. *Nutritional Review*, **61**: 376–383.
- Viswanath, V., Urooj, A., and Malleshi, N.G. (2009) Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*). *Food Chemistry*, **114**: 340–346.
- Voutilainen, S., Nurmi, T., Mursu, J., and Rissanen, T.H. (2006) Carotenoids and cardiovascular health. *American Journal of Clinical Nutrition*, **83**: 1265–1271.
- Wang, I.K., Lin-Shiau, S.Y., and Lin, J.K. (1999) Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase-9 and caspase-3 in leukaemia HL-60 cells. *European Journal of Cancer*, **35**: 1517–1525.
- Wang, X., Hao, M.W., Dong, K., Lin, F., Ren, J.H., and Zhang, H.Z. (2009) Apoptosis induction effects of EGCG in laryngeal squamous cell carcinoma cells through telomerase repression. *Archive of Pharmacal Research*, **32**: 1263–9.
- Wilkinson, W.S., Barbee, C., and Knox, F.E. (1968) Nutrient content of dehydrated coastal Bermuda grass and pearl millet. *Journal of Agricultural Food Chemistry*, **16**: 665–668.
- Wu, X. and Prior, R.L. (2005) Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts, and grains. *Journal of Agricultural Food Chemistry*, **53**: 3101–3113.
- Yang, L., Browning, J.D., and Awika, J.M. (2009) sorghum 3-deoxyanthocyanins possess strong phase ii enzyme inducer activity and cancer cell growth inhibition properties. *Journal of Agricultural Food Chemistry*, **57**: 1797–1804.
- Zhang, G. and Hamaker, B.R. (1998) Low  $\alpha$ -amylase starch digestibility of cooked sorghum flour and the effect of protein. *Cereal Chemistry*, **75**: 710–713.

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# 13 Nutraceutical and health properties of common beans (*Phaseolus vulgaris*)

Hoda Kadouh and Kequan (Kevin) Zhou

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## 13.1 Introduction

The common bean (*Phaseolus vulgaris* L.), an herbaceous annual legume, is among the world's oldest cultivated crops (Mensack *et al.*, 2010). The cultivated common bean is a genetically diverse plant of which several races are known to exist, originating from Middle America and Andean South America (Singh *et al.*, 1991). The bean has evolved from a wild-growing vine into a major dietary legume no longer restricted to these areas (Schoonhoven and Voysest, 1991). Like other legumes, it can acquire nitrogen through mutualistic symbiosis (Doyle and Luckow, 2003). Its varieties are known as nitrogen-fixing plants nodulated by a range of rhizobial strains, thus serving as a major source of protein for certain populations, namely Latin Americans (Vásquez-Arroyo *et al.*, 1998). Today, dry and green beans are produced worldwide with a geographically diverse list of top production sites (FAO, 2009). Approximately 18 million metric tons were produced globally in 2008, rendering the common bean the most important economic variety of the genus *Phaseolus* (Yang *et al.*, 2010). Indeed, among the food legume crops known as pulses, it is the most extensive and widely consumed (Blair *et al.*, 2009). Common varieties of *P. vulgaris* include black beans, white beans, pinto beans, and kidney beans, which are considered to be primary dietary protein sources in many developing countries.

## 13.2 Health beneficial effects of *Phaseolus vulgaris*

Consumption of common bean extract has been linked to the amelioration of signs of chronic diseases, particularly diabetes and obesity (Preuss, 2009). Hypocholesterolemic activities of the dietary common bean and its effects on the reduction of coronary heart disease risk have also been reported (Anderson *et al.*, 1984). Moreover, *P. vulgaris* was shown to exert anti-carcinogenic effects in animal models (Hughes *et al.*, 1997). These benefits associated with

common bean consumption may be attributable to the presence of multiple functional components in the bean, such as diverse phytochemicals including phenolic compounds (Cardador-Martínez *et al.*, 2002), dietary fiber (Hughes, 1991), and alpha-amylase inhibitor(s) (Le Berre-Anton *et al.*, 1997). Phenolic compounds are known to possess both anti-carcinogenic and antioxidant properties (Hollman and Katan, 1999). Antioxidants are strong scavengers of free radicals and reactive oxygen species and play a vital role in inhibiting oxidative mechanisms that lead to chronic diseases (Koleckar *et al.*, 2008). Thus, we start with natural antioxidants in the common bean.

### 13.2.1 Natural antioxidants in the common bean

Multiple studies have revealed a high concentration of antioxidants in different common bean varieties and compartments, as shown in this section. Antioxidants in the common bean are chiefly concentrated in seed coats (Cardador-Martínez, Loarca-Piña, and Oomah, 2002). Total phenolic contents of hull, whole, and dehulled beans were computed and ranged from 0.6 to 78.2 mg of catechin equivalents per gram of sample (Cardador-Martínez *et al.*, 2002). Bean hulls appeared to have a much higher phenolic content and antioxidant activity than their corresponding whole beans (Oomah *et al.*, 2010). For instance, the manually separated hull displayed 37-fold greater phenolic content than the whole bean flour and dehulled beans (Cardador-Martínez *et al.*, 2002). Most antioxidants in the common bean are phenolic compounds, including quercetin, kaempferol, flavonols, daidzein, anthocyanins, and tannins (Akond *et al.*, 2011; Aparicio-Fernandez *et al.*, 2005; Beninger and Hosfield, 2003). Quercetin and kaempferol contents in the whole bean ranged from 6.9–23.5 and 13.8–204 µg/g bean, respectively (Díaz-Batalla *et al.*, 2010). Four phenolic acids were also detected in the common bean including *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid with ferulic acid being predominant, ranging from 17 to 36 µg/g bean (Díaz-Batalla *et al.*, 2006). Anthocyanin and polyphenol content varies within different genotypes of common bean, which corresponds to varying degrees of antioxidant activity (Akond *et al.*, 2011). Mutants enriched with antioxidants have been produced. For instance, NaN<sub>3</sub>-induced mutants were shown to accumulate significantly higher amounts of total phenols, anthocyanins, and preanthocyanidins and, therefore, higher antioxidant capacity when compared to the classic common bean (Jeng *et al.*, 2010). It is also worth pointing out that different treatments of bean products may also influence their antioxidant capacity. For instance, tempeh flour obtained from fermentation of common bean flour exhibits higher antioxidant and antiradical activity as well as higher phenol content than regular unfermented bean flour (Reyes-Bastidas *et al.*, 2010).

These bioactive ingredients drive a variety of beneficial health effects. For instance, tannins, which are mostly found in red-colored beans (Reed, 1995), are polyphenolic compounds with diverse bioactivities such as anti-inflammatory, antiproliferative, and antimicrobial functions (Dos Santos *et al.*, 2006; Taguri *et al.*, 2006; Matito *et al.*, 2003). They are acknowledged antioxidants with free radical-scavenging capacity, metal-chelating activity, and an ability to inhibit lipid peroxidation and prooxidative enzymes (Koleckar *et al.*, 2008). Anthocyanins, another class of antioxidants found in beans, are pigments that do not only color the inhabited beans but also possess antioxidant properties that further lead to secondary health benefits such as anti-carcinogenic and anti-inflammatory functions in addition to beneficial roles in the management of diabetes and obesity and the prevention of cardiovascular disease (He and Giusti, 2010). Last but not least, flavonols in beans have been shown to have an antioxidant effect as well as a protective

**Table 13.1** TDF content (% seed weight or g TDF in 100g) in selected cooked common beans (*Phaseolus vulgaris*)

Common bean	TDF (%)	References
Black bean	48.1, 10.97	Campos-Vega <i>et al.</i> , 2009, Panlasigui <i>et al.</i> , 1995
White bean	15.44	Panlasigui <i>et al.</i> , 1995
Kidney bean	45.4	Kanaya <i>et al.</i> , 2007
Pinto bean	36.5	Campos-Vega <i>et al.</i> , 2009
French bean	24.45	Khatoon & Prakash, 2004
Carilla	25.2	Martin-Cabrejas <i>et al.</i> , 2004

TDF=total dietary fiber.

effect against chronic disorders (Hollman and Katan, 1999) such as vascular disease (Woodman and Chan, 2004) and some cancers (Nothlings *et al.*, 2007; Hirvonen *et al.*, 2001).

### 13.2.2 Dietary fiber in the common bean

The dry common bean is rich in both soluble and insoluble fiber (Hughes, 1991). Dietary fiber is known to be associated with a number of health benefits, especially in relation to cardiovascular disease and digestive disorders. Interestingly, the health beneficial effects of fiber seem to be modulated by its coupling with phytochemicals in most food sources (Slavin and Jacobs, 2010). This is likely true in the case of *Phaseolus vulgaris*. Studies testing the content of different nutrients in beans have found considerable amounts of dietary fiber in different types of common bean. According to the results summarized in Table 13.1, the total dietary fiber content in a variety of processed beans ranges between 10.97 and 48.1% of the total weight of the bean, suggesting that these legumes are an important source of dietary fiber.

Research has rendered adequate fiber intake an imperative factor in preventing and managing chronic diseases. Higher intakes of dietary fiber in general and soluble fiber specifically were associated with lower risks for coronary heart disease in an adult population that participated in the NHANES I study (Bazzano *et al.*, 2003). Soluble fiber intake of 10–15 g daily was shown to lower blood total cholesterol and LDL-cholesterol fraction levels (Brown *et al.*, 1999) and soluble fibers that are suggested to have a cholesterol-lowering effect are found in a variety of food sources including beans (Marlett, 2001). Furthermore, higher intakes of dietary fiber have been proven directly proportional to a reduction in blood pressure (Streppel *et al.*, 2005; Whelton *et al.*, 2005).

Several studies agree that increasing the intake of dietary fiber (soluble or insoluble) induces an increase in postprandial satiety and a reduction in subsequent hunger. This may, therefore, contribute to body weight reduction (Howarth *et al.*, 2001). A review of literature indicated that the physical and chemical properties of different types of dietary fiber evoke a significant control of energy intake via increasing satiety, satiation, and gastric fullness and aiding with compliance to reduced calorie diets (Burton-Freeman, 2000).

Viscous fiber in the diet is thought to aid in blood glucose control by slowing the rates of gastric emptying, digestion, and glucose absorption (Slavin, 2008). Studies have declared beneficial effects of fiber in controlling both type 1 and type 2 diabetes. Giacco *et al.* revealed an evident improved glycemic control in type 1 diabetic patients following a six-month ingestion of 50 g of dietary fiber daily (Giacco *et al.*, 2000). The effect of dietary fiber in controlling type 2 diabetes has also long been established. It was reported

in 1981 that high fiber intake assists with blood sugar control in type 2 diabetics and reduces insulin demand in these patients (Simpson *et al.*, 1981).

Of particular significance it has been proposed that dietary fiber consumption aids in the prevention of certain cancers. For instance, several prospective cohort studies have established an inverse relationship between fiber intake and the development of colorectal cancer (Park *et al.*, 2005). Similarly, an inverse relationship was observed between fiber intake and the risk of developing breast cancer in post-menopausal women in a long-term study (Park *et al.*, 2009).

Legumes, including the common bean, were suggested to be considered as functional foods due to their cholesterol-lowering effect, which is likely attributable to their high fiber content (Trinidad *et al.*, 2010).

### 13.2.3 Anti-hyperglycemic effect

The hypoglycemic effect of the common bean has long been established and, in fact, bean pods are known as traditional remedies for diabetes mellitus (Helmstadter, 2010). Several animal studies have been published (presented below); however, there is a lack of human studies where a pure extract of the bean (or the whole bean itself) is administered and tested against signs of diabetes, namely hyperglycemia. As early as 1987, administration of a common bean complex to an experimental rabbit model of diabetes was followed by a significant reduction in glycemia (Khaleeva *et al.*, 1987). When tested on healthy rabbits, gastric administration of a *Phaseolus vulgaris* preparation weekly over four weeks was associated with a decrease in hyperglycemic peak and the area under the glucose tolerance curve following dextrose infusion, suggesting an anti-hyperglycemic effect of the plant (Roman-Ramos *et al.*, 1995). An aqueous common bean extract administered orally to diabetic rats was associated with a remarkable reduction in blood glucose levels (Venkateswaran *et al.*, 2002) and improvement of other blood diabetes parameters and hepatic carbohydrate enzyme levels. This effect was significant even when compared to an antihyperglycemic agent that belongs to the sulfonylurea class of oral diabetes medications (Pari and Venkateswaran, 2003). Animal research in the field has continued to be conducted over the last five years. Tormo *et al.*, for instance, administered a purified pancreatic alpha-amylase inhibitor (alpha-AI) extracted from white beans to diabetic rats and healthy controls and reported that glycemia was significantly reduced in response to treatment, not only in the diabetic group but also in the non-diabetic animals (Tormo *et al.*, 2006). Shortly after, Preuss *et al.* found that the ingestion of a dry bean (*P. vulgaris*) extract before and during both a sucrose and a starch challenge was associated with a significant reduction in blood glucose concentration in experimental diabetic rats (Preuss *et al.*, 2007). In healthy rats, a reduction in glycemia was also seen when a starch-rich diet was accompanied by daily supplementation with an alpha-AI-containing *P. vulgaris* dry extract for ten days (Fantini *et al.*, 2009).

Table 13.2 summarizes results from the above animal studies investigating the anti-hyperglycemic effects of *P. vulgaris* extracts. It is evident that the hypoglycemic effect is more powerful in diabetic than in healthy animals. These findings are promising; however, this is a limited body of evidence. In fact, further investigation is required and clinical studies are needed to establish a solid relationship between common bean intake and the regulation of glycemia. Only a few human studies examining the hypoglycemic and/or the carbohydrate absorption-reducing effect of common beans have been published to date.

**Table 13.2** Hypoglycemia effects of *Phaseolus vulgaris* extract in animal models

Animal model	<i>P. vulgaris</i> extract	Duration	Serum/Plasma glucose concentration decrease (%)		Serum/Plasma insulin concentration change (%)		References
			Cmax	AUC	Cmax*	AUC*	
Wistar rats	500 mg/kg/day	10 days	20 <sup>2</sup>	NR	ND	ND	Fantini <i>et al.</i> , 2009
STZ-D rats	200 mg/kg/day	45 days	62.2 <sup>1,2</sup>	NR	+59.6 <sup>1,2</sup>	NR	Pari <i>et al.</i> , 2003
STZ-D rats	100 mg/kg/day	22 days	55.4 <sup>1</sup>	NR	-42.8	NR	Tormo <i>et al.</i> , 2006
Sprague-Dawley rats	0.5 g × 2/day	4 hours	NR	60 <sup>1,2</sup> /49 <sup>1,2</sup>	ND	ND	Preuss <i>et al.</i> , 2007
New Zealand rabbits	4 mL/kg (×4)	4 weeks	22.6 <sup>1,2</sup>	20.8 <sup>1,2</sup>	ND	ND	Roman-Ramos <i>et al.</i> , 1995

<sup>1</sup> Statistical significance was P < 0.05.

<sup>2</sup> Compared to placebo/control.

Cmax, maximum serum/plasma glucose concentration; AUC, area under glucose curve; Cmax\*, maximum serum/plasma insulin concentration; AUC\*, area under insulin curve.

NR = not reported;

ND = not determined;

STZ-D = Streptozotocin-induced diabetic.

When compared to bread, a 50 g carbohydrate-equivalent serving of cooked common bean caused a significantly lower glycemic response in healthy subjects receiving a carbohydrate-rich diet, represented by the area under the glucose curve of 82.5% (Panlasigui *et al.*, 1995). *Phaseolus vulgaris* seems to demonstrate its antihyperglycemic activity through an  $\alpha$ -amylase inhibitor that has been identified. Kidney bean seeds were found to contain two isoforms of the inhibitor (major form Alpha-AI) exhibiting a mixed non-competitive human pancreatic  $\alpha$ -amylase inhibition activity (Le Berre-Anton *et al.*, 1997). The white kidney bean extract containing alpha-AI may serve as an agent to prevent or ameliorate “diabesity” through its glycemia-reducing ability as well as its calorie-restricting effect where 1 g of Phase 2 starch neutralizer (which inhibits  $\alpha$ -amylase; previously known as Phaseolamin 2250) would inhibit 2250 calories from starch. The extract is recognized as safe for human consumption (Preuss, 2009).

Babish *et al.* recently found the common bean to be among a range of botanical products that are associated with an increase in lipogenesis in differentiating adipocytes, an activity similar to that exhibited by troglitazone, an anti-diabetic pharmaceutical agent (2010). This in-vitro trial partly explains the anti-diabetic role *Phaseolus vulgaris* is thought to play.

### 13.2.4 Weight loss

The potential weight-reducing effect of the common bean has been investigated by a few groups of researchers over the last decade. Even though several trials have utilized herbal preparations including, but not limited to, *Phaseolus vulgaris*, a few randomized, double-blinded, placebo-controlled trials involving the common bean as the only functional ingredient have been published. In a study employing 60 overweight subjects, a 450 mg dose of common bean extract previously identified as  $\alpha$ -amylase inhibitor was given to the treatment group as one tablet per day for 30 days before a carbohydrate-rich meal. These individuals experienced a significant reduction in body weight, fat mass, and anthropometric measures as compared to those receiving placebo, while maintaining lean body mass. In 30 days, they experienced average reductions of 6.45 lbs in their body weight, 10.45% in their body fat, 2.93 cm in their waist circumference and 1.48 cm in their hip circumference, where as the corresponding reductions in the placebo group were 0.77 lbs, 0.16%, 0.46 cm, and 0.11 cm, respectively (Celleno *et al.*, 2007). Udani *et al.* conducted two studies, three years apart, observing the effect of common bean extract on carbohydrate absorption and weight loss. In the first study consisting of 27 participants, obese adults received 1500 mg of Phase 2 twice a day with meals, while consuming a high fiber, low fat diet that provides 100–200 g of carbohydrates daily. After eight weeks, the results demonstrated that these subjects lost an average of 3.79 lbs while individuals receiving placebo lost 1.65 lbs. This difference, however, was not statistically significant. The mentioned dose appeared to be safe as no adverse effects were seen (Udani *et al.*, 2004). In the second study consisting of 25 participants, healthy subjects received 1000 mg of a white bean extract twice daily before meals, while following a weight loss program consisting of diet and exercise. After four weeks, they lost an average of 6 lbs while those receiving placebo lost 4.7 lbs. This difference was not statistically significant. However, when the subjects were grouped by total carbohydrate intake from the diet, a statistical significance was seen in weight loss between the recipients of bean extract (8.7 lbs) and the recipients of placebo (1.7 lbs), in the group consuming the most carbohydrates (Udani and Singh, 2007). These results support the

possibility of considering *Phaseolus vulgaris* extract among the natural food sources that potentially aid in weight loss. However, given the limited amount of literature and the lack of statistical significance in some of the weight loss data, longer term clinical studies involving a large amount of subjects must be pursued to establish a solid basis for the utilization of this seed and/or its extracts in weight loss programs. Moreover, it would be beneficial if the effect of the common bean (or extract) would be compared to that of a widely-used weight loss pharmaceutical product, taking not only weight loss, but also safety and adverse effects into consideration.

### 13.2.5 Cardiovascular disease prevention

Studies conducted over the last three decades establish a protective effect of *Phaseolus vulgaris* against cardiovascular disease by assuming a hypolipidemic role. Venkateswaran *et al.* found in the study cited previously that these rats experienced a significant reduction in circulating blood lipids in response to common bean intake for 45 days, namely total cholesterol, very-low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, serum triglycerides, free fatty acids, and phospholipids (Venkateswaran *et al.*, 2002). Pari and Venkateswaran again verified that this treatment had a hypolipidemic effect in these animals and prevented the fatty acid changes that are usually triggered by diabetes (Pari and Venkateswaran, 2004). Rosa *et al.* had previously suggested that hypercholesterolemic rats fed whole beans may experience a reduction in cholesterol; however, it is likely retained in enterohepatic circulation (1998). On the other hand, these rats had a higher cholesterol output when given beans without hulls (Rosa *et al.*, 1998). In 1989, Shutler *et al.* found that the consumption of the baked bean was associated with a reduction of total blood cholesterol in young male adult subjects, which may be related to the reduction in fat intake when consuming a bean-rich diet (Shutler *et al.*, 1989).

### 13.2.6 Cancer prevention

A few animal studies connecting common bean intake with cancer prevention have been published. Hangen and Bennink found that rats consuming common beans had a lower incidence of lab-induced colon cancer and significantly less tumor multiplicity than their controls (2002). Another study later suggested that the ingestion of a polysaccharide extract from the common bean was inversely related to the development of the same cancer in rats, likely due to the production of short chain fatty acids in the colon, namely butyrate (Feregrino-Perez *et al.*, 2008).

With the known richness of *Phaseolus vulgaris* in antioxidants and the generally known protective effects of the latter against cancer, it is not surprising that common beans may have some chemopreventive functions; however, evidence-based information is still needed and this is only attainable through studies, most importantly clinical studies. Further investigations would establish a clear relationship between the common bean and cancer, as well as the components responsible for this relationship, given the diversity of functional compounds in a bean. For instance, haemagglutinins have been of interest and they have been tested in *in vitro* studies yielding promising results over the last decade. Wong *et al.*, for example, recently purified a haemagglutinin from Japanese Hokkaido



red beans and found that it demonstrated a strong anti-proliferative effect on human hepatoma cells. However, this compound is not stable at temperatures above 90 °C (Wong *et al.*, 2010).

### 13.3 Possible adverse effects

Several varieties of common bean are a source of the lectin phytohaemagglutinin which, in high doses, is a toxic compound as it alters cell metabolism, thus causing reversible poisoning. This, however, can be prevented by soaking and cooking the beans at high temperature where most of the compound is lost or destroyed (FDA, 2009). On the other hand, phytohaemagglutinin extracted from beans is medically used as a mitogen to enhance T-lymphocyte cell division (Marko *et al.*, 2010) and may be a potent inhibitor of cancer cell proliferation as shown above.

### 13.4 Conclusion

The common bean, *Phaseolus vulgaris*, is indeed not only a dietary element that provides basic nutrition, but also a functional food item that potentially influences health and well-being. As aforesaid it may be beneficial for managing diabetes, preventing cardiovascular disease, preventing cancers, and providing dietary fiber and a variety of antioxidants that likely contribute to the mentioned functions and provide additional health advantages. Given these benefits, in addition to the known nutritional value of common beans, it may be useful to include them in abundance in the diet and encourage their consumption. Nevertheless, the amount of available literature is very limited and more studies are needed, namely clinical studies to establish a strong relationship and develop guidelines for the human diet. Governmental and academic institutions can easily invest in studies involving this crop that is widely abundant, relatively inexpensive, and generally safe to consume.

### References

- Akond, G.M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., and Hossain, K. (2011) Anthocyanin, total polyphenols and antioxidant activity of common bean. *American Journal of Food Technology*, **6**: 385–394.
- Anderson, J., Story, L., Sieling, B., Chen, W., Petro, M., and Story, J. (1984) Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *The American Journal of Clinical Nutrition*, **40**: 1146–1155.
- Aparicio-Fernandez, X., Manzo-Bonilla, L., and Loarca-Pina, G.F. (2005) Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B1. *Journal of Food Science*, **70**: S73–S78.
- Bazzano, L.A., He, J., Ogden, L.G., Loria, C., Vupputuri, S., Myers, L., and Whelton, P.K. (2001) Legume consumption and risk of coronary heart disease in US men and women: NHANES I Epidemiologic Follow-up Study. *Archives of Internal Medicine*, **161**: 2573–2578.
- Bazzano, L.A., He, J., Ogden, L.G., Loria, C.M., and Whelton, P.K. (2003) Dietary fiber intake and reduced risk of coronary heart disease in US men and women – The National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Archives of Internal Medicine*, **163**: 1897–1904.

- Beninger, C.W., and Hosfield, G.L. (2003) Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of Agricultural and Food Chemistry*, **51**: 7879–7883.
- Blair, M., Torres, M., Giraldo, M., and Pedraza, F. (2009) Development and diversity of Andean-derived, gene-based microsatellites for common bean (*Phaseolus vulgaris* L.). *BMC Plant Biology*, **9**: 100.
- Brown, L., Rosner, B., Willett, W.W., and Sacks, F.M. (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition*, **69**(1): 30–42.
- Burton-Freeman, B. (2000) Dietary fiber and energy regulation. *Journal of Nutrition*, **13**: 272s–275s.
- Campos-Vega, R., Reynoso-Camacho, R., Pedraza-Aboytes, G., Acosta-Gallegos, J.A., Guzman-Maldonado, S.H., Paredes-Lopez, O., Oomah, B.D., and Loarca-Pina, G. (2009) Chemical composition and in vitro polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, **74**: T59–65.
- Cardador-Martínez, A., Loarca-Piña, G., and Oomah, B.D. (2002) Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, **50**: 6975–6980.
- Celleno, L., Tolaini, M.V., D'Amore, A., Perricone, N.V., and Preuss, H.G. (2007) A dietary supplement containing standardized *Phaseolus vulgaris* extract influences body composition of overweight men and women. *International Journal of Medical Sciences*, **4**: 45–52.
- Center for Food Safety and Applied Nutrition. (2009) *The Bad Bug Book Foodborne Pathogenic Microorganisms and Natural Toxins Handbook*. College Park, Md.: U.S. Food and Drug Administration.
- Diaz, A.M., Caldas, G.V., and Blair, M.W. (2010) Concentrations of condensed tannins and anthocyanins in common bean seed coats. *Food Research International*, **43**: 595–601.
- Díaz-Batalla, L., Widholm, J.M., Fahey, G.C., Castaño-Tostado, E., and Paredes-López, O. (2006) Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, **54**: 2045–2052.
- Dos Santos, M.D., Almeida, M.C., Lopes, N.P., and de Souza, G.E.P. (2006) Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biological and Pharmaceutical Bulletin*, **29**: 2236–2240.
- Doyle, J.J., and Luckow, M.A. (2003) The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. *Plant Physiology*, **131**: 900–910.
- Fantini, N., Cabras, C., Lobina, C., Colombo, G., Gessa, G.L., Riva, A., Donzelli, F., Morazzoni, P., Bombardelli, E., and Carai, M.A. (2009) Reducing effect of a *Phaseolus vulgaris* dry extract on food intake, body weight, and glycemia in rats. *Journal of Agricultural and Food Chemistry*, **57**: 9316–9323.
- Feregrino-Perez, A.A., Berumen, L.C., Garcia-Alcocer, G., Guevara-Gonzalez, R.G., Ramos-Gomez, M., Reynoso-Camacho, R., Acosta-Gallegos, J.A., and Loarca-Pina, G. (2008) Composition and chemopreventive effect of polysaccharides from common beans (*Phaseolus vulgaris* L.) on azoxymethane-induced colon cancer. *Journal of Agricultural Food Chemistry*, **56**: 8737–8744.
- Food and Agricultural Organization of the United Nations. (2009) *FAOSTAT*.
- Giacco, R., Parillo, M., Rivellese, A.A., Lasorella, G., Giacco, A., D'Episcopo, L., and Riccardi, G. (2000) Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. *Diabetes Care*, **23**: 1461–1466.
- Hangen, L., and Bennink, M.R. (2002) Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Nutrition and Cancer*, **44**: 60–65.
- He, J.A., and Giusti, M.M. (2010) Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, **1**: 163–186.
- Helmstadter, A. (2010) Beans and diabetes: *Phaseolus vulgaris* preparations as antihyperglycemic agents. *Journal of Medicinal Food*, **13**: 251–254.
- Hirvonen, T., Virtamo, J., Korhonen, P., Albanes, D., and Pietinen, P. (2001) Flavonol and flavone intake and the risk of cancer in male smokers (Finland). *Cancer Causes and Control*, **12**: 789–796.
- Hollman, P.C.H., and Katan, M.B. (1999) Health effects and bioavailability of dietary flavonols. *Free Radical Research*, **31**: S75–S80.
- Howarth, N.C., Saltzman, E., and Roberts, S.B. (2001) Dietary fiber and weight regulation. *Nutrition Reviews*, **59**: 129–139.
- Hughes, J.S. (1991) Potential contribution of dry bean dietary fiber to health. *Food Technology*, **45**: 122 ff.
- Hughes, J.S., Ganthavorn, C., and Wilson-Sanders, S. (1997) Dry beans inhibit azoxymethane-induced colon carcinogenesis in F344 rats. *Journal of Nutrition*, **127**: 2328–2333.

- Jeng, T.L., Shih, Y.J., Lai, C.C., Wu, M.T., and Sung, J.M. (2010) Anti-oxidative characterisation of NaN<sub>3</sub>-induced common bean mutants. *Food Chemistry*, **119**: 1006–1011.
- Kanaya, K., Tada, S., Mori, B., Takahashi, R., Ikegami, S., Kurasawa, S., Okuzaki, M., Mori, Y., and Innami, S. (2007) A simplified modification of the AOAC official method for determination of total dietary fiber using newly developed enzymes: preliminary interlaboratory study. *Journal of AOAC International*, **90**: 225–237.
- Khaleeva, L.D., Maloshtan, L.N., and Sytnik, A.G. (1987) [Comparative evaluation of the hypoglycemic activity of the vegetal complex of *Phaseolus vulgaris* and chlorpropamide in experimental diabetes]. *Probl Endokrinol (Mosk)*, **33**: 69–71.
- Khatoun, N. and Prakash, J. (2004) Nutritional quality of microwave-cooked and pressure-cooked legumes. *International Journal of Food Sciences and Nutrition*, **55**: 441–448.
- Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L., and Opletal, L. (2008) Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini Reviews in Medicinal Chemistry*, **8**: 436–447.
- Le Berre-Anton, V., Bompard-Gilles, C., Payan, F., and Rouge, P. (1997) Characterization and functional properties of the alpha-amylase inhibitor (alpha-AI) from kidney bean (*Phaseolus vulgaris*) seeds. *Biochimica Et Biophysica Acta-Protein Structure and Molecular Enzymology*, **1343**: 31–40.
- Marko, A.J., Miller, R.A., Kelman, A., and Frauwirth, K.A. (2010) Induction of glucose metabolism in stimulated T lymphocytes is regulated by mitogen-activated protein kinase signaling. *PLoS One*, **5**: e15425.
- Marlett, J.A. (2001) Dietary fiber and cardiovascular disease. In Cho, S., & Dreher, M.L. (eds.), *Handbook of dietary fiber* (pp. 17–30). New York: M. Dekker.
- Martin-Cabrejas, M.A., Sanfiz, B., Vidal, A., Molla, E., Esteban, R., and Lopez-Andreu, F.J. (2004) Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural Food Chemistry*, **52**: 261–266.
- Matito, C., Mastorakou, F., Centelles, J.J., Torres, J.L., and Cascante, M. (2003) Antiproliferative effect of antioxidant polyphenols from grape in murine Hepa-1c1c7. *European Journal of Nutrition*, **42**(1): 43–49.
- Mensack, M.M., Fitzgerald, V.K., Ryan, E.P., Lewis, M.R., Thompson, H.J., and Brick, M.A. (2010) Evaluation of diversity among common beans (*Phaseolus vulgaris* L.) from two centers of domestication using “omics” technologies. *BMC Genomics*, **11**: 686.
- Nothlings, U., Murphy, S.P., Wilkens, L.R., Henderson, B.E., and Kolonel, L.N. (2007) Flavonols and pancreatic cancer risk - The multiethnic cohort study. *American Journal of Epidemiology*, **166**: 924–931.
- Okada, Y., Okada, M., and Sagesaka, Y. (2010) Screening of dried plant seed extracts for adiponectin production activity and tumor necrosis factor-alpha inhibitory activity on 3T3-L1 adipocytes. *Plant Foods for Human Nutrition*, **65**: 225–232.
- Oomah, B.D., Corbe, A., and Balasubramanian, P. (2010) Antioxidant and anti-inflammatory activities of bean (*Phaseolus vulgaris* L.) hulls. *Journal of Agricultural and Food Chemistry*, **58**: 8225–8230.
- Panlasigui, L.N., Panlilio, L.M., and Madrid, J.C. (1995) Glycaemic response in normal subjects to five different legumes commonly used in the Philippines. *International Journal of Food Sciences and Nutrition*, **46**: 155–160.
- Pari, L., and Venkateswaran, S. (2003) Effect of an aqueous extract of *phaseolus vulgaris* on plasma insulin and hepatic key enzymes of glucose metabolism in experimental diabetes. *Pharmazie*, **58**: 916–919.
- Pari, L., and Venkateswaran, S. (2004) Protective role of *Phaseolus vulgaris* on changes in the fatty acid composition in experimental diabetes. *Journal of Medicinal Food*, **7**: 204–209.
- Park, Y., Brinton, L.A., Subar, A.F., Hollenbeck, A., and Schatzkin, A. (2009) Dietary fiber intake and risk of breast cancer in postmenopausal women: the National Institutes of Health-AARP Diet and Health Study. *The American Journal of Clinical Nutrition*, **90**(3): 664–671.
- Park, Y., Hunter, D.J., Spiegelman, D., Bergkvist, L., Berrino, F., van den Brandt, P.A., Buring, J.E., Colditz, G.A., Freudenheim, J.L., Fuchs, C.S., Giovannucci, E., Goldbohm, R.A., Graham, S., Harnack, L., Hartman, A.M., Jacobs, D.R., Kato, I., Krogh, V., Leitzmann, M.F., McCullough, M.L., Miller, A.B., Pietinen, P., Rohan, T.E., Schatzkin, A., Willett, W., Wolk, A., Zeleniuch-Jacquotte, A., Zhang, S.M.M., and Smith-Warner, S.A. (2005) Dietary fiber intake and risk of colorectal cancer – A pooled analysis of prospective cohort studies. *Jama-Journal of the American Medical Association*, **294**: 2849–2857.
- Preuss, H.G. (2009) Bean amylase inhibitor and other carbohydrate absorption blockers: effects on diabetes and general health. *Journal of the American College of Nutrition*, **28**: 266–276.
- Preuss, H.G., Echard, B., Bagchi, D., and Stohs, S. (2007) Inhibition by natural dietary substances of gastrointestinal absorption of starch and sucrose in rats and pigs: 1. Acute studies. *International Journal of Medical Sciences*, **4**: 196–202.

- Reed, J.D. (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science*, **73**: 1516–1528.
- Reyes-Bastidas, M., Reyes-Fernandez, E.Z., Lopez-Cervantes, J., Milan-Carrillo, J., Loarca-Pina, G.F., and Reyes-Moreno, C. (2010) Physicochemical, nutritional and antioxidant properties of tempeh flour from common bean (*Phaseolus vulgaris* L.). *Food Science and Technology International*, **16**: 427–434.
- Roman-Ramos, R., Floressaenz, J.L., and Alarconaguilar, F.J. (1995) Anti-hyperglycemic effect of some edible plants. *Journal of Ethnopharmacology*, **48**: 25–32.
- Rosa, C.O., Costa, N.M., Nunes, R.M., and Leal, P.F. (1998) [The cholesterol-lowering effect of black, cari-quinha and red beans (*Phaseolus vulgaris*, L.) in hypercholesterolemic rats]. *Archives in Latinoam Nutrition*, **48**: 306–310.
- Rosa, C.O., Costa, N.M., Leal, P.F., and Oliveira, T.T. (1998) [The cholesterol-lowering effect of black beans (*Phaseolus vulgaris*, L.) without hulls in hypercholesterolemic rats]. *Archives in Latinoam Nutrition*, **48**: 299–305.
- Tormo, M.A., Gil-Exojo, I., Romero de Tejada, A., and Campillo, J.E. (2006) White bean amylase inhibitor administered orally reduces glycaemia in type 2 diabetic rats. *The British Journal of Nutrition*, **96**: 539–544.
- Slavin, J., and Jacobs, D.R. (2010) Dietary fiber: all fibers are not alike. In Wilson, T. (ed.), *Nutrition guide for physicians* (pp. 13–24). Totowa, N.J.: Humana.
- Schoonhoven, A.V., Voysset, O., and Centro Internacional de Agricultura Tropical. (1991) *Common Beans: Research for Crop Improvement*. Wallingford, Oxon, UK; Tucson, AZ, USA: C.A.B. International in association with Centro Internacional de Agricultura Tropical.
- Shutler, S.M., Bircher, G.M., Tredger, J.A., Morgan, L.M., Walker, A.F., and Low, A.G. (1989) The effect of daily baked bean (*Phaseolus vulgaris*) consumption on the plasma lipid levels of young, normo-cholesterolaemic men. *British Journal of Nutrition*, **61**: 257–265.
- Simpson, H.C.R., Lousley, S., Geekie, M., Simpson, R.W., Carter, R.D., Hockaday, T.D.R., and Mann, J.I. (1981) A high-carbohydrate leguminous fiber diet improves all aspects of diabetic control. *Lancet*, **1**: 1–4.
- Singh, S.P., Gepts, P., and Deboucq, D.G. (1991) Races of common bean (*Phaseolus-Vulgaris*, Fabaceae). *Economic Botany*, **45**: 379–396.
- Slavin, J. (2008) Position of the American Dietetic Association: Health implications of dietary fiber. *Journal of the American Dietetic Association*, **108**: 1716–1731.
- Streppel, M.T., Arends, L.R., van't Veer, P., Grobbee, D.E., and Geleijnse, J.M. (2005) Dietary fiber and blood pressure - A meta-analysis of randomized placebo-controlled trials. *Archives of Internal Medicine*, **165**: 150–156.
- Stukelj, M., Valencak, Z., Krsnik, M., and Svete, A.N. (2010) The effect of the combination of acids and tannin in diet on the performance and selected biochemical, haematological and antioxidant enzyme parameters in grower pigs. *Acta Veterinaria Scandinavica*, **52**: 19.
- Taguri, T., Tanaka, T., and Kouno, I. (2006) Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biological and Pharmaceutical Bulletin*, **29**: 2226–2235.
- Trinidad, T.P., Mallillin, A.C., Loyola, A.S., Sagum, R.S., and Encabo, R.R. (2010) The potential health benefits of legumes as a good source of dietary fibre. *British Journal of Nutrition*, **103**: 569–574.
- Udani, J., Hardy, M., and Madsen, D.C. (2004) Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2 brand proprietary fractionated white bean extract. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, **9**: 63–69.
- Udani, J., and Singh, B.B. (2007) Blocking carbohydrate absorption and weight loss: a clinical trial using a proprietary fractionated white bean extract. *Alternative Therapies in Health and Medicine*, **13**: 32–37.
- United States Department of Health and Human Services, United States Department of Agriculture, and United States Dietary Guidelines Advisory Committee. (2005) *Dietary Guidelines for Americans, 2005*. Washington, D.C.: G.P.O.
- U.S. Department of Agriculture, Agricultural Research Service. (2010) USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, <http://www.ars.usda.gov/ba/bhnrc/ndl>.
- Vásquez-Arroyo, J., Sessitsch, A., Martinez, E., and Pena-Cabriales, J.J. (1998) Nitrogen fixation and nodule occupancy by native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). *Plant and Soil*, **204**: 147–154.
- Venkateswaran, S., Pari, L., and Saravanan, G. (2002) Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in rats with streptozotocin-induced diabetes. *Journal of Medicinal Food*, **5**: 97–103.

- Whelton, S.P., Hyre, A.D., Pedersen, B., Yi, Y., Whelton, P.K., and He, J. (2005) Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *Journal of Hypertension*, **23**: 475–481.
- Williams, C.L., Bollella, M., and Wynder, E.L. (1995) A new recommendation for dietary fiber in childhood. *Pediatrics*, **96**: 985–988.
- Wong, J.H., Wan, C.T., and Ng, T.B. (2010) Characterisation of a haemagglutinin from Hokkaido red bean (*Phaseolus vulgaris* cv. Hokkaido red bean). *Journal of the Science of Food and Agriculture*, **90**: 70–77.
- Woodman, O.L., and Chan, E.C. (2004) Vascular and anti-oxidant actions of flavonols and flavones. *Clinical and Experimental Pharmacology and Physiology*, **31**: 786–790.
- Yang, S.S., Valdes-Lopez, O., Xu, W.W., Bucciarelli, B., Gronwald, J.W., Hernandez, G., and Vance, C.P. (2010) Transcript profiling of common bean (*Phaseolus vulgaris* L.) using the GeneChip Soybean Genome Array: optimizing analysis by masking biased probes. *BMC Plant Biology*, **10**: 85.

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# 14 Health benefits and bioactive compounds in field peas, faba beans, and chickpeas

Margaret Udahogora

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## 14.1 Introduction

Legumes are good sources of plant based proteins and selected micronutrients, have a high concentration of certain carbohydrates, antioxidants, and dietary fiber (López-Amoro's *et al.*, 2006), and are low in fat and calories (Marinangeli and Jones, 2011). Table 14.1 summarizes selected nutrient content in some legumes. Although legumes have been used as a staple food by mankind since earliest civilization, pulses are often advocated in diet due to their beneficial nutritional effects and low cost (Craig, 2009). More importantly, many diets in developing countries are based on legume and cereal products and there is an increasing interest in strictly vegetarian diets in Western societies (Craig and Mangels, 2009). In a plant based balanced diet containing legume protein, the high intake of legumes may imply a risk of inadequate mineral supply with a low absorption of Fe and Zn. However, the levels of Fe and Zn, and probably other minerals, can be improved by food processing operations such as fermentation, soaking, germination, use of degrading enzymes, and a well planned vegetarian diet (Sandberg, 2002; Craig and Mangels, 2009).

Pulses are produced worldwide. Field pea (*Pisum sativum* L.), a native of Southwest Asia, is grown on over 25 million acres worldwide (Oelke, 1991; Schatz and Endres, 2009). The major producers include Russia and China, followed by Canada, Europe, Australia, and the United States. Current field pea production is primarily for human consumption and also serves as a livestock feed (Schatz and Endres, 2009). Faba bean is considered in some areas to be superior to field pea and other legumes (Muehlbauer *et al.*, 1997). Its production is concentrated in Northern Europe, the Mediterranean, the Nile Valley, Ethiopia, Central Asia, East Asia, Oceania, Latin America, and North America. China is the largest producer of faba bean in the world. The crop has a special place in the diets of populations in Europe's Mediterranean regions, the Middle East, and China ([http://www.agric.wa.gov.au/PC\\_92109.html?s=1001](http://www.agric.wa.gov.au/PC_92109.html?s=1001)). Contrary to the pea consumption, faba bean is used mainly as human food in developing countries and as a livestock feed in

**Table 14.1** Food composition of cooked chickpeas, peas, and faba beans

Component	Chickpeas, cooked 1.00 cup	Peas, cooked 1.00 cup	Faba beans, cooked 1.00 cup
Calories	268.96	231.28	187
Calories from fat	38.23	6.88	5.7
Protein	14.53 g	16.35 g	13.0 g
Carbohydrate	44.95 g	41.38 g	33.4 g
Total fiber	12.46 g	16.26 g	9.2 g
Soluble fiber	3.87 g	5.04 g	–
Insoluble fiber	8.59 g	11.23 g	–
Zinc	3.51 mg	1.96 mg	1.7 mg
Iron	4.74 mg	2.53 mg	2.5 mg
potassium	477 mg	709 mg	456 mg
Folate	282 mcg	127 mcg	177 mcg

<http://whfoods.org/genpage.php?tname=nutrientprofile&dbid=15>

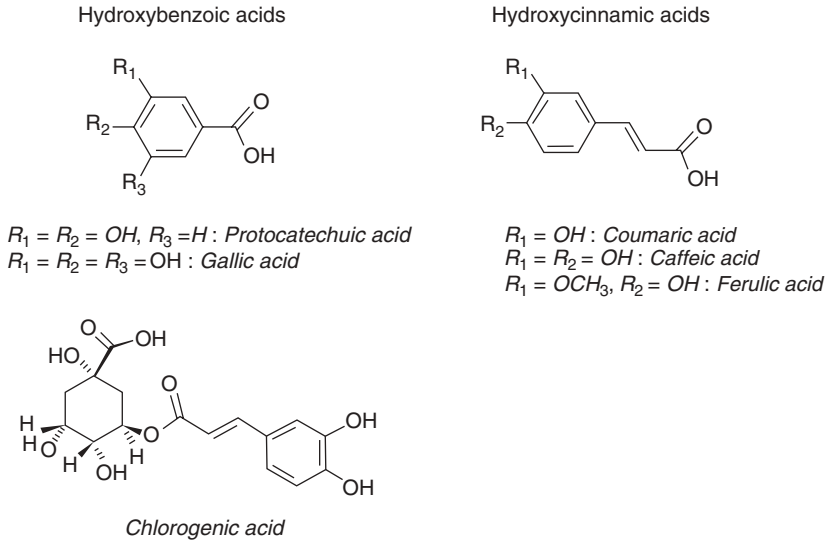
<http://nutritiondata.self.com/facts/legumes-and-legume-products/4322/2>

industrialized countries (Muehlbauer *et al.*, 1997; <http://www.fao.org/docrep/t0646e/T0646E0s.htm>). Moreover, varieties free of bitter ingredients, sweet, and of mild taste are reserved for human consumption, while varieties without those characteristics are fed to animals (<http://www.fao.org/docrep/t0646e/T0646E0s.htm>). In climates that are too dry, faba bean production is substituted with that of chickpeas (*Cicer arietinum*). The dual use of chickpeas in human and animal feed is characterized by a clear variety preference. The white or cream-colored seeds, very large and wrinkled, are mainly for human consumption, while the smaller ones, of various color, size, and shape, are reserved for animal consumption particularly in Western Mediterranean countries (<http://www.fao.org/docrep/t0646e/T0646E0s.htm>).

Experimental, clinical, and epidemiological research has been interested in the role these annual leguminous crops play in the reduction of cardiovascular diseases, type 2 diabetes mellitus, and cancer (López-Amoro's *et al.*, 2006). Findings on their protective effect, when dietary intake is significant, are emerging (Carratù and Sanzini, 2005). This chapter summarizes the potential biological properties of selected legumes, the modification of phytochemical bioavailability, and potential allergic and adverse effects related to the bioactive compounds.

## 14.2 Phenolic compounds in field peas, chickpeas, and faba beans

Phenolic phytochemicals are the largest category of phytochemicals. They comprise important dietary phenolics including flavonoids, tannins (Yadahally *et al.*, 2010), polyphenols, and phenolic acids (King and Young, 1999; D'Archivio *et al.*, 2007). Phenolic acids are the most important and the largest group of antioxidants occurring in peas. They consist of two subgroups: hydroxybenzoic and hydroxycinnamic acids (Klepacka *et al.*, 2011) (Figure 14.1). Hydroxycinnamic acids consist chiefly of coumaric, caffeic, and ferulic acids (D'Archivio *et al.*, 2007). The forms of coumaric – trans *p*-coumaric acid and cis *p*-coumaric acid, and chlorogenic acid have been identified in peas (Klepacka *et al.*,

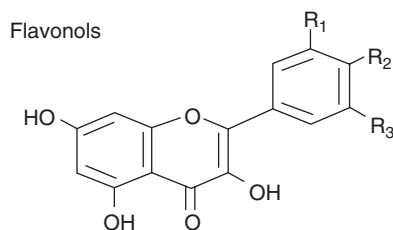


**Figure 14.1** Hydroxybenzoic and hydroxycinnamic acids.  
 Source: Manach *et al.* (2004).

2011; Xu and Chang, 2009). Peas contain trace amounts of ferulic acid. Other phenolic compounds in peas include benzoic acid derivatives (vanillic, gallic, syringic, protocatechuic acid, and *p*-hydroxybenzoic acid (López-Amoro's *et al.*, 2006). Chlorogenic and gallic acids are the predominant phenolic acids among the compounds detected in both raw and cooked green peas (Xu and Chang, 2009). The total amount of phenolics in peas is estimated to be 16.2–42.1 mg/100 mg (Klepacka *et al.*, 2011). The percentage of phenolic acids with respect to the total content of phenolics in the peas ranges between 87 and 92% and is variety dependent.

The examination of chickpeas has revealed variation in the distribution and concentration of phenolic compounds in the cotyledon, embryonic axes, and seed coat fractions of the seed. The highest concentration of total phenolics and condensed tannins were in the seed coat and are easily removed by dehulling. The flavonoids present include quercetin, kaempferol, myricetin, daidzein, and genistein (Figure 14.2). These flavonoids are mainly concentrated in the embryonic axe, however, genistein and daidzein are present in small amounts compared to those in soybean, which has the highest content, 15 and 28 times more, respectively, of these isoflavones. Additional phenolic acids in chickpeas comprise benzoic acid derivatives (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, and syringic) and cinnamic acid derivatives (caffeic, chlorogenic, ferulic, sinapic, and *p*-coumaric acid) (Yadahally *et al.*, 2010). Chlorogenic, gallic, *p*-coumaric acid, and protocatechualdehyde are the predominant phenolic acids among the compounds detected in both raw and cooked chickpeas (Xu and Chang, 2009). However, quantification of phenolics in food is influenced by the type of extraction condition and analytical methods employed. In addition, climatic conditions, agrotechnical procedures, harvesting, maturation stage, storage, industrial or domestic processing, and genetic factors influence the structures and properties of phenolics, thus affecting the determined amounts (Klepacka *et al.*, 2011).



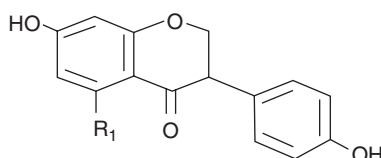


$R_2 = OH; R_1 = R_3 = H$  : Kaempferol

$R_1 = R_2 = OH; R_3 = H$  : Quercetin

$R_1 = R_2 = R_3 = OH$  : Myricetin

Isoflavones



$R_1 = H$  : Daidzein

$R_1 = OH$  : Genistein

**Figure 14.2** Flavonoids in chickpeas.

Source: Manach *et al.* (2004).

Phenolics are biologically active compounds, and epidemiological and experimental studies have been used to assess their disease-preventive properties (D'Archivio *et al.*, 2007). Much of the evidence on the protective effects of polyphenols has been derived from *in vitro* studies or animal models with the use of concentrations higher than those consumed in the human diet. There has been a rapid increase in the studies investigating the bioavailability and the protective effects of the phenolic compounds in humans. Prior to the wide use of these polyphenols in specific food, pharmaceutical, and cosmetic industries, there is still a need to develop efficient procedures for their extraction. Proper analysis and characterization of phenolic compounds remains a challenging task due to their structural diversity, and interaction with cellular components (Ajila *et al.*, 2010).

## 14.3 Health benefits of compounds in field peas, chickpeas, and faba beans

### 14.3.1 Antioxidant activities

Phenolics have been considered as powerful antioxidants *in vitro* (Fernandez-Panchon *et al.*, 2008). Rice-Evans and Miller (1997), and others, have characterized them as more potent antioxidants than vitamins E, C, and carotenoids. The antioxidant properties of

compounds of interest are related to their ability to prevent the chain initiation, to break the free radical chain through the donation of a hydrogen atom or an electron, and to delocalize unpaired electrons within the aromatic structure. Additional mechanisms of action of antioxidants include decomposition of peroxides, reductive capacity and radical scavenging (Yadahally *et al.*, 2010).

Studies have shown that the available antioxidant activity of phenolic compounds in plant foods can be enhanced by improving agricultural practices, post-harvest treatments, and food formulation and processing conditions. In peas and beans, the antioxidant activity could be significantly increased after germination, whereas a decrease has been observed in lentils (López-Amoro's *et al.*, 2006). The various post germination changes in phenolic compounds are dependent on the presence of light, germination time, and the type of seeds. In peas, for instance, the germination increased *p*-hydroxybenz aldehyde, *cis p*-coumaric acid, and *trans* ferulic acid (López-Amoro's *et al.*, 2006) in the early stage of germination with a higher increase observed after four days in the presence of light. This increase in antioxidant activity was measured by free radical scavenging capability. On the other hand, the thermal processing of legumes (chickpea, peas, lentils) was found to decrease ferric reducing antioxidant power, in peroxy radical scavenging capacities, and in cell-based antioxidant capacities of the legumes in all processing treatments (Xu and Chang, 2009) compared to the raw legumes. However, these changes were dependent on the type of legume and processing conditions. Steaming emerged as the best method to preserve phenolic and antioxidant components of peas and chickpeas.

In the Yadahally *et al.* (2010) study, the antioxidant properties of chickpeas were tested using a combination of several tests for a more reliable and complete examination of the antioxidant properties of the extract. The methods included 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, hydrogen peroxide scavenging capacity (PRSC), ferric reducing antioxidant power (FRAP), and chelating activity of metal ions (CAMI). The authors reported a dose dependent scavenging capacity for the phenolic compounds from different fractions of the extracts. A strongest scavenging activity was observed with the chickpea seed coat extracts followed by the embryonic axe extracts. Similar findings were reported with dark peas and more antioxidant activity was observed in the seed coat fraction (Duenas *et al.*, 2006). The authors attributed the higher antioxidant activities to the higher content of phenolic compounds in the seed coat and embryonic axe. These results suggest that all the three fractions of legumes are quite effective in scavenging free radicals. The scavenging ability of phenolic compounds could be considered as the rationale for health benefits attributed to a diet rich in phenolics and there is an increasing interest in their use as ingredients in functional foods (Yadahally *et al.*, 2010).

Numerous other clinical and epidemiologic studies have reported that isoflavones and phenolic antioxidants possess the ability to avert some cholesterol induced and oxidative stress related chronic diseases, such as cardiovascular diseases, obesity, cancer, and diabetes. Isoflavones, high in concentration in chickpeas embryonic axe fraction, particularly genistein and daidzein have been associated with a reduction of breast cancer, prostate cancer, and cardiovascular diseases (Yadahally *et al.*, 2010; Anisha and Prema, 2008). Apart from their phytoestrogenic effect, these isoflavones have antioxidant properties. Genistein has been found as an inhibitor of protein tyrosine kinases and DNA topoisomerases I and II and can hinder signal transduction pathways, thus halting the cell growth (Anisha and Prema, 2008).

### 14.3.2 Hypocholesterolemic activity

Serum cholesterol concentration in humans is reported to be influenced by the dietary carbohydrates and proteins of legumes. Table 14.2 summarizes the hypocholesterolemic effect of peas, chickpeas, and faba beans. In 1965, Grande and others investigated the effect of carbohydrates and proteins from leguminous seeds (peas, beans) and other foods on serum cholesterol levels. A serving of 48 g/day of protein and 115 g of carbohydrate from leguminous seeds was provided to adult male participants. The study resulted in a significant decrease in serum cholesterol concentration of 19 mg/100 mL in the group fed leguminous carbohydrate in comparison to other sources of carbohydrates (bread, potatoes, sucrose) (Grande *et al.*, 1965).

Faba beans also demonstrated a beneficial impact on lipid profiles. An earlier study (Weck *et al.*, 1983) reported that faba bean proteins had a comparable effect of reducing cholesterol, similar to soybean proteins, in human studies. Fruhbeck and others (1997) confirmed the hypocholesterolemic effect of faba beans in young men (18–21 years of age) who supplemented a basic diet with 90 g of cooked or raw faba beans. Total low-density-lipoprotein (LDL), cholesterol, and triacylglycerols decreased significantly compared to initial values in all subjects on the faba bean supplement. Decreases of 0.25–0.71, 0.18–0.35, and 0.13–0.49 mmol/L were recorded for triacylglycerols, LDL, and total cholesterol, respectively. High density lipoprotein and glucose also showed some improvement. The authors concluded that the hypocholesterolemic effect observed could be associated with the combined effects of several factors such as phytate, tannins, fiber, and saponins being present in the seed flour (Fruhbeck *et al.*, 1997).

The cholesterol lowering effect has also been evaluated in 27 subjects using chickpeas compared to wheat based diet of similar fiber content. The intervention was based on the daily intake of 140 g of chickpeas with bread made with 30% chickpea flour. The authors found a low but significant reduction in serum total cholesterol and LDL by 0.25 and 0.20 mmol/L, respectively, following 5 weeks of consuming a chickpea supplemented diet. The observed change in cholesterol could also be explained by an increase in polyunsaturated fatty acid consumption among subjects on a chickpea diet. It was also concluded from this study that soluble dietary fiber, as well as other factors such as oligosaccharides, isoflavones, phospholipids, fatty acids, phytosterols, saponins, vitamins, and minerals, may have played an important role in the resultant hypocholesterolemic effect. Thus, the sum of the factors rather than individual components could have contributed to the observed changes (Pittaway *et al.*, 2007). Some studies have shown the mechanism of action of dietary fiber. The fiber is fermented in the colon into short chain fatty acids (SCFA) such as acetic acid, propionic acid, and butyric acid. These SCFA have the ability to enhance water absorption in the colon, and propionic acid, in particular, has been found to inhibit the activity of the hydroxy-3-methylglutaryl-CoA reductase, the limiting enzyme in cholesterol synthesis. The additional health benefit of dietary fiber is the ability to bind bile acids. Thus, partially interrupting the enterohepatic circulation of bile results in an increase in cholesterol use in bile synthesis, which impacts the lowering of cholesterol in plasma (Trinidad *et al.*, 2010).

In 2007, Pittway and colleagues evaluated the effect of consumption of four 300 g cans of chickpeas per week over a period of 12 weeks followed by a four-week habitual diet. Consumption of chickpeas increased participants' intake of fiber and polyunsaturated fat. The chickpea phase resulted in the reduction of serum cholesterol and low-density lipoprotein cholesterol of 7.7 and 7.3 mg/dL, respectively. Improvement in fasting insulin and insulin

**Table 14.2** Hypocholesterolemic effects of legumes: peas, chickpeas, and beans

<b>Subjects</b>	<b>Health status</b>	<b>Diet</b>	<b>period</b>	<b>Blood cholesterol response</b>	<b>References</b>
<b>40</b>	Borderline high or High cholesterol	Basic diet Supplement of 90g cooked or raw faba beans flour/d	30 days	Reduction of : TG : 0.25–0.71 mmol/L LDL : 0.18–0.35 mmol/L TC : 0.13–0.49 mmol/L	Fruhbeck <i>et al.</i> , 1997
<b>27</b>	<70 years, No medication. Free living adults	Chickpeas & shortbread (30%) chickpea flour vs. wheat diet	Randomized cross over 5 week each	Reduction of: TC : 0.25 mmol/L LDL : 0.20 mmol/L	Pittaway <i>et al.</i> , 2007
<b>45</b>	Free living adults Mildly hypercholesterolemic	Four 300 g cans chickpeas/wk Followed by habitual diet without chickpeas	12 weeks 4 weeks Cross over design	Chickpea phase: TC : 7.7 mg/dL less LDL : 7.3 mg/dL less	Pittaway <i>et al.</i> , 2008
<b>20</b>	Moderately raised cholesterol (30–60yrs)	50 g carbohydrate Chickpeas, peas, & other legumes	22 weeks Cross over design	A decreased trend of Greens peas chickpeas TC : 7 mg/dL 5 mg/dL LDL : 1 mg/dL 1 mg/dL	Trinidad <i>et al.</i> , 2010

TC = total cholesterol.

LDL = lipoprotein cholesterol.

resistance were also recorded. Although the dietary intake of PUFA had a significant effect on the observed changes in metabolic parameters, the study did not determine the contributing effect of other dietary changes that occurred (Pittaway *et al.*, 2008).

A recent study by Trinidad *et al.* (2010) assessed the potential health benefits of various legumes, including chickpeas and peas, among moderately raised serum cholesterol subjects. The authors showed a decreased trend in total and LDL cholesterol for chickpeas and peas, while kidney beans resulted in a significant reduction in blood level of total cholesterol and LDL (6% and 9%, respectively). The authors attributed the lack of conclusive results to the short-term nature of their study.

Lipid lowering effects of legumes have also been reported in animal models. The use of the faba bean in a hypercholesterolemia inducing diet in rats led to a significant decrease in serum cholesterol levels after two weeks (Bonilla *et al.*, 1998). Another study that assessed the effect of faba bean protein isolates and the intact legumes on serum lipids in rats showed a significant reduction in triacylglycerols, and plasma LDL/VLDL. Hepatic triacylglycerols and cholesterol levels also decreased. In addition, rats fed on faba bean-based diets had a lower body weight compared to those on casein-based diets, probably due to the difference in energy intake. The effectiveness of the whole seeds was higher than that of the protein isolate, which might be an indication that other components of faba beans contributed to lowering of serum cholesterol. The primary mechanisms involved in cholesterol reduction were related to the increase in steroid fecal excretion due to an enhanced biliary cholesterol excretion. The authors found no reduction in cholesterol synthesis as assessed from HMG-CoA reductase activity (Macarulla *et al.*, 2001).

### 14.3.3 Hypoglycemic activity

Differences exist in the degree to which various starch containing foods affect the level of blood glucose of both diabetic and non-diabetic individuals (Throne *et al.*, 1983; Thompson *et al.*, 1984). In general, foods containing potentially available, slowly digestible carbohydrate are likely to elicit a lower concentration of blood glucose after consumption compared to foods containing similar levels of rapidly digestible starch. The differences in digestibility are related to the nature of the starch, the interaction of the starch with fiber, the presence of antinutritive factors and protein in the food, and food processing and preparation (Hawkins *et al.*, 2005).

An early study using a rat model revealed increased fecal loss of carbohydrate in animal fed raw amylose starch compared to those on a diet high in raw amylopectin starch (Thorne *et al.*, 1983). In another study, rats were fed corn and legume starch and the former was less digestible. *In vitro*, it was observed that the rate of hydrolysis of legume starch was less than corn starch (Geervani and Theophilus, 1981; Shurpalekar *et al.*, 1979). Legumes contain 5–10% more amylose compared to cereals. For instance chickpeas contain 30–40% amylose, which is resistant to total and rapid hydrolysis by enzymes in the small intestine (Botham *et al.*, 1996, Nestel *et al.*, 2004). Thus, this contributes to the lower availability of glucose as a substrate for colonic fermentation, which improves the bowel health (Pittway *et al.*, 2007).

The role of peas, chickpeas, and faba beans in improving insulin resistance and post-meal blood glucose levels has been evaluated in a number of studies and are summarized in Table 14.3. In 1980, Jenkins and colleagues assessed the glycemic effect of eight varieties of dried legumes and 24 common foods based on grains and tuberous vegetables. Healthy

**Table 14.3** Hypoglycemic effects of legumes: peas, chickpeas, and beans

<b>Subjects</b>	<b>Health status</b>	<b>Diet</b>	<b>period</b>	<b>Blood glucose response</b>	<b>References</b>
<b>25</b>	Healthy women & men	50 g of a single food beans & peas, & other sources of carbohydrates	Post fasting study	45% lower peak glucose concentrations	Jenkins <i>et al.</i> , 1980
<b>15</b>	Type 2 diabetes inadequately controlled blood glucose	Peas and beans vs. a control diet	3 weeks Randomized cross over	Lower mean postprandial glucose & lower mean urine glucose excretion	Karlstrom <i>et al.</i> , 1987
<b>19</b>	Healthy middle age men & women	50 g carbohydrate From chickpeas or wheat bread	6 weeks each Cross over	21% & 27% Lower plasma glucose (30,60min) 54% Lower plasma insulin & HOMA-IR* (120min)	Nestel <i>et al.</i> , 2004
<b>27</b>	Healthy men & women (<70yr)	140 g of chickpeas/daily & short bread made of 30% chickpea flour	5 week duration Randomized cross over study	No significant difference in glucose level but improved total cholesterol & LDL	Pittaway <i>et al.</i> , 2007
<b>25</b>	Men & women – Overweight Hypercholesterolemic	Muffins/d containing WPF, FPF, WF (50g/day of pulse)	28 days Cross over design	20–25% – Lower insulin concentration for the FPF and WPF vs. WF	Christopher <i>et al.</i> , 2010

\* HOMA-IR = plasma insulin (mU/l) / plasma glucose (mmol/l) / 22.5.

\*\* WPF = whole pea flour; FPF = fractionated pea flour; WF = wheat flour.

volunteers consumed 50 g portions of carbohydrate. The findings show a 45% lower peak rise in plasma glucose concentration from dried legumes.

In 1984, Thompson and others examined five leguminous foods, including chickpeas, and eight non-leguminous products considering their polyphenol concentrations and the blood glucose response in normal and diabetic volunteers. Their findings showed that for both normal and diabetic individuals, the leguminous foods resulted in lower blood glucose response, measured as the glycemic index, when compared to non-leguminous foods. The presence of large polymeric polyphenols in legumes showed a higher correlation with glycemic index response (Thompson *et al.*, 1984). The negative relationship between polyphenols and blood glucose, although not clear, could be explained by the binding of tannins onto legume starches, thus reducing starch digestibility due to this binding (Thompson *et al.*, 1984). The inhibition of amylase activity has also been reported and may affect starch digestibility. Other studies have suggested a reduction in protein–tannin complex digestibility in legumes, which may indirectly affect starch digestibility. A tight association between protein and starch has been found in legumes (Throne *et al.*, 1983; Hawkins *et al.*, 2005). Legumes contain twice as much protein compared to cereals, thus a possible protein–starch interaction in leguminous seeds contributes to their decreased glycemic response compared to cereals. In terms of antinutritive factors, they include enzyme inhibitors, phytates, and possibly lectins. Enzyme inhibitors such as amylase and sucrose have been shown to decrease the rate of carbohydrate digestion and absorption. However, these inhibitors can be deactivated by heat treatment (Throne *et al.*, 1983).

A study by Nestel *et al.* (2004) reported lower plasma glucose 30 and 60 minutes after a single chickpea based meal compared to a standard wheat based meal. In addition, after two hours of consuming the chickpea meal, the plasma insulin and the estimated homeostasis model assessment of insulin resistance (HOMA-IR) were (54%) significantly lower than other dietary treatments. At the end of the six-week study, the investigation did not show any long-term improvement in plasma glucose and insulin sensitivity and this could be related to the use of subjects who had no apparent insulin resistance at the beginning of the study (Nestel *et al.*, 2004). A later study by Pittway *et al.* (2007), did not confirm the effect of chickpeas in comparison to a wheat grain diet. Both diets had comparable nutrient and fiber content. Significant reductions in low density lipoprotein and total cholesterol were observed in the group consuming the chickpea diet. The lack of glucose improvement could be related to the normoglycemic state of participants.

In 2005, Hawkins and others assessed the rate of digestibility and glucose availability in chickpeas using an in vitro stimulation of the human gastrointestinal tract. They reported the different effect of precooked/vacuum compared with boiled seeds. The rapidly available sugar was lowest in boiled whole chickpeas (26.7 g/100 mg) compared to the canned chickpeas (38.9–43.3 g/100 mg). In addition, the domestically boiled chickpeas showed a higher proportion of slowly digestible starch content compared to canned chickpeas, and the values were ~50% vs. ~40%. The authors concluded that domestically boiled chickpeas would have a higher beneficial glycemic index compared to precooked/vacuum packages chickpea products.

Recently, Christopher *et al.* (2010) reported the health benefits of consuming peas in a human clinical trial with a controlled diet. The subjects consumed muffins made with whole (WPF) or fractionated pea flour (FPF) equivalent to half a cup per day of the pulse. The results showed lower (20%–25%), insulin concentrations among the WPF and FPF compared to those who received wheat based muffins. Other additional benefits were a significant

reduction in HOMAR-IR and android: gynoid fat ratio in women on a pea based diet. The authors concluded that under a controlled diet, the USDA's recommended intake of 50 g/d of pulse or half a cup of WPF and FPF decreased the fasting insulin concentrations and insulin resistance in hypercholesterolemic and overweight individuals.

Numerous studies have assessed the glycemic effects of consuming legumes and other complex carbohydrates. They have illustrated an improvement in glucose metabolism, a reduction in insulin or oral hypoglycemic agent utilization, lowering of fasting blood glucose, a reduction in glycosylated protein concentration, and lowering of urinary glucose and c-peptide output (Venn and Mann, 2004). However, due to the combination of food items, it was not possible to determine to what extent the changes in whole grain foods and legumes intake might have contributed to these observations.

#### **14.4 Antinutritional factors in peas, chickpeas, and faba beans**

Legumes were originally considered as having low nutritive value due to the presence of antinutritive compounds such as phenolic compounds, phytic acid, protease inhibitors, saponins, and plant sterols. However, more recent findings suggest health benefits for consumers (Xu and Chang, 2009). The antinutritive elements in chickpeas include trypsin inhibitor, hemagglutinin, tannins, phytic acid, and saponins. The cotyledon fraction of chickpeas is the main source of proteins and carbohydrates and contains substantial quantities of phytic acid, flatulence factors, and the proteinaceous enzyme inhibitors (Yadahally *et al.*, 2010). The seed coat phenolic compounds have been reported as possessing distinct inhibitory mechanism against the enzymatic action of trypsin and  $\alpha$ -amylase (Shahidi *et al.*, 2001).

Germination of chickpeas induces the formation of enzymes that eliminate or decrease the antinutritive factors (Bau *et al.*, 1997) and is more effective in reducing phytic acid, stachyose, and raffinose and results in the retention of all mineral and B vitamins compared to raw seeds. It is recognized that additional benefits of germination include increase in the levels of free amino acids, carbohydrates availability, dietary fiber, and bioactive compounds (Vidal-Valverde *et al.*, 2003; López-Amoro's *et al.*, 2006). Germination also decreases the total carbohydrate and fat content, which could be attributed to hydrolysis of those components for their usage as an energy source to start germination. Raffinose, stachyose, and verbascose were completely eliminated during germination. Hemagglutinin was almost completely eliminated with any type of food processing of chickpeas and faba beans, while others compounds were decreased (Bau *et al.*, 1997).

Apart from germination, thermal food processing has been shown to contribute to a decrease in antinutritive compounds in legumes (chickpeas, peas). Traditionally, the presence of saponins and phytic acid had been considered to be antinutritional. These were described as impairing the digestibility of protein, and the bioavailability of iron, zinc, and calcium, thus reducing the nutritional value of pulses. However, recent research has indicated that low levels of phytic acid are beneficial as an antioxidant (Graf and Eaton, 1990). Consumption of phytate in food or use of purified sodium phytate has been associated with a reduction in glycemic response to starchy foods as well as a decrease in plasma triacylglycerol and cholesterol. Therefore, reduction of phytic acid through food processing suggests dual benefits of enhancing the bioavailability of proteins and dietary micronutrient (vitamins,



minerals) of legumes, and promoting health by controlling hypercholesterolemia and atherosclerosis (Thompson *et al.*, 1997). An additional benefit of phytic acid in rodents' model is its anticancer effect for colon, mammary gland, and other tumor cells (Shamsuddin *et al.*, 2002).

## 14.5 Bioactive peptides

Legumes have an abundance of protein and peptides involved in plant defense (Hao *et al.*, 2009). Chickpeas, dry peas, and faba beans are high in protein, approximately 20–30%, on a dry weight basis (Roy *et al.*, 2010). These percentages may vary depending on the plant species, variety, maturity, and growing conditions. The proteins include many antinutritional proteins, referred to as antimicrobial peptides, such as lectins or agglutinins, proteases enzyme inhibitors (trypsin and chymotrypsin inhibitors), ribosome-inactivating proteins, and the non-antinutritional compound, angiotensin I-converting enzyme (ACE) inhibitor (Roy *et al.*, 2010). Other antimicrobial peptides present in legumes include arcelins, chitinases,  $\beta$ -1, 3-glucanases, defensins, and  $\alpha$ -amylase inhibitors (Hao *et al.*, 2009).

The majority of proteins in legumes are storage proteins, which are classified as glutelins, albumin, and globulins, based on their solubility properties. In legumes, globulins (vicilin or legumin) represent ~70% of the total protein in pulses. Allergic reactions associated with the ingestion of vicilin and convicilin in peas and/or chickpeas have been reported, particularly in the Mediterranean area and certain Asian countries including India (Sanchez-Monge *et al.*, 2004; Ireneo *et al.*, 2008).

Despite the good content of protein, pulses lack some essential amino acids and are relatively low in leucine, tryptophan, threonine, and valin as well as total sulfur containing amino acids such as methionine and cysteine (El-Adawy, 2002; Roy *et al.*, 2010). However, chickpea protein is rich in essential amino acids such as isoleucine, lysine, tryptophan, and total aromatic amino acids (El-Adawy, 2002). Peas are generally a good source of lysine, histidine, and tyrosine. The limiting amino acids in pea protein include methionine and cysteine (Zdunczyk *et al.*, 1997). Thus, plant protein sources are often mixed with cereals for complementary feeding.

In terms of the digestibility of the legume proteins, *in vitro* studies have reported different values ranging from 78 to 95.6%, with some varieties showing the highest digestibility (Bhatty and Christison, 1984; Vidal-Valverde *et al.*, 2003). Bhatty and Christison (1984) found a protein digestibility of 84% for faba beans. However, the *in vitro* digestibility was improved with germination and cooking. The improvement may be associated with destruction of trypsin inhibitor, reduction of tannins and phytic acids, and denaturation of protein (El-Adawy, 2002).

An early study in 1952 reported interrelationships between consumption of faba beans and the agglutination of erythrocytes due to the presence of lectins in the seeds (Creger *et al.*, 1952). The ingestion of raw pulse seeds or flours of other legumes has been associated with similar health issues. Additional toxic effects include pancreatic enlargement, bloating, and vomiting, which have been observed and related to the presence of antinutritional compounds (ANC) (Roy *et al.*, 2010). However, these ANC, when properly processed, possess beneficial properties in the prevention and treatment of diseases. The interest in biological activity and potential use of ANC as nutraceuticals has increased in recent years.

### 14.5.1 Nutraceutical potential of peptides

Different peptides released *in vitro* or *in vivo* from plant proteins have been studied for their bioactivity and ability to regulate certain functions in the human body. Some of the health benefits attributed to those peptides include blood pressure and cholesterol lowering, antioxidant and antimicrobial properties, and enhancement of mineral bioavailability/absorption, among others.

A protein cyclophilin-like antifungal has been isolated from chickpea seeds. The *in vitro* study revealed the inhibitory effect of the compound on the growth of certain fungi, the human immunodeficiency virus-1 reverse transcriptase, and exhibited an anti-mitogenic activity on mouse splenocytes (Ye and Ng, 2002). Other researchers have extracted the proteinaceous alpha amylase inhibitor from chickpeas and measured its activity. The antinutritional protein inhibited 73.6% of the activity of human saliva sample, comparable to the  $\alpha$ -amylase from kidney bean, which has been used as a dietary supplement in body weight management. Thus, the ability of the chickpea extract to hinder the digestion of complex carbohydrates could potentially be useful in controlling obesity in humans (Hao *et al.*, 2009).

Faba bean properties against candida induced oral thrush have been reported. In order to study other potential antimicrobial activity of the plant, Zhang and Lewis (1997) extracted from faba beans the defensins peptides and named them as fabatins. A moderate bactericidal effect was observed against gram-negative *Escherichia coli* and gram-positive *Enterococcus hirae* when incubated with the peptides. However, a higher antibacterial activity was recorded against the gram-negative *pseudomonas aeruginosa* with a lower concentration of 1  $\mu$ g/ml resulting in a six-fold killing effect. The mechanism of action of various defensins is not well understood and some defensins' toxicity against gram-negative and gram-positive bacteria could be related to the initial binding of the positively charged peptides to the surfaces of lipopolysaccharides in gram-negative and teichoic acid in gram-positive (Zhang and Lewis, 1997; Carvalho and Gomes, 2009). Isolated antimicrobial peptides have shown potential to combat fungal and bacterial strains that are becoming multi-drug resistant. The abilities and usefulness of the various plant based peptides are a new opportunity for exploring how plant defensins could be developed into drugs for human use and present an opportunity for crop improvement (Wong *et al.*, 2006; Carvalho and Gomes, 2009).

Although research on the role of lectins remains in its infancy, studies based on animal models have reported the effect of lectins in preventing certain cancers, in activation of certain innate defense mechanisms and as an agent for preventing or controlling obesity (Ewen *et al.*, 2006; Hartmann and Meisel, 2007; Roy *et al.*, 2010). The mechanisms of action of lectins on tumor have been suggested to be the reduction in cell division; the lectins can bind to cell membranes or receptors, thus causing cytotoxicity and apoptosis. Some studies have also suggested their ability to increase the number of macrophages, thus increasing the tumor cells susceptibility to attack. The immunomodulatory properties of pulses have been mainly based on pea; in mice, the lectins activate the spleen lymphocytes. In human studies, lentil lectins have shown a robust effect on decreasing the onset of human hepatoma (Wang *et al.*, 2000) and Merkel skin carcinomas (Sames *et al.*, 2001). More research is still needed to analyze the potential of immunomodulatory agents in pulses that could enhance immune system activity.

When pulses or flour are consumed raw, protease inhibitors have the ability to interfere with digestion due to their resistance to pepsin and acidic pH of the human digestive tract.

The resultant suppression of the negative feedback regulating the pancreatic secretion stimulates its enlargement. The inadequate hydrolysis of dietary protein leads to a decrease in amino acid absorption and protein synthesis. Some food processing methods such as germination, cooking, dehulling, and extrusion cooking are used to deactivate protease inhibitors (Roy *et al.*, 2010). Denatured protease inhibitors have anti-inflammatory properties or therapeutic cancer agents; however, most studies have been performed with soybean extract (Roy *et al.*, 2010). Human and animal cells have been shown to contain some protease inhibitors, which could be an indication of the resistance of protease inhibitors to digestion and transport to various tissues (Moy and Bilings, 1994). Further studies are needed to assess the mechanism of anti-inflammation and anti-carcinogenicity of these compounds.

Angiotensin I converting enzyme (ACE) plays a role in the regulation of blood pressure and cardiovascular function. The enzyme activates the Angiotensin I into Angiotensin II, a vasoconstrictor, and also deactivates the bradykinin, which is a vasodilator. The ACE inhibition results in reduced high blood pressure. Synthetic ACE inhibitors are widely used; however, due to their side effects there is now an interest in inhibitory peptides from natural sources (Hong *et al.*, 2006). Peptides with these inhibitory activities have been isolated in peas, chickpeas, and other plant and animal proteins (Roy *et al.*, 2010). Many of these peptides have been discovered from the enzymatic hydrolysates of various food proteins, in the hope of being useful as a potential replacement to the synthetic nutraceutical ACE component. Although legume peptides have been shown in animal and human models to be effective in the prevention and treatment of high blood pressure and other heart related diseases *in vitro* (Aluko, 2008), the *in vitro* activity may not result in the same effect *in vivo*. Orally ingested ACE inhibitory peptides are exposed to gastrointestinal enzymes, which could result in inactivation or activation of peptides (Roy *et al.*, 2010).

## References

- Ajila, C.M., Brar, S.K., Verma, M., Tyagi, R.D., Godbout, S., and Valéro, J.R. (2010) *Extraction and Analysis of Polyphenols: Recent Trends*. Critical Reviews in Biotechnology, Boca Raton, Fla.
- Aluko E. Rotimi (2008) Determination of Nutritional and Bioactive Properties of Peptides. In Enzymatic Pea, Chickpea, and Mung Bean Protein Hydrolysates. *Journal of AOAC International*, **91**(4): 947–956.
- Anisha, G.S. and Prema, P. (2008) Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude R-galactosidase from *Streptomyces griseoalbus*. *Food Chemistry*, **106**: 1175–1179.
- Ann-Sofie Sandberg (2002) Bioavailability of minerals in legumes. *British Journal of Nutrition*, **88**: 3, S281–S285.
- Bau, H.M., Villanme, C., Nicolas, J.P., and Méjean, L. (1997) Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soy bean (*Glycine max*) seeds. *Journal of Science and Food Agriculture*, **73**: 1–9.
- Bhatty, R.S. and Christison, G.I. (1984) Composition and nutritional quality of pea (*Pisum sativum* L), faba bean (*Vicia faba* L spp minor) and lentil (*Lens culinaris* Medik) meals, protein concentrates and isolates. *Plant Foods for Human Nutrition*, **34**: 41–51.
- Bonilla, S., Noel-Suberville, C., Higuere, P., Puy Portillo, M., Macarulla, M.T., Martinez, A., and Garcin, H. (1998) Inclusion of a legume in a saturated fat-rich diet affects the cholesterol status but not the expression of triiodothyronine and retinoic acid receptors in rat liver. *Annals of Nutrition and Metabolism*, **42**: 297–303.
- Botham, R.L., Morris, V.J., Noel, T.R., and Ring, S.G. (1996) A study of the *in vivo* digestibility of retro-graded starch. *Carbohydrate Polymers*, **29**: 347–352.

- Carratù, B. and Sanzini, E. (2005) Biologically-active phytochemicals in vegetable food. *Ann its Super Sanita*, **41**(1): 7–16.
- Carvalho, A. deO. and Gomes, V.M. (2009) Plant defensins—Prospects for the biological functions and biotechnological. *Properties Peptides*, **30**: 1007–1020.
- Craig, W.J. (2009) Health effects of vegan diets. *The American Journal of Clinical Nutrition*, **89**(5): 1627S–1633S.
- Craig, W.J. and Mangels, A.R. (2009) Position of the American Dietetic Association: vegetarian diets. *Journal of the American Dietetic Association*, **109**(7): 1266–1282.
- Creger, W.P. and Gifford, H. (1952) Some interrelationships of blood and the faba beans. *Principle in vitro. Blood Journal*, **7**: 721–728.
- D'Archivo, M., Carmela, F., Di Benedetto, R., Raffaella, G., Claudio, G., and Masella, R. (2007) Polyphenols, dietary sources and bioavailability *Ann Ist Super Sanità*, **43**(4): 348–361.
- Duenas, M., Hernandez, T., and Estrella, I. (2006) Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, **98**: 95–103.
- El-Adawy, T.A. (2002) Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, **57**: 83–97.
- Ewen, S.S.W.B., Bardocz, S., Puztai, A., and Pryme, I.F. (2006) Suppression of growth of tumor cell lines in vitro and tumors in vivo by mistletoe lectins. *Histology and Histopathology*, **21**(3): 285–299.
- Fernandez-Pancho, M.S., D. Villano, A.M. Troncoso, and M.C. Garcia-Parrilla (2008) Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. *Critical Reviews in Food Science and Nutrition*, **48**: 649–671.
- Frühbeck, G., Monreal, I., and Santidrian, S. (1997) Hormonal implications of the hypocholesterolemic effect of intake of field beans (*Vicia faba* L.) by young men with hypercholesterolemia. *American Journal of Clinical Nutrition*, **66**: 1452–1460.
- Geervani, P. and Theophilus, F. (1981) Influence of legume starches on protein nutrition and availability of lysine and methionine to albino rats. *Journal of Food Science*, **46**: 817–828.
- Graf, E. and Eaton, J.W. (1990) Antioxidant functions of phytic acid. *Free Radical Biology And Medicine*, **8**: 61–69.
- Grande, F., Anderson, J.T., and Keys, A. (1965) Effect of carbohydrates of leguminous seeds, wheat and potatoes on serum cholesterol concentration in man. *Journal of Nutrition*, **86**: 313–317.
- Hong, G.L., Ju-Zhen Wan, Guo-Wei Le, and Yong-Hui Shi (2006) Novel angiotensin I-converting enzyme inhibitory peptides isolated from Alcalase hydrolysate of mung bean protein. *Journal of Peptide Science*, **12**: 509–514.
- Hao, X., Jianguai, L., Qinghua, S., Jusong, Z., Xiaoling, H.E., and Hao, M.A. (2009) Characterization of a novel legumin apha-amylase inhibitor from chickpeas (*Cicer arietinum* L.) seeds biosci. *Biotechnology and Biochemistry*, **73**(5): 1200–1202.
- Hartmann, R. and Meisel, H. (2007) Food-derived peptides with biological activity: From research to food applications. *Current Opinion in Biotechnology*, **18**: 163–169.
- Hawkins, A. and Johnson, S.K. (2005) In vitro carbohydrate digestibility of whole-chickpea and chickpea bread products. *International Journal of Food Sciences and Nutrition*, **56**(3): 147–155.
- Jenkins, D.J., Wolever, T.M., Taylor, R.H., Barker, H.M., and Fielden, H. (1980) Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *British Medical Journal*, **281**(6240): 578–580.
- Karlstrom, B., Vessby, B., Asp, N.G., Boberg, M., Lithell, H., and Berne, C. (1987) Effects of leguminous seeds in a mixed diet in noninsulin-dependent diabetic patients. *Diabetes Research*, **5**: 199–205.
- Klepacka, J., Gujska, E., and Michalak, J. (2011) Phenolic compounds as cultivar and variety distinguishing factors in some plant products. *Plant Foods for Human Nutrition*, DOI 10.1007/s11130-010-0205-1 Springerlink.com
- King, A. and Young, G. (1999) Characteristics and occurrence of phenolic phytochemicals. *Journal of the American Dietetic Association*, **99**(2): 213–218.
- López-Amoro's, M.L., Hernandez, T., and Estrella, I. (2006) Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, **19**: 277–283.
- Macarulla, M.T., Medina, C., De Diego, M.A., Chavarri, M., Zulet, M.A., Martinez, J.A., Noel-Suberville, C., Higuera, P., and Portillo, M.P. (2001) Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolaemic rats. *British Journal of Nutrition*, **85**: 607–614.

- Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004) Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**: 727–747.
- Marinangeli, C.P. and Jones, P.J. (2011) Whole and fractionated yellow pea flours reduce fasting insulin and insulin resistance in hypercholesterolaemic and overweight human subjects. *British Journal of Nutrition*, **105**(1): 110–117.
- Martínez Mercedes, Ireneo San, Dolores María, Enrique Ibáñez, Carnés Jerónimo Fernández-Caldas (2008). In vitro and in vivo cross-reactivity studies of legume allergy in a Mediterranean population. *Int Arch Allergy Immunol* **147**: 222–230.
- Moy, L.Y. and Bilings, P.C. (1994) A proteolytic activity in human breast cancer cell line which is inhibited by the anticarcinogenic Bowman-Birk protease inhibitor. *Cancer Letters*, **85**: 205–210.
- Muehlbauer, F.J. and Tullu, A. (1997) Vicia Faba L. <http://www.hort.purdue.edu/newcrop/cropfactsheets/fababean.html> <http://www.fao.org/docrep/t0646e/T0646E0s.htm> [http://www.agric.wa.gov.au/PC\\_92109.html?s=1001](http://www.agric.wa.gov.au/PC_92109.html?s=1001)
- Nestel, P., Cehun, M., and Chronopoulos, A. (2004) Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. *American Journal of Clinical Nutrition*, **79**: 390–395.
- Oelke, E.A., Oplinger, E.S., Hanson, C.V., Davis, D.W., Putnam, D.H., Fuller, E.I., and Rosen, C.J. (1991) Dry Field Pea, <http://www.hort.purdue.edu/newcrop/afcm/drypea.html>
- Pittaway, J.K., Ahuja, K.D.K., Robertson, I.K., and Ball, M.J. (2007) Effects of a controlled diet supplemented with chickpeas on serum lipids, glucose tolerance, satiety and bowel function. *Journal of the American College of Nutrition*, **26**(4): 334–340.
- Pittaway, J.K., Robertson, I.K., and Ball, M.J. (2008) Chickpeas may influence fatty acid and fiber intake in the ad libitum diet, leading to small improvements in serum lipid profile and glycemic control. *Journal of the American Dietetic Association*, **108**(6): 1009–1013.
- Rice-Evans, C., and Miller, N. (1997) Measurement of the antioxidant status of dietary constituents, low density lipoproteins and plasma. *Prostaglandins Leukot Essent Fatty Acids*, **57**(4–5): 499–505.
- Roy, A.F., Boye, J.I., and Simpson, B.K. (2010) A review bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International*, **43**: 432–442.
- Sanchez-Monge, R.G., Lopez-Torrejón, N., Pascualw, C.Y., Varela, J., Martín-Esteban, M., and Salcedo, G. (2004) Vicilin and convicilin are potential major allergens from pea. *Clinical and Experimental Allergy*, **34**: 1747–1753.
- Sames, K., Shumacher, U., Halata, Z., Van Damme, E.J., Peumans, W.J., Asmus, B., et al. (2001) Lectins as bioactive plant proteins: A potential in cancer treatment. *Critical Reviews in Food Science and Nutrition*, **45**: 425–445.
- Schatz, B. and Endres, G. (2009) Field pea production <http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/al166.pdf>
- Shahidi, F., Chava, U.D., Nacz, M., and Amarowicz, R. (2001) Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). *Journal of Agricultural Food Chemistry*, **49**: 926–933.
- Shurpalekar, K.S., Sundaravalu, D.E., and Rao, M.N. (1979) In vitro and in-vivo digestibility of legume carbohydrate. *Nutr Rep Review*, **19**: 111–117.
- Thorne, J.M., Thompson, U.L., and Jenkins, J.A.D. (1983) Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Journal of Clinical Nutrition*, **38**: 481–488.
- Trinidad, P., Mallillin, A.C., Loyola, A.S., Sagum, R.S., and Encabo, R.R. (2010) The potential health benefits of legumes as a good source of dietary fibre. *British Journal of Nutrition*, **103**: 569–574.
- Venn, B.J. and Mann, J.I. (2004) Cereal grains, legumes and diabetes. *European Journal of Clinical Nutrition*, **58**: 1443–1461.
- Vidal-Valverde, C., Frias, J., Hernández, A., Martín-Alvarez, P., Sierra, I., Rodríguez, C., Blázquez, I., and Vicente, G. (2003) Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) Seeds. *Journal of Scientific Food Agriculture*, **83**: 298–306.
- Wang, H., Ng, T.B., Ooi, V.E., and Liu, W.K. (2000) Effects of lectins with different carbohydrate-binding specificities on hepatoma, choriocarcinoma, melanoma and osteosarcoma cell lines. *International Journal of Biochemistry and Cell Biology*, **32**(3): 365–372.
- I Wong, J.H., Zhang, X.Q., Wang, H.X., and Ng, T.B. (2006) A mitogenic defensin from white cloud beans (*Phaseolus vulgaris*). *Peptides*, **27**(9): 2075–2081.

- Xu, B.J. and Chang, S.K.C. (2009) Effects of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*, **110**: 1–13.
- Yadahally, N., Sreerama, V., Sashikala, B., and Pratapa, V.M. (2010) Variability in the distribution of phenolic compounds in milled fractions of chickpea and horse gram: evaluation of their antioxidant properties. *Journal of Agricultural Food Chemistry*, **58**: 8322–8330.
- Ye, X.Y. and Ng, T.B. (2002) Isolation of a new cyclophilin-like protein from chickpeas with mitogenic, antifungal and anti-HIV-1 reverse transcriptase activities. *Life Sciences*, **70**: 1129–1138.
- Zhang, Y. and Lewis, K. (1997) Fabatins: new antimicrobial plant peptides. *FEMS Microbiology Letters*, **149**: 59–64.
- Zdunczyk, Z., Godyck, I.A., and Amarowicz, R. (1997) Chemical composition and content of antinutritional factors in Polish cultivars of peas. *Plant Foods for Human Nutrition*, **50**: 37–45.

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## **15 Bioactives and health benefits of lentils (*Lens culinaris L.*)**

D. Dan Ramdath and Rong Tsao

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### **15.1 Introduction**

Several epidemiological studies have demonstrated strong associations between a diet rich in plant-based foods and reductions in the risk of developing several types of non-communicable chronic diseases (NCDs), such as coronary heart disease, cancer, and type 2 diabetes mellitus. Additionally, these studies have also reported that diets rich in plant-based foods are associated with significant lowering of mortality rates from NCDs (Joshiyura *et al.*, 2001; Bazzano *et al.*, 2001, 2003; Rissanen *et al.*, 2003). Some of the purported benefits of consuming a varied diet rich in fruits and vegetables have been attributed to the phytochemicals contained within the plant matrix. The term phytochemical encompasses a large group of non-nutrient secondary metabolites that are found in plants, and are thought to play a significant role in the health effects associated with consuming a diet that is rich in plant-based foods. Although phytochemicals such as anthocyanins and polyphenols are commonly found in colored fruits and vegetables (Rissanen *et al.*, 2003; Rochfort and Panozzo, 2007), there is growing evidence that grain legumes make significant contributions to dietary phytochemical intakes, which may play an important role in the well known health benefits of legumes (Leterme, 2002; Tharanathan and Mahadevamma, 2003). The health promoting role of phytochemicals, as well as that of the various vitamins and minerals found in pulse, specifically lentils, will be examined in this chapter.

### **15.2 Epidemiology: pulses and chronic diseases**

Legumes, commonly referred to as “pulses” are grown in all parts of the world and are typically marketed as dry products (Schneider, 2002). Pulses are grain legumes and include lentils, peas, chickpeas, and beans, which are rich in protein and starch (Table 15.1). However, this definition excludes leguminous seeds that are used primarily

**Table 15.1** Summary of nutritional characteristics of Canadian green and red lentils

Composition (g/100g dry matter)	Canadian green lentils		Canadian red lentils	
	Mean	Range	Mean	Range
Protein	26.3	21.3–30.2	25.7	21.6–29.0
Starch	45.0	41.9–48.5	44.0	41.5–46.9
Amylose (% of total starch)	25.4	22.5–27.4	26.5	24.3–28.3
Fat	1.1	1.0–1.3	1.1	1.0–1.3
Minerals (mg/100g dry matter)				
Calcium	77.2	48.4–97.0	89.9	48.4–107.7
Copper	1.0	0.7–1.3	1.1	0.7–1.6
Iron	7.6	5.4–11.4	8.1	5.4–13.1
Potassium	964.7	550.8–1286.5	1104.0	550.8–1233.1
Magnesium	126.8	104.1–167.1	127.5	104.1–150.0
Manganese	1.6	0.6–2.9	1.8	0.6–2.9
Phosphorus	455.8	260.3–725.8	503.7	260.3–611.2
Zinc	3.9	2.9–5.9	4.3	2.9–5.1
Vitamins (mg/100g)				
Retinol (A)	ND	ND	ND	ND
C	0.71	0.26–2.18	0.73	0.45–0.89
D	ND	ND	ND	ND
Thiamin (B1)	0.29	0.13–0.51	0.34	0.28–0.41
Riboflavin (B2)	0.33	0.23–0.45	0.31	0.29–0.36
Niacin	2.57	0.57–3.56	1.73	1.28–2.47
Panthenic acid (B5)	1.32	0.71–2.06	1.10	0.87–1.96
Pyridoxine (B6)	0.23	0.16–0.33	0.28	0.17–0.34
Cyanocobalamin (B12)	ND	ND	ND	ND
Biotin	ND	ND	ND	ND
γ-tocopherol	5.08	3.10–6.36	5.84	4.95–6.37
α-tocopherol	0.60	0.36–0.88	0.49	0.42–0.70
Folic acid (μg/100g)	138.11	100.0–217.0	139.6	101.1–177.0
Fatty Acid (% in oil)				
Palmitic (C16:0)	12.66	10.79–15.36	14.74	13.25–15.77
Oleic (C18:1)	20.86	17.04–25.63	19.82	17.05–22.17
Linoleic (C18:2)	44.38	40.97–46.14	44.29	42.91–45.23
Linolenic (C18:3)	14.15	11.93–16.23	13.51	12.68–14.66
Sugars (g/100g dry matter)				
Sucrose	2.01	1.68–2.48	1.80	1.64–1.97
Raffinose	0.43	0.33–0.49	0.42	0.34–0.51
Stachyose	2.09	1.66–2.63	1.94	1.67–2.29
Verbascose	0.56	0.36–0.76	0.52	0.39–0.69
Phytic acid (g/100g dry matter)	0.79	0.30–1.20	1.02	0.77–1.20
Tannins (g/100g dry matter)	0.54	0.30–1.02	0.61	0.44–0.79
TIA (mg/g dry matter)	2.57	1.94–3.38	2.74	2.46–3.31

ND = not detected.

TIA = trypsin inhibitor activity.

Source: Modified from Wang and Daun, 2004.



for oil extraction, such as soybeans (Schneider, 2002). Although previously considered primarily to be an economically viable substitute for meat, there is increasing recognition that pulses are an important component of a healthy diet. In addition to being an important source of essential nutrients, the consumption of pulses is associated with a reduced risk for developing several types of chronic diseases, including coronary heart disease, diabetes, and obesity (Geil and Anderson, 1994; Bazzano *et al.*, 2001). Early epidemiological data from the Seven Country Study suggested that flavonoid intake may partly contribute to differences in coronary heart disease mortality across populations, but does not seem to be an important determinant of cancer mortality (Hertog *et al.*, 1995). More recently, however, the American Institute for Cancer Research has promoted the inclusion of pulses in two of their 15 recommendations for dietary measures to prevent cancer (Leterme, 2002). Regular intake of pulse foods (particularly beans and lentils) is also associated with a plasma cholesterol-lowering effect, which can reduce the risk of developing heart disease (Anderson *et al.*, 1984; Shutler *et al.*, 1989; Kingman, 1991).

Regular consumption of pulses is a major feature of the Mediterranean diet and is a long-standing tradition of many Asian cultures, especially the Indian sub-continent. However, in North America where much of the world's pulse production occurs there is very limited local consumption. Increasing evidence that support the health benefits of pulses has provoked a renewed interest by the industry to promote increased consumption of pulses. For example, Kabagambe and others (2005) reported that daily consumption of one serving of beans is associated with a 38% lower risk of myocardial infarction (Kabagambe *et al.*, 2005). However, there are many gaps in the evidence currently available and, as such, regulatory agencies have been reluctant to approve a pulse health claim. The American Heart Association has included in its recommendation for a heart healthy diet the consumption of four to five servings per week of pulses, nuts, and seeds, where one serving is equal to half a cup of cooked dry beans or peas (AHA, 2010). The Canada Food Guide recommends minimizing dietary saturated fat intake by eating meat alternatives such as beans, lentils, and tofu on a regular basis (Health Canada, 2007).

In appraising the available evidence Anderson and Major (2002) performed a meta-analysis of eleven clinical trials that examined the effects of pulses (excluding soybeans) on serum lipids. They found that intake of non-soya pulses was associated with modest but significant changes in total cholesterol (−7.2%), LDL-cholesterol (−6.2%), and HDL-cholesterol, triacylglycerols, and body weight (Anderson and Major, 2002). It was suggested that part of the cholesterol lowering effects of pulses was attributable to their content of isoflavones, phospholipids and fatty acids, and saponins among other factors. Table 15.2 summarizes the available evidence for the health promoting properties of lentils along with possible modes of action. It seems evident that regular intakes of lentils may reduce the risk for CVD by favorable effects on blood pressure, and glycaemia and risk for diabetes. Collectively, the available evidence suggests that regular consumption of pulses may have important protective effects on risk for CVD, type 2 diabetes and certain types of cancers. However, neither the active components that confer these health benefits nor the mode of action is known.

**Table 15.2** Summary of health benefits associated with nutrients found in lentils

<b>Evidence for the disease preventing properties of lentils and possible mode of action</b>		
	<b>Protective effect</b>	<b>Property of lentils</b>
CVD	<p>Reduce serum lipids (total and LDL-cholesterol, triacylglycerols)<sup>1</sup>.</p> <p>The food form appears to affect cholesterol-lowering ability, where whole pulses are observed to lower LDL-cholesterol more significantly than ground pulses<sup>4</sup>.</p>	<p>Mainly soluble fibre, may also be due to synergistic action of protein, resistant starch, oligosaccharides, vitamins, minerals and phytochemicals<sup>2,3</sup>.</p>
Diabetes	<p>Observed to be a low glycemic food (GI ranges from 28–52)<sup>2,5,6</sup>.</p>	<p>Low GI attributed to nonstarch polysaccharides, resistant starch and oligosaccharides – delay gastric emptying rate and slow digestion of starch in small intestine.</p> <p>Decreased blood glucose may also be due to the presence of phytic acid, lectins, amylase inhibitors, or polyphenols compounds<sup>2,5,6</sup>.</p>
Cancer	<p>Non-digestible carbohydrates may act as prebiotics, stimulating the growth of bifidobacteria and lactobacilli, resulting in increased formation of the short chain fatty acid butyrate (antitumour and anti-inflammatory activity)<sup>8,9,10,11,12</sup>.</p> <p>Inverse correlation between pulse consumption and colon cancer mortality, reduced risk of prostate, gastric, pancreatic cancer in epidemiological studies<sup>13,14</sup>.</p> <p>Can have a protective effect against colorectal, prostate and lung cancer through its antioxidant effect<sup>15,16</sup>.</p> <p>Contain saponins, which exhibit direct and selective cytotoxic action against cancer cells; act as immune stimulants and regulate cell proliferation<sup>17</sup>.</p>	<p>Insoluble dietary fibre Oligosaccharides Resistant starch</p> <p>Dietary fibre, oligosaccharides, folate selenium, protease inhibitors, phytic acid, lignans, phenolic acids, saponins, isoflavones<sup>7</sup>.</p> <p>Pulses grown selenium-rich soil (e.g. Canada) are good sources of selenium, which is a useful antioxidant in biological systems<sup>15,16</sup>.</p> <p>Saponins<sup>17</sup>.</p>

Source: Data sources:

<sup>1</sup>Anderson and Major, 2002; <sup>2</sup>Rizkalla *et al.*, 2002; <sup>3</sup>Schulze *et al.*, 2007; <sup>4</sup>Jarvi *et al.*, 1999; <sup>5</sup>Foster-Powell *et al.*, 2002; <sup>6</sup>Messina, 1999; <sup>7</sup>Mathers, 2002; <sup>8</sup>Lanza *et al.*, 2006; <sup>9</sup>Gonzalez de Mejia *et al.*, 1999; <sup>10</sup>Hangen and Bennink, 2002; <sup>11</sup>Jain *et al.*, 1999; <sup>12</sup>Marchand *et al.*, 1991; <sup>13</sup>Patterson and Levander, 1997; <sup>14</sup>Rao and Sung, 1995; <sup>15</sup>Dueñas *et al.*, 2003; <sup>16</sup>Dilis and Trichopolou, 2009; <sup>17</sup>Han and Baik, 2008.

### 15.3 Health effects of pulse carbohydrates

Pulses contain numerous compounds with demonstrated benefits towards improving glycaemic control and reduction of CHD risk factors (Rizkalla *et al.*, 2002; Wolever *et al.*, 2008; Sievenpiper *et al.*, 2009; Pulse Canada, 2009). Pulse-rich diets are associated with decreases in both serum glucose and HbA<sub>1c</sub> (Sievenpiper *et al.*, 2009; Pulse Canada, 2009) and this is potentially very important in the search for adjuncts to improve the management of T2DM, and thereby reduce CVD risk. Inclusion of lentils as part of a healthy diet can confer a significant reduction of the dietary glycaemic load (GL), which reflects dietary carbohydrate quality and quantity, and is directly related to the increased risk for type 2 diabetes (Liu *et al.*, 2000) and other chronic diseases (Barclay *et al.*, 2008). There is also evidence to suggest that a low GL diet might reduce exposure of endothelial cells to fluctuating glucose concentrations, increased oxidative stress, and apoptosis; these if left unchecked may trigger proinflammatory responses and hepatic damage (Risso *et al.*, 2001). As such a low GL diet that incorporates lentils and other pulses may have significant implications for minimizing the tissue and organ damage associated with chronic elevation of circulating blood glucose.

Lentils and other pulses are well known for their high fiber content (total and soluble), low glycaemic load, anti-nutrients, and polyphenols and have been recommended as key components of weight loss strategies (Wylie-Rosett *et al.*, 2004). The fiber components have been considered one of the primary reasons for the positive effects of legumes on plasma lipid levels (Delzenne and Cani, 2005; Pulse Canada, 2009). Fiber is one type of non-protein bioactive found in high quantities in lentils and has received much attention for its ability to lower blood pressure and serum cholesterol, and to improve insulin sensitivity in diabetics and non-diabetics alike (Festa *et al.*, 2006). Lentils are known to be high in fiber, containing approximately 8 g of fiber per 100 g of cooked lentils (USDA, 2009). The high total and soluble fiber contents of pulses have been associated with a low glycemic response, which is very useful in the dietary management of diabetes (Kreisberg, 1998). In a prospective observational study, an inverse relationship was found between dietary fiber intake and increased risk of developing diabetes (Castro *et al.*, 2005). Further, a systematic review of 41 clinical trials assessing the effect of pulses on glycaemic control revealed that pulses alone, or in low-glycaemic index (GI) or high fiber diets were able to improve fasting blood glucose, insulin, and HbA<sub>1c</sub> (Sievenpiper *et al.*, 2009). An inverse association between fiber intake and cardiovascular death has also been observed in both men and women, and increasing dietary fiber was observed to protect against cardiovascular disease and its risk factors (Brown *et al.*, 1999; Pereira *et al.*, 2004; Whelton *et al.*, 2005; Schulze *et al.*, 2007).

In an attempt to elucidate the mechanism of action of pulse starch on glycaemic response, Liu and others (2008) isolated and characterized the starch fractions of different cultivars of pea, lentil and chickpea grown in Canada under identical environmental conditions. The *in vitro* digestibility and physicochemical properties were investigated and the correlations between physicochemical properties and starch digestibility were determined. Lentil starch exhibited lower rapidly digestible starch (RDS) content and hydrolysis rate. Consequently the expected GI of the starch fraction from lentils was the lowest among the tested starch fractions (Liu *et al.*, 2008).

## 15.4 Health promoting vitamins and minerals in lentils

The benefits of consuming lentils extend beyond their most well-recognized characteristic of being low in fat and high in protein; indeed their non-protein portion, especially starch quality and quantity, may confer significant health benefits (Salas-Salvadó *et al.*, 2006; Campos-Vega *et al.*, 2009). Table 15.1 shows that lentils also contain useful amounts of mineral nutrients such as iron, potassium, magnesium, manganese, copper, phosphorous, and zinc. Although the bioavailability of iron in pulses may be affected by the presence of phytic acid, parallel intakes of vitamin C rich beverages, and foods could promote iron absorption, thereby making it more bioavailable (Hallberg *et al.*, 1989). Iron deficiency is the most common nutrient deficiency globally, and lentils could play a useful role as a source of iron. Given their potassium content, lentils represent a good source of this mineral and in light of the growing global efforts to reduce dietary sodium intakes of lentils should be encouraged. Several studies have shown that unlike sodium, increase dietary potassium has a protective effect against risk of stroke and high blood pressure (Ascherio *et al.*, 1998; Larsson *et al.*, 2011). Complementing this is the fact that lentils are an excellent source of folic acid; on average one serving (half a cup) of cooked lentils provides the 45% of the recommended intake of folic acid for an adult – 400 µg/day (AHA, 2010). Prior to conception and during pregnancy women are encouraged to increase their folic acid intake since inadequate intake of folic acid has been linked to neural tube defects in newborn infants (Wolff *et al.*, 2009). These defects have been attributed to the fact that folic acid is essential for one-carbon methylation reaction, including DNA synthesis (McKay *et al.*, 2004). During the activation of dietary folic acid, tetrahydrofolic acid is converted to methylene-tetrahydrofolate by the addition of one-carbon donors (such as serine and glycine), which is used for DNA synthesis and other one-carbon addition reactions. Similarly, when folate levels are low in the presence of normal B<sub>12</sub> there is insufficient methyl tetrahydrofolic and the conversion of homocysteine to methionine by methionine synthase is impaired, resulting in high levels of homocysteine. It has been suggested that elevated blood homocysteine may cause damage to the endothelial cell lining of arteries and promote blood clots (AHA, 2010).

As shown in Table 15.1, there are useful amounts of the essential fatty acids linoleic and linolenic acid; however, there are very little fat soluble vitamins in lentils. Some varieties (red gram) may be a useful source of lutein. Mamatha *et al.* (2011) have recently reported values of between 185 and 200 µg/100 g dry weight in split red gram (Mamatha *et al.*, 2011).

## 15.5 Health promoting phenolic compounds in lentils

As mentioned earlier, like other pulses, lentils are not only an excellent source of macronutrients such as protein, fatty acids, fibers, and carbohydrates but also contain phytochemicals that are increasingly being recognized for their potential benefits to human health. However, unlike other legumes such as soybean, the phytochemical profiles of lentils and their potential health benefits have not been well studied. While phenolic compounds are known to be the major phytochemicals in pulses including lentils, due to the lack of

direct information, they were not part of the recent review by Rochfort and Panozzo (2007). These authors examined the chemistry, biochemistry, and effect of processing and cooking on the health promoting phytochemicals, phytosterols, resistant starch, bioactive carbohydrates, alkaloids and saponins, and isoflavones. Apart from isoflavones, which are only found in significant amount in soybeans, no phenolic compounds were discussed (Rochfort and Panozzo, 2007).

Phenolic compounds are strong antioxidants and those from other foods, particularly fruits and vegetables, have been shown to be associated with the reduced risk of many chronic diseases (Tsao, 2010; Bazzano *et al.*, 2001, 2003). However, little is known about the phenolic composition of lentils and how they contribute to health. Red lentils are primarily consumed after the seed coat is removed by abrasive dehulling, while green lentils are usually eaten whole. Recent studies have indicated that the majority of phenolics in lentils are essentially located in their seed coat or hull (Dueñas *et al.*, 2002), therefore, milling along with other processing methods can significantly change the phenolic composition of lentils. The seed coat was found to be very rich in catechins, procyanidins dimers and trimers, but contained small amounts of glycosides of quercetin, myricetin, luteolin, and apigenin, whereas the cotyledon contained mainly hydroxybenzoic and hydroxycinnamic acids in low concentration. Hydroxycinnamic acid esters were identified in the cotyledon, and interestingly a stilbene trans-resveratrol-5-glucoside was found for the first time in the seed coat of lentils (Dueñas *et al.*, 2002). The same researchers also found that in the seed coat of lentils the main polyphenols group was proanthocyanidins, including monomeric, oligomeric, and polymeric forms. The most abundant proanthocyanidins in the seed coat of lentils were the polymers (65–75%), with a mDP (mean degree of polymerization) of 7–9, followed by the oligomers (20–30%), with a mDP of 4–5 (Dueñas *et al.*, 2003). The major monomeric flavan-3-ol was (+)-catechin-3-glucose, with lesser amounts of (+)-catechin and (–)-epicatechin. Various dimer, trimer, and tetramer proanthocyanidins constituted of catechin, gallocatechin, and catechin gallate units were also identified, as well as several procyanidins and prodelphinidins from pentamers to nonamers (polymers) (Dueñas *et al.*, 2003). In black lentils, the major anthocyanin is delphinidin 3-O-(2-β-D-glucopyranosyl-α-L-arabinopyranoside). Many other phenolic compounds have also been identified in lentils.

Recently, Amarowicz and others (2009) identified 24 phenolic compounds including phenolic acids and flavonoids in the extract of lentils. *p*-Hydroxybenzoic acid was found to be the dominant phenolic acid, and quercetin diglycoside, catechin, and digallate procyanidin were the main flavonoids identified. Fractions containing flavonoids and procyanidins were most responsible for the antioxidant activities (Amarowicz *et al.*, 2009). The lentil seed coat with higher flavonoid compounds was found to have superior antioxidant activity than that of other pulses such as peas (Dueñas *et al.*, 2006). They also found that flavones, flavonols, and proanthocyanidins from methanolic extract of lentils contributed the most antioxidant capacity to the seed coat, whereas the flavonoid catechin was the major antioxidant contributor of the cotyledon (Dueñas *et al.*, 2006). Others have also reported on the importance of proanthocyanins (condensed tannins) of lentils. The fraction of the lentil extract enriched in condensed tannins exhibited significantly higher values of total phenolic and total condensed tannin contents, and antioxidant activity as compared to the crude extract and semi purified fractions (Zou *et al.*, 2011). Eighteen compounds including kaempferol glycoside, and other flavonoid glycosides and proanthocyanins, were identified and found to contribute the most to the antioxidant activities *in vitro* (Zou *et al.*, 2011).

Many factors can affect the phenolic composition, and therefore the bioactivities found in lentils after harvest. The total phenolic content, total flavonoids content, and condensed tannins content (proanthocyanins) of lentils were found to be greatly dependent on the extraction solvent used, leading to significantly altered antioxidant activities *in vitro* (Xu and Chang, 2007). Processing can significantly affect the phytochemical content and profiles, and the antioxidant capacities in pulses including lentils. In lentils, 29% of proanthocyanidins were removed during soaking in water at 30 °C for three hours, and the same amount at 100 °C for 30 minutes. Tannin contents were further reduced during cooking (Rehman and Shah, 1996). More recently, four thermal processing methods (conventional boiling, conventional steaming, pressure boiling, and pressure steaming) have been found to cause significant ( $P < 0.05$ ) reductions in total phenolic, procyanidin, total saponin, phytic acid, chemical antioxidant capacities (ferric reducing antioxidant power and peroxy radical scavenging capacity), and cellular antioxidant activity as well as antiproliferation capacities of cool-season food legumes, compared to the original raw legumes. Different cooking methods have varied effects on total phenolics, saponins, phytic acids, and individual phenolic compounds. All thermal processing methods (except conventional steaming) caused significant ( $P < 0.05$ ) decreases in gallic, chlorogenic, p-coumaric, sinapic, subtotal benzoic, subtotal cinnamic acid, and total phenolic acid of lentil. (Xu and Chang, 2009). Exogenous enzyme treatment also causes changes to the phenolic composition of lentil flours, particularly those of the hydroxycinnamic compounds and proanthocyanidins. However, quercetin 3-O-rutinoside and luteolin increased and reached the highest concentration with tannase. Enzyme treatments with viscozyme,  $\alpha$ -galactosidase, or tannase produced an increase in the antioxidant activity when compared to raw lentils. Quercetin 3-O-rutinoside appeared to be the compound with the greatest influence on the antioxidant activity (Dueñas *et al.*, 2007). Soaking and cooking studies on chickpeas and lentils suggest that 2–5% of saponin content can be lost from chickpeas during cooking, but a much larger 6–14% can be lost from lentils (Ruiz *et al.*, 1996).

While antioxidant activities of the phytochemicals, particularly the phenolic compounds in lentils may play important roles in reducing risks of many chronic diseases, other activities such as the inhibitory effects against important gastrointestinal enzymes of starch digestion,  $\alpha$ -glucosidase,  $\alpha$ -amylase and aldose reductase have also attracted much attention in the last few years. Strong inhibitors of these key enzymes have the potential to lower the incidence of long-term complications of diabetes mellitus by tempering the rate of increase of post prandial blood glucose levels. Flavonoids such as quercetin, kaempferol, and their glycosides have been shown as potent inhibitors of  $\alpha$ -glucosidase *in vitro* (Li *et al.*, 2009; Habtemariam, 2011). Kaempferol-3-O-rutinoside, for example, was found most recently to have over eight times more potency towards  $\alpha$ -glucosidase than the reference drug, acarbose, which is used as an adjunct to manage persons with type 2 diabetes. Furthermore, it displayed a synergistic effect with a less potent flavonoid aglycones, kaempferol, and quercetin (Habtemariam, 2011). Termentzi and others (2008) have also attributed the aldose reductase inhibitory activity of the *Sorbus domestica* fruit to the high content of flavonoids and hydroxycinnamoyl esters, both of which have been found in lentils, as mentioned above.

The health benefits of lentils are likely to be the collective effects of all the bioactive components discussed above, including macromolecules such as resistant starch and protein, and micronutrients such as vitamins and phenolic compounds. The roles of phenolic compounds, i.e. phenolic acids, flavonoids, and proanthocyanins, in lentils will become clearer as more research is conducted. In addition, human studies underway presently will add to

these findings and provide further insights into the mechanism of action of the health promoting components of lentils. Studies on the dose response of lentil consumption are required as are studies to examine the bioavailability and specific health benefits associated with the various polyphenols that are present in lentils. Collectively, it is anticipated that this evidence will better inform health promotion messages for the increased consumption of lentils and other pulses.

## References

- AHA, American Heart Association. (2010) How Do I Follow a Healthy Diet? [www.heart.org](http://www.heart.org).
- Amarowicz, R., Estrella, I., Hernández, T., Dueñas, M., Troszyńska, A., Agnieszka, K., and Pegg, R.B. (2009) Antioxidant activity of a red lentil extract and its fractions. *International Journal of Molecular Sciences*, **10**: 5513–27.
- Anderson, J., Story, L., Sieling, B., Chen, W., Petro, M., and Story, J. (1984) Hypocholesterolaemic effects of oat bran or bean intake for hypercholesterolaemic men. *American Journal of Clinical Nutrition*, **40**: 1146–1155.
- Anderson, J.W., and Major, A.W. (2002) Pulses and lipaemia, short- and long-term effect: potential in the prevention of cardiovascular disease. *British Journal of Nutrition*, **88**: 263–271.
- Ascherio, A., Rimm, E.B., Hernán, M.A., Giovannucci, E.L., Kawachi, I., Stampfer, M.J., and Willett, W.C. (1998) Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. *Circulation*, **98**: 1198–204.
- Barclay, A.W., Petocz, P., McMillan-Price, J., Flood, V.M., Prvan, T., Mitchell, P., and Brand-Miller, J.C. (2008) Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. *American Journal of Clinical Nutrition*, **87**: 627–637.
- Bazzano, L.A., He, J., Ogden, L.G., Loria, C., Vupputuri, S., Myers, L., and Whelton, P.K. (2001) Legume consumption and risk of coronary heart disease in US men and women. *Archives on Internal Medicine*, **161**: 2573–2578.
- Bazzano, L.A., Serdula, M.K., and Liu, S. (2003) Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Current Atherosclerosis Reports*, **5**: 492–499.
- Boushey, C.J., Beresford, S.A., Omenn, G.S., and Motulsky, A.G. (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease probable benefits of increasing folic acid intakes. *JAMA*, **274**: 1049–1057.
- Brown, L., Rosner, B., Willett, W.W., and Sacks, F.M. (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition*, **69**: 30–42.
- Campos-Vega, R., Raynoso-Camacho, R., Pedraza-Aboytes, G., Acosta-Gallegos, J.A., Guzman-Maldonado, S.H., Paredes-Lopez, O., Oomah, B.D., and Loarca-Pina, G. (2009) Chemical composition and in vitro polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, **74**: T59–T65.
- Chung, H.-J., Liu, Q., Donner, E.A., Hoover, R., Warkentin, T.D., and Vandenberg, A. (2008) Composition, molecular structure, thermal properties, pasting characteristics and in vitro digestibility of starches from newly released canadian cultivars of pea, lentil and chickpea. *Cereal Chemistry*, **85**: 473–781.
- Castro, I.A., Barroso, L.P., and Sinnecker P. (2005) Functional foods for coronary heart disease risk reduction: a meta-analysis using a multivariate approach. *American Journal of Clinical Nutrition*, **82**: 32–40.
- Delzenne, N.M. and Cani, P.D. (2005) A place for dietary fibre in the management of the metabolic syndrome. *Current Opinion in Clinical Nutrition and Metabolic Care*, **8**: 636–640.
- Dilis, V. and Trichopoulou, A. (2009) Nutritional and health properties of pulses. *Mediterranean Journal of Nutrition and Metabolism*, **1**: 149–157.
- Dueñas, M., Hernández, T., and Estrella, I. (2002) Phenolic composition of the cotyledon and the seed coat of lentils (*Lens culinaris* L.). *European Food Research Technology*, **215**: 478–483.
- Dueñas, M., Hernández, T., and Estrella, I. (2006) Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, **98**: 95–103.

- Dueñas, M., Hernández, T., and Estrella, I. (2007) Changes in the content of bioactive polyphenolic compounds of lentils by the action of exogenous enzymes. *Effect on their antioxidant activity. Food Chemistry*, **101**: 90–97.
- Dueñas, M., Sun, B., Hernández, T., Estrella, I., and Spranger, M.I. (2003) Proanthocyanidin composition in the seed coat of lentils (*Lens culinaris* L.). *Journal of Agricultural Food Chemistry*, **51**: 7999–8004.
- Festa, A., Williams, K., D'Agostino, R., Wagenknecht, L.E., and Haffner, S.M. (2006) The natural course of  $\beta$ -cell function in nondiabetic and diabetic individuals. *Diabetes*, **55**: 1114–1120.
- Foster-Powell, K., Holt, S.H.A., and Brand-Miller, J.C. (2002) International table of glycemic load values: 2002. *American Journal of Clinical Nutrition*, **76**: 5–56.
- Geil, P.B. and Anderson, J.W. (1994) Nutrition and health implications of dry beans: a review. *Journal of the American College of Nutrition*, **13**: 549–558.
- Gonzalez de Mejia, E., Castano-Tostado, E., and Loarca-Pina, G. (1999) Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research*, **441**: 1–9.
- Habtemariam, S. (2011)  $\alpha$ -glucosidase inhibitory activity of kaempferol-3-O-rutinoside. *Natural Product Communications*, **6**: 201–3.
- Hallberg, L., Brune, M., and Rossander, L. (1989) The role of vitamin C in iron absorption. *International Journal for Vitamin and Nutrition Research*, **30**: 103–8.
- Han, H. and Baik, B.K. (2008) Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *International Journal of Food Science Technology*, **43**: 1971–1978.
- Hangen, L. and Bennink, M.R. (2002) Consumption of black beans and navy beans (*phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Nutrition and Cancer*, **44**: 60–65.
- Health Canada. (2007) Eating Well with Canada's Food Guide. [www.hc-sc.gc.ca](http://www.hc-sc.gc.ca)
- Hertog, M.G.L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B.S., Toshima, H., Feskens, E.J.M., Hollman, P.C.H., and Katan, M.B. (1995) Flavanoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine*, **155**: 381–386.
- Jain, M.G., Hislop, G.T., Howe, G.R., and Ghadirian, P. (1999) Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. *Nutrition and Cancer*, **34**: 173–184.
- Jarvi, A.E., Karlstrom, B.E., Granfeldt, Y.E., Bjorck, I.E., Asp, N.G.L., and Vessby, B.O.H. (1999) Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care*, **22**: 10–18.
- Joshiyura, K.J., Hu, F.B., Manson J.E., Stampfer, M.J., Rimm, E.B., Speizer, F.E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D., and Willett, W.C. (2001) The effect of fruit and vegetable intake on risk for coronary heart disease. *Annals of Internal Medicine*, **134**: 1106–1114.
- Kabagambe, E.K., Baylin, A., Ruiz-Narvarez, Siles, X., and Campos, H. (2005) Decreased consumption of dried mature beans is positively associated with urbanization and nonfatal acute myocardial infarction. *Nutritional Epidemiology*, **135**: 1770–1775.
- Kingman, S. (1991) The influence of legume seeds on human plasma lipid concentrations. *Nutrition Research Review*, **4**: 97–123.
- Kreisberg, R.A. (1998) Diabetic dyslipidemia. *American Journal of Cardiology*, **82**: 67U–73U.
- Lanza, E., Hartman, T.J., Albery, P.S., Shields, R., Slattery, M., Caan, B., Paskett, E., Iber, F., Kikendall, J.W., Lance, P., Daston, C., and Schatzkin, A. (2006) High dry bean intake and reduced risk of advanced colorectal adenoma recurrence among participants in the polyp prevention trial. *Journal of Nutrition*, **136**: 1896–903.
- Larsson, S.C., Virtamo, J., and Wolk, A. (2011) Potassium, calcium, and magnesium intakes and risk of stroke in women. *American Journal of Epidemiology*, May 3. [Epub ahead of print] doi: 10.1093/aje/kwr051
- Leterme, P. (2002) Recommendations by health organizations for pulse consumption. *British Journal of Nutrition*, **88**(Suppl. 3): S239–242.
- Li, H., Song, F., Xing, J., Tsao, R., Liu, Z., and Liu, S. (2009) Screening and structural characterization of alpha-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MS(n) and SORI-CID FTICR MS. *Journal of the American Society of Mass Spectrometry*, **20**: 1496–1503.
- Liu, S., Willett, W.C., Stampfer, M.J., Hu, F.B., Franz, M., Sampson, L., Hennekens, C.H., and Manson, J.E. (2000) A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *American Journal of Clinical Nutrition*, **71**: 1455–1461.



- Mamatha, B.S., Sangeetha, R.K., and Baskaran, V. (2011) Provitamin-A and xanthophyll carotenoids in vegetables and food grains of nutritional and medicinal importance. *International Journal of Food Science and Technology*, **46**: 315–323.
- Marchand, L.L., Hankin, J.H., Kolonel, L.N., and Wilkens, L.R. (1991) Vegetable and fruit consumption in relation to prostate cancer risk in hawaii: a reevaluation of the effect of dietary beta-carotene. *American Journal of Epidemiology*, **133**: 215–219.
- Mathers, J.C. (2002) Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. *British Journal of Nutrition*, **88**(Suppl. 3): S273–S279.
- McKay, J.A., Williams, E.A., and Mathers, J.C. (2004) Folate and DNA methylation during in utero development and aging. *Biochemical Society Transactions*, **32**: 1006–1007.
- Messina, M.J. (1999) Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*, **70**(Suppl.): 439S–50S.
- Patterson, B.H. and Levander, O.A. (1997) Naturally occurring selenium compounds in cancer chemoprevention trials: a workshop summary. *Cancer Epidemiology Biomarkers and Prevention*, **6**: 63–69.
- Pereira, M.A., P'Reilly, E., and Augustsson, K. (2004) Dietary fibre and risk of coronary heart disease: a pooled analysis of cohort studies. *Archives in Internal Medicine*, **164**: 370–376.
- Pulse Canada. (2009) The health benefits of pulses: clinical trial findings, dry beans, peas, lentils, chickpeas. <http://www.pulsecanada.com/uploads/9f/96/9f9658890a7180395b648f83f26978c5/09-Apr-20-Clinical-Trial-release.pdf> Accessed July 30, 2009.
- Rao, A.V. and Sung, M.K. (1995) Saponins as anticarcinogens. *Journal of Nutrition*, **125**(Suppl. 3): 717S–724S.
- Rehman, Z. and Shah, W.H. (1996) Effect of different domestic processing and cooking methods on the tannin contents of lentils (*Lens esculenta*), *Pakistan Journal of Scientific and Industrial Research*, **39**: 60–63.
- Rissanen, T.H., Voutilainen, S., Virtanen, J.K., Venho, B., Vanharanta, M., Mursu, J., and Salonen, J.T. (2003) Low intake of fruits, berries and vegetables is associated with excess mortality in men: the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. *Nutritional Epidemiology*, **33**(1): 199–204.
- Risso, A., Mercuri, F., Quagliari, L., Damante, G., and Ceriello, A. (2001) Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. *American Journal of Physiology - Endocrinology and Metabolism*, **281**: E924–930.
- Rizkalla, S.W., Bellisle, F., and Slama, G. (2002) Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. *British Journal of Nutrition*, **88**(Suppl. 3): S255–S262.
- Rochfort, S., and Panozzo, J. (2007a) Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry*, **55**: 7981–94.
- Rochfort, S. and Panozzo, J. (2007b) Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry*, **55**: 7798–7994.
- Ruiz, R.G., Price, K.R., Arthur, A.E., Rose, M.E., Rhodes, M.J.C., Fenwick, and R.G. (1996) Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). *Journal of Agricultural and Food Chemistry*, **44**: 1526–1530.
- Salas-Salvadó, J., Bulló, M., Pérez-Heras, A., and Ros, E. (2006) Dietary fibre, nuts and cardiovascular diseases. *British Journal of Nutrition*, **96**(Suppl. 2): S46–51.
- Schneider, A.V.C. (2002) Overview of the market and consumption of pulses in Europe. *British Journal of Nutrition*, **88**: 243–250.
- Schulze, M.B., Schulz, M., Heidemann, C., Schienkiewitz, A., Hoffmann, K., and Boeing, H. (2007) Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis. *Archives in Internal Medicine*, **167**: 956–965.
- Shutler, S., Bircher, G., Tredger, J., Morgan, L., Walker, A., and Low, A. (1989) The effect of daily baked bean (*Phaseolus vulgaris*) consumption on the plasma lipid levels of young, normocholesterolaemic men. *British Journal of Nutrition*, **61**, 257–265.
- Sievenpiper, J.L., Kendall, C.W.C., Esfahani, A., Wong, J.M.W., Carleton, A.J., Jiang, H.Y., Bazinet, R.P., Vidgen, E., and Jenkins, D.J.A. (2009) Effect of non-oil pulses on glycaemic control: a systemic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia*, **52**: 1479–1495.
- Takeoka, G.R., Dao, L.T., Tamura, H., and Harden, L.A. (2005) Delphinidin 3-O-(2-β-D-glucopyranosyl-α-L-arabinopyranoside): a novel anthocyanin identified in Beluga black lentils. *Journal of Agricultural and Food Chemistry*, **53**: 4932–4937.

- Termentzi, A., Alexiou, P., Demopoulos, V.J., and Kokkalou, E. (2008) The aldose reductase inhibitory capacity of *Sorbus domestica* fruit extracts depends on their phenolic content and may be useful for the control of diabetic complications. *Pharmazie*, **63**: 693–696.
- Tharanathan, R.N. and Mahadevamma, S. (2003) Grain legumes – a boon to human nutrition. *Trends in Food Science Technology*, **14**: 507–518.
- Tsao, R. (2010) Chemistry and biochemistry of dietary polyphenols. *Nutrients*, **2**: 1231–1246.
- USDA (United States Department of Agriculture). (2009) Agriculture Research Services. Products and Services SR 20 [Online] Available at: <http://www.ars.usda.gov/Services/docs.htm?docid%41747>. Accessed on 06 May 2011.
- Wang, N. and Daun, J.K. (2004) *The Chemical Composition and Nutritive Value of Canadian Pulses*. Canadian Grain Commission, Grain Research Laboratory. Winnipeg, MB, CN.
- Whelton, S.P., Hyre, A.D., Pedersen, B., Yi, Y., Whelton, P.K., and He, J. (2005) Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *Journal of Hypertension*, **23**: 475–481.
- Wolever, T.M.S., Mehling, C., Chiasson, J.L., Josse, R.G., Leiter, L.A., Maheux, P., Rabasa-Lhoret, R., Rodger, N.W., and Ryan, E.A. (2008) Low glycaemic index diet and disposition index in type 2 diabetes (the Canadian trial of Carbohydrates in Diabetes): a randomised controlled trial. *Diabetologia*, **51**: 1607–1615.
- Wolff, T., Witkop, C.T., Miller, T., and Syed, S.B. (2009) Preventive Services Task Force. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. *Preventive Services Task Force. Annals of Internal Medicine*, **150**: 632–639.
- Wylie-Rosett, J., Segal-Isaacson, C.J., and Segal-Isaacson, A. (2004) Carbohydrates and increases in obesity: does the type of carbohydrate make a difference? *Obesity Research*, **12**: 124S–129S.
- Xu, B., and Chang, S.K. (2009) Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. *Journal of Agricultural and Food Chemistry*, **57**: 10718–10731.
- Xu, B.J. and Chang, S.K. (2007) A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science*, **72**: S159–166.
- Zou, Y., Chang, S.K., Gu, Y., and Qian, S.Y. (2011) Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. Morton) extract and its fractions. *Journal of Agricultural and Food Chemistry*, **59**: 2268–2276.

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# 16 Soy isoflavones and bone health

Rong Tsao

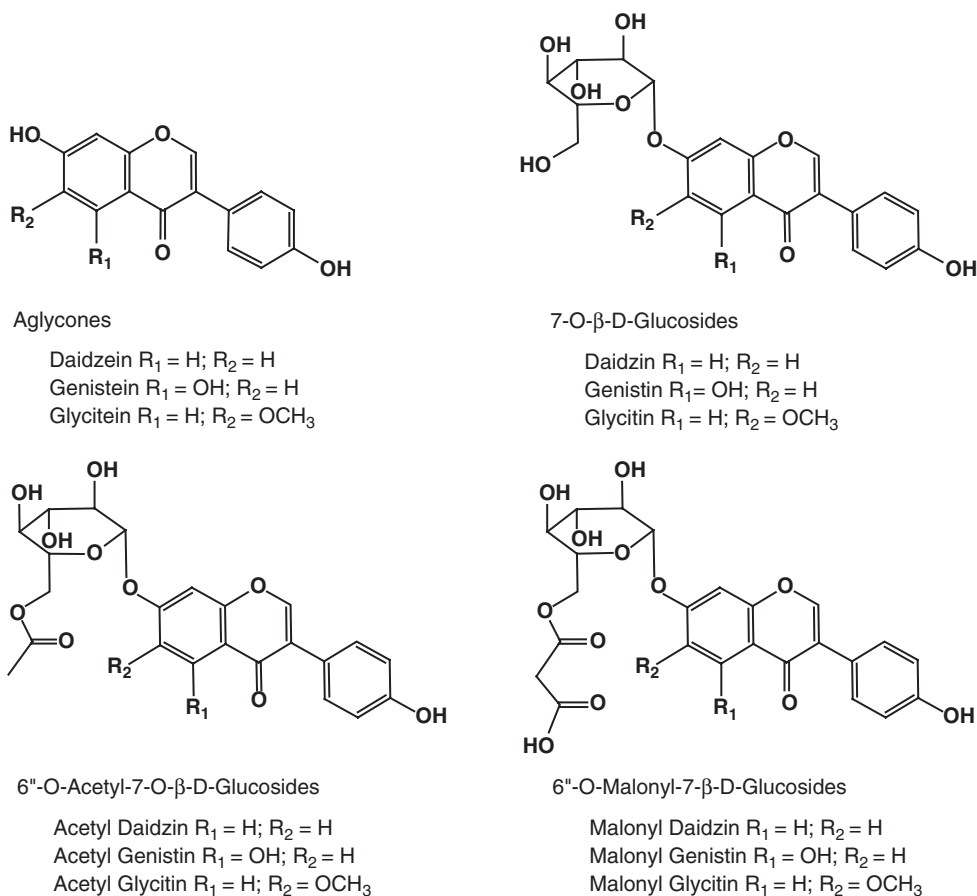
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## 16.1 Introduction

Soybeans have been consumed in East Asian cultures for centuries, whereas in the West, the eating of soybean and soy-based foods only became a recent event due largely to the potential health benefits that these foods might offer. The various health benefits of soybean seem to come from components other than the macronutrients such as proteins, carbohydrates, and fats in soybeans, rather, increasing evidence suggests that the “nonnutritive” secondary metabolites or phytochemicals might play important roles. Different phytochemicals have been identified in soy and investigated for their bioactivities in health promotion and disease prevention. Proteins, or their hydrolysis products – peptides such as lunasin – have been found to contribute to reduced serum cholesterol level and blood pressure in animal and human trials (Jenkins *et al.*, 2010; Messina, 2010). Phytochemicals including saponins and flavonoids have also been attributed to different health related activities (Messina, 1999, 2010; Tsai *et al.*, 2010). However, what really makes soybean unique and a subject of much scientific research is undoubtedly the isoflavones. Generally speaking, isoflavones are found primarily in leguminous plants. Soybean, being the most widely consumed legume, and its high isoflavone contents are believed to contribute to lowering risk of several chronic diseases, as well as having the ability to relieve menopausal symptoms and increase bone density in women (Messina, 2010); reducing risks associated with heart and cardiovascular diseases (Torres *et al.*, 2006; Jenkins *et al.*, 2010); and lowering incidences of cancer (Lampe, 2010; Messina, 2010; Tsai *et al.*, 2010). Isoflavones have also been shown as potent antioxidants, which can reduce the long-term risks of diseases caused by oxidative stresses from excess free radicals (Mann *et al.*, 2007). More importantly, among foods commonly consumed by humans, only isoflavones in soybeans and soy-based foods are found in physiologically relevant amounts (Franke *et al.*, 1998). This chapter will focus on the chemistry and biochemistry of soy isoflavones and how they contribute to bone health.

## 16.2 Biosynthesis and composition of isoflavones in soybeans

Isoflavones are a unique subgroup of flavonoids; the latter contain anywhere from 2000 to 6500 compounds. It is generally accepted that these phytochemicals, including the isoflavones, frequently serve as plants' chemical defense against invading insects and microorganisms (Harborne and Williams, 2000; de Rijke *et al.*, 2006; Tsao and McCallum, 2010). The general structure of isoflavones has a common C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> 3-ring feature similarly seen in all flavonoids, i.e. two phenolic rings A and B, and a middle pyran C ring (Figure 16.1). The only difference between the isoflavones and other flavonoids is the position of the B ring, which in isoflavones are attached to the C3 position (Figure 16.1). Like all flavonoids, the B ring and central 3 carbon bridge forming the C ring originate from the amino acid phenylalanine produced in the shikimate pathway. In this phenylpropanoid pathway, phenylalanine is converted by phenylalanine ammonia lyase (PAL) to cinnamate, a key precursor of C<sub>6</sub>-C<sub>3</sub> structures. The A ring is condensed from 3 units of malonyl-CoA



**Figure 16.1** Isoflavones in soybean.

through the activity of a cytosolic Acetyl-CoA Carboxylase (ACC) from citrate precursors (Fatland *et al.*, 2004). The two parts are then condensed to yield the first flavonoid structure naringenin chalcone by the enzyme chalcone synthase (CHS). Chalcone is isomerized by chalcone flavanone isomerase (CHI) to a flavanone. Chalcone is where isoflavones are branched out through different enzymes including CHI and isoflavones synthase (IFS) (Tsao and MaCallum, 2010). Understanding the biosynthetic pathways of isoflavones can help in breeding soybean varieties with desired isoflavone contents as ingredients of functional foods (Shao *et al.*, 2010).

There are 12 major isoflavones in soybean and soy products; three aglycones genistein, daidzein and glycitein, their respective 7-O- $\beta$ -D-glucosides (genistin, daidzin and glycitin), 6''-O-acetyl-7-O- $\beta$ -D-glucosides (acetylgenistin, acetyldaidzin, and acetylglycitin), and 6''-O-malonyl-7-O- $\beta$ -D-glucosides (malonylgenistin, malonyldaidzin and malonylglycitin) (Figure 16.1). The concentrations of these forms vary in soybean and soy products depending on cultivar, geographic region, agronomical practice, environmental conditions of plant growth, and processing conditions (Shao *et al.*, 2010; Shao *et al.*, 2011).

### **16.3 Separation, characterization, and analysis of isoflavones**

While the predominant natural forms of isoflavones are glycosides (Shao *et al.*, 2011), accurate assessment of the composition of all of these isoflavone forms in soybean and soy-based foods is important in quality control of soy products, and in understanding the effect of processing conditions on isoflavone content of soy foods and food supplements, and how the different forms of isoflavones are absorbed by humans (Song *et al.*, 1998; Delmonte and Rader, 2006; Shao *et al.*, 2010).

The earlier AOAC official method 2001.10 was developed to analyze aglycones due to the lack of standard references of the glycosides (AOAC, 2005). In this method, the isoflavones were extracted with 80% methanol from soy and soy-based foods at 65°C for 2 hours, saponified at ambient temperature with NaOH solution, then acidified, filtered, and centrifuged before being analyzed by high performance liquid chromatography (HPLC). Quantification of the isoflavone aglycones was based on the standard dilution method. However, the new AOAC official method, as a result of a collaborative study used a different solvent system for extraction of all native forms of the 12 isoflavones (aglycones and glycosides) (Collison, 2008). The extraction solvent was a mixture consisting of acetonitrile–water–dimethylsulfoxide (DMSO) at 58.5/39.0/2.5, v/v/v, and the extraction was done at room temperature for 1 hour. An internal standard apigenin was used for quantification of all 12 isoflavones by HPLC (Collison, 2008).

Although different chromatographic methods have been used for separation and quantification of isoflavones, as mentioned above, HPLC is advantageous over other separation techniques because it requires no derivatization prior to analysis, which thus allows rapid and accurate quantification of the native forms of isoflavones. Certainly HPLC coupled with mass spectrometer is an ideal and powerful tool for the identification of isoflavones (Wu *et al.*, 2004; Tsao *et al.*, 2006). However, an ultraviolet (UV) or diode array detector (DAD), with a C18 reversed-phase column (particle size varies from 3–5  $\mu$ m) and a binary gradient using mobile phases consisted of acidified water and acidified acetonitrile is still the most frequently used system. Great advances have been made to obtain complete

profiles of all 12 isoflavones in soybean and soy products (Zhang *et al.*, 2006; Hsieh *et al.*, 2004): however, mostly with prolonged running time. Delmonte and Rader (2006) succeeded in resolving 20 isoflavones within 90 minutes including the 12 soy isoflavones. The new official AOAC method runs for 60 minutes (Collison, 2008). Only a few HPLC separations showed great resolution using a standard column within a reasonable time (150×4.6 mm, 5 μm) (Luthria *et al.*, 2007). The method of Zhang and others (2006) resolved all 12 isoflavones in less than 20 minutes using a C18 column and a gradient mobile phase consisted of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water, although the particle size of the column was not clear. This is perhaps the best method used thus far. Internal standards (IS) have been used and strongly suggested, however, as far as the authors are aware, no IS are preferred, and there is no common IS agreed among users (Song *et al.*, 1998; Delmonte *et al.*, 2006). A recent method developed in the author's laboratory not only avoided the use of an internal standard, but the 12 native isoflavones were also completely separated in significantly shorter time (30 minutes). In this method, all 12 compounds were quantified individually from their own standard curves (Shao *et al.*, 2011).

The development of ultra high performance liquid chromatography (U-HPLC or UPLC) has changed drastically the way isoflavones are analyzed. Separation done by UPLC can significantly enhance the efficiency and drastically reduce the analytical time (to <1/10 of the time of a conventional HPLC). Churchwell *et al.* (2005) compared UPLC–MS with conventional HPLC–MS for the determination of isoflavones and found that in general, UPLC–MS produced significant improvements in method sensitivity, speed, and resolution. More sensitive detection techniques such as fluorescence and electrochemical detectors have been particularly useful in analyzing minute amounts of isoflavones and their metabolites in biological samples, when a MS detector is not available (Wang *et al.*, 2002; Saracino and Raggi, 2010).

## 16.4 Soy isoflavones and bone health

Estrogen deficiency plays a key role in osteoporosis and other menopause-related chronic diseases. As shown in Figure 16.1, the isoflavone structures, particularly the 4'-hydroxyl group, are similar to estrogen, thus important for their ability to bind to estrogen receptors, as are many substances, including antiestrogens like the drug tamoxifen used successfully to treat breast cancer (Setchell, 2001). There are two estrogen receptors that isoflavones are known to bind to, the classic estrogen receptor alpha (ER $\alpha$ ) and the newly discovered ER $\beta$ . Distribution of these two receptors depends on tissue types (Anderson *et al.*, 1999). Reproductive cells, especially those of the uterus and breast, are abundant in ER $\alpha$  (Anderson *et al.*, 1999; Messina, 1999; Scheiber and Rebar, 1999), whereas bone tissue has greater amounts of ER $\beta$  (Messina, 1999). The different tissue distribution of  $\alpha$  and  $\beta$ -receptors points to the possibility of tissue-selective effects of the isoflavones as these phytoestrogens appear to have different effects in different types of tissues (Kuiper *et al.*, 1997; Scheiber and Rebar, 1999). Genistein has been found to bind with a much greater affinity to ER $\beta$  than to ER $\alpha$  (Kuiper *et al.*, 1997; Anderson *et al.*, 1999; Messina, 1999; Scheiber and Rebar, 1999). The different binding capacities of isoflavones to the different ER receptors suggest that they can affect biological processes controlled by estrogen. Further, the preferential binding of isoflavones to ER $\beta$  compared with ER $\alpha$  means these compounds can influence bone metabolism, thus benefiting bone health (Potter *et al.*, 1998; Kuiper *et al.*, 1997; Kuiper

*et al.*, 1998). This has led to the recent recognition of isoflavones as Selective Estrogen Receptor Modulators (SERMs) (Setchell, 2001). An ideal SERM is “a compound that acts as a potent anti-estrogen in the breast and uterus to prevent estrogen-driven cell proliferation and, at the same time, has strong estrogenic effects in bone, the cardiovascular system, and the central nervous system, where hormones can help a variety of postmenopausal conditions” (McNeil, 1998). For isoflavones, this means they are likely to have the beneficial effects of estrogen without the negatives. The natural hormone estrogen contributes significantly to the regulation of skeletal metabolism via constraining and balancing effects on bone remodeling cycles, so isoflavones, known as phytoestrogens, can be expected to behave similarly. The outcome of such effects is to keep bone cell activity adequately balanced so that the resorptive activity of osteoclasts does not progressively exceed the anabolic activity of osteoblasts. Osteoclasts and osteoblasts are, therefore, instrumental in controlling the amount of bone tissue: osteoblasts form bone, osteoclasts resorb bone. Imbalance between the two activities can therefore lead to risks of bone health such as osteoporosis.

### **16.4.1 Cell culture and biomarkers (*in vitro*)**

For this reason, cell lines used for *in vitro* studies are mainly based on either osteoblasts or osteoclasts. Several cell lines have been used in studying the effect of soy isoflavones on cell proliferation, differentiation and associated biomarkers. Receptor activator of nuclear factor-kappaB ligand (RANKL) is the essential factor for osteoclast formation and activation and enhances bone resorption. On the other hand, osteoprotegerin (OPG), which is produced by osteoblastic lineage cells, acts as a decoy receptor that neutralizes RANKL and prevents bone loss. These biomarkers are frequently used in various studies on isoflavones and bone health.

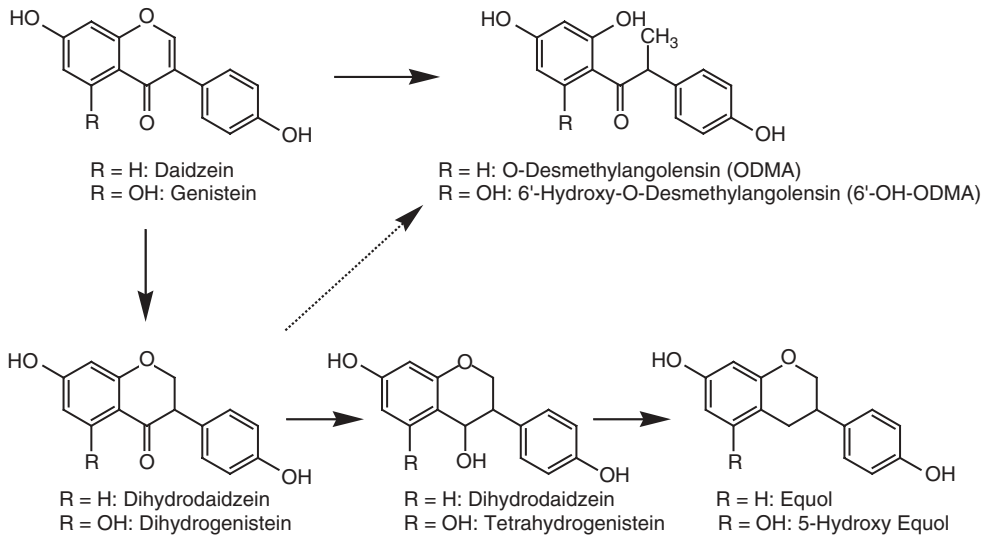
Osteoblastic MC3T3-E1 cells cultured in the presence of daidzein or genistein showed a significant increase in the activity of a key marker enzyme, alkaline phosphatase (ALP), which is important in osteoblastic cell differentiation, an indicator of bone formation. Daidzein and genistein also significantly increased in protein and DNA contents of the MC3T3-E1 cells at 0.1–10 nM (Sugimoto and Yamaguchi, 2000; Yamaguchi and Sugimoto, 2000). Viereck *et al.* (2002) assessed the effects of genistein on OPG mRNA steady state levels and protein production in primary human trabecular osteoblasts obtained from healthy donors. Genistein increased OPG mRNA levels and protein secretion by osteoblast cells by up to two- to six-fold in a dose- ( $P < 0.0001$ ) and time-dependent ( $P < 0.0001$ ) fashion with a maximum effect at  $10^{-7}$  M. They concluded that genistein was capable of up regulating the production of OPG by human osteoblasts. Thus, dietary sources of phytoestrogens may help to prevent bone resorption and bone loss by enhanced osteoblastic production of OPG. The anti-resorptive effects of estrogen on bone metabolism are thought to be mediated through modulation of paracrine factors produced by osteoblastic lineage cells that act on osteoclastic lineage cells. Chen and others (2003) used the osteoblastic MC3T3-E1 cells and examined the possible roles of estrogen receptors in the actions of genistein on osteoblastic cells. They concluded that the beneficial skeletal effects of genistein, at achievable dietary levels, appear to be mediated, at least in part, by interleukin-6 and OPG, and estrogen receptors play important roles in the inhibition of interleukin-6 synthesis by genistein in osteoblastic MC3T3-E1 cells. A study using the human cell line MG63 osteoblasts revealed that genistein-treated osteoblasts possessed a more organized cytoskeleton, and genistein's inhibitory effect

upon cell proliferation was associated with exposure of phosphatidylserines on the external plasmalemma surface. Genistein-treated osteoblasts synthesized relatively high levels of collagen and ALP and, even in a nonosteogenic growth medium, formed mineralized bone noduli (Morris *et al.*, 2006). Using other human osteoblastic SaOS-2 cells, Chen and Wang (2006) provided first evidence that genistein could modulate the actions of parathyroid hormone (PTH) in these cells in an estrogen-depleted condition. They found that genistein (at  $10^{-8}$ – $10^{-6}$  M) induced ALP activity and OPG expression in a dose-dependent manner. Genistein (at 1 microM) was found to stimulate the mRNA expression of RANKL. These effects could be completely abolished by co-treatment with oestrogen antagonist ICI 182780 (Chen and Wang, 2006). Isoflavones can induce estrogen response element-mediated transcription in osteoblastic cells. For example, Tang and others (2010a) investigated whether isoflavones genistein and daidzein regulate target gene transcription through cAMP regulatory element (CRE) using human osteoblastic cell line MG-63 cells. They concluded that isoflavones genistein and daidzein might modulate bone remodeling through ERs by regulating target gene expression through the CRE motifs.

Kanno *et al.* (2004) investigated the effects of phytoestrogens on osteoclast differentiation using ER $\alpha$ -transfected RAW264.7 (RAW264.7-ER $\alpha$ ) cells. When the cells were cultured with the RANKL, both formation of tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells and TRAP activity were increased compared with control cells that were cultured in the absence of RANKL. Their results suggest that coumestrol has an inhibitory effect on the differentiation of osteoclasts. Tang and others (2010b) also found that isoflavone derivatives markedly inhibited the RANKL plus macrophage colony stimulating factor (M-CSF)-induced osteoclastic differentiation from bone marrow stromal cells and RAW264.7 macrophage cells, but not the cell proliferation and differentiation of human cultured osteoblasts.

Although isoflavones exist in soy and soy-food as glycosides, the actual activities may be from the aglycones and their metabolites. The effect of isoflavones on bone health, therefore, may depend at least partially on the estrogenic activities of the metabolites. Isoflavone glycosides including glucosides, malonylglucosides, and acetylglucosides are first hydrolyzed by  $\beta$ -glucosidase to produce aglycones in the digestive tract. These aglycones are then metabolized by intestinal microorganisms to different metabolites (Figure 16.2). Hwang and others (2006) investigated the modulatory effects of daidzein, genistein, and their metabolites dihydrogenistein (DGTN), dihydrodaidzein (DDZ), tetrahydrodaidzein (TDZ), O-desmethylangolensin (ODMA), and equol (EQL), in the presence of estrogen using several *in vitro* systems, and found the intermediate metabolites, such as DGTN, DDZ, and TDZ, bound much more weakly to both ER $\alpha$  and ER $\beta$  and induced less potently in transcriptional activity, gene expression, and mammary cell proliferation than their precursors. EQL had the strongest binding affinities and estrogenic activities especially for ER $\beta$  among the daidzein metabolites and showed the ability to suppress osteoclast formation at high doses. They concluded that isoflavones may exert their effects as estrogen antagonists in a high estrogen environment, or they may act as estrogen agonists in a low estrogen environment (Hwang *et al.*, 2006). Most recently, Bitto and others (2010) reviewed the current literature and found that aglycone genistein appears to be the most effective isoflavone in preserving bone health. Genistein aglycone, through a peculiar anti-osteoporotic dual mode of action, can positively regulate bone cell metabolism rebalancing bone turnover towards bone formation. The OPG-RANKL system was considered by the authors as the mechanism through which genistein stimulates osteoblasts and inhibits osteoclast function.





**Figure 16.2** Metabolic pathway of the major soy isoflavones aglycones, daidzein, and genistein, which are first cleaved from their glycosidic conjugates by intestinal glucosidases.

### 16.4.2 Animal studies (*in vitro*)

Results from *in vitro* studies using cell lines provide information on how soy isoflavones may act at the molecular level. However, the ultimate ability to prevent estrogen-mediated bone loss relies on *in vivo* studies using animal and human subjects. Many animal models, particularly ovariectomized (OVX) rodent models, have been used to study the potential skeletal effects of isoflavones. The OVX rodent models have been accepted by the US Food and Drug Administration for studying osteoporosis (Messina and Messina, 2000). Many animal studies have shown positive roles of isoflavone-containing diets in preventing bone loss (Arjmandi *et al.*, 1996, 1998; Brynin, 2002). The first of such studies using a diet containing isoflavone-rich soy protein was conducted by Arjmandi and others (1996) who found that isoflavones were responsible for improved bone mineral density in an OVX rat model. The same group later found that diet containing soy isoflavones prevented decrease in bone density, and the rate of bone formation was higher in the OVX groups than the sham group, and was not significantly different in the OVX plus soy groups, indicating that isoflavones did not slow the increased rate of bone turnover with ovariectomy in this study population (Arjmandi *et al.*, 1998). Reduction of the bone mineral density (BMD) in OVX mice was found to be significantly restored with genistein treatment (Fanti *et al.*, 1998; Ishimi *et al.*, 1999).

In a recent study, Zhang and others (2009) found that consumption of diets containing soy isoflavone extract, but not purified genistein, significantly preserved trabecular bone mass, bone volume, and trabecular bone separation in the proximal tibial metaphysis of the OVX mice. Purified genistein decreased mRNA expression of RANKL, carbonic anhydrase II, and cathepsin K and enhanced the ratio of OPG:RANKL mRNA expression in the tibial head of the OVX mice. However, the diet containing soy extract suppressed the OVX-induced increase in serum ALP but did not alter bone-specific gene expression of tibia. They

therefore concluded that a soy extract containing a similar level of genistein could be more effective than purified genistein in improving tibial trabecular bone quality in OVX mice, but that the mechanism of action might be distinct from that of genistein.

The effects of isoflavones on bone health depend on the life-stage of the animals. For example, genistein suppressed the femoral bone loss in OVX young rats (Fanti *et al.*, 1988; Anderson *et al.*, 1998), but isoflavones had no beneficial effects in older, adult animal models (Picherit *et al.*, 2001; Register *et al.*, 2003). The effects may also depend on how isoflavones are metabolized. Similar to the recent *in vitro* studies, the effects of isoflavone metabolites in bone health seem to be of greater importance *in vivo*. Using OVX rats, Kolios and others (2009) compared the various biomechanical and histological changes in rats supplemented with 17 $\beta$ -estradiol (estrogen), equol, and genistein over a period of five weeks. They found that estrogen and equol were able to improve the elasticity of callus formation significantly in postmenopausal osteoporotic bone. The effects of estrogen were more anabolic than those of equol and were visible in changes to the trabecular bone. However, in terms of the whole body, equol seemed to induce less of an adverse reaction than estrogen. Genistein as an osteoclast inhibitor influenced callus stiffness and negatively impacted trabecular structure in severely osteoporotic bones. Estrogen and equol were able to improve fracture healing in ovariectomy-induced osteoporotic bones, and the extent of callus formation played only a minor role (Kolios *et al.*, 2009). The *in vivo* efficacy of isoflavones may also depend on the metabolism and bioavailability of the various glycosides compared to aglycones, and the ratios or combinations of isoflavone components (Reinwald and Weaver, 2006). Weaver and Cheong (2005) have attributed the variable results to: 1) life-stage dependent, estrogen dependent; 2) compound dependent, dose dependent, interactions—synergistic or opposing?; 3) equol vs. nonequol producers; 4) lack of dietary control (Weaver and Cheong, 2005).

### 16.4.3 Human clinical trials

As phytoestrogens, isoflavones seem to be effective when estrogen is deficient, i.e., during menopause, and not effective when estrogen is in sufficient supply (Weaver and Cheong, 2005). This is supported by clinical trials such as those reported by Mei and others (2001) who found that high dietary isoflavones was associated with higher bone mineral density (BMD) of the spine and the hip in postmenopausal, but not premenopausal, women. The first clinical study on the effect of isoflavones on bone health was carried out with postmenopausal women, whose bone mineral content and density in the lumbar spine both increased significantly after consuming 90 mg isoflavones for six months (Potter *et al.*, 1998). Since then, more than 30 trials have examined the effects of isoflavone-containing products on BMD in postmenopausal women (Ma *et al.*, 2008; Liu *et al.*, 2009; Messina, 2010). Results from the many short-term studies (less than one year) have been inconclusive, however, there seems to be a general tendency that isoflavones can attenuate bone loss in perimenopausal and in younger postmenopausal women (Messina *et al.*, 2004; Reinwald and Weaver, 2006). A double-blind randomized clinical trial (RCT) by Alekel and others (2010) revealed that bone loss from the lumbar spine was attenuated in perimenopausal women receiving 80.4 mg/day soy isoflavone components for 24 weeks, but not in women on an isoflavone poor (4.4 mg/day) or isoflavone-free diet. Furthermore, use of soy protein supplement containing isoflavones did not improve the BMD in healthy postmenopausal women when

started at the age of 60 years or later (Kreijkamp-Kaspers *et al.*, 2004; Reinwald and Weaver, 2006). There have indeed been many short- and long-term human intervention trials reporting positive effects of isoflavones in reducing bone loss and other biomarkers (Uesugi *et al.*, 2002; Harkness *et al.*, 2004; Lydeking-Olsen *et al.*, 2004; Roudsari *et al.*, 2005; Huang *et al.*, 2006; Marini *et al.*, 2007; Ma *et al.*, 2008). The most recent positive results were from Atteritano and others (2009) who found, in a study of young postmenopausal women selected for osteopenia, an increase in spine and hip BMD with genistein compared with a loss in the placebo group.

On the other hand, many recent human trials with skeletal-related outcomes have not been supportive of these findings. Brink and others (2008) found in a one-year randomized, double-blind, placebo-controlled, clinical trial that the consumption of isoflavone-enriched food did not affect BMD in a group of early postmenopausal women. Most recently, Kenny and others (2009) found that addition of dietary soy protein and or isoflavone did not affect the change in BMD (or markers of bone turnover) over a one year period in a group of late postmenopausal women, and concluded that because soy protein and isoflavones (either alone or together) did not affect BMD, they should not be considered as effective interventions for preserving skeletal health in older women. They hypothesized that individuals who produce isoflavone metabolites such as equol might show the largest benefits in bone health, however, they failed to find differences in BMD dependant on equol production (Kenny *et al.*, 2009). Another recent study showed that treatment with 120 mg soy hypocotyl isoflavones reduced whole-body bone loss but did not slow bone loss at common fracture sites in healthy postmenopausal women over a period of two years (Wong *et al.*, 2009). An even longer, three year clinical trial in postmenopausal women supplemented with isoflavones also showed no bone-sparing effect of extracted soy isoflavones, except for a modest effect at the femoral neck (Alekel *et al.*, 2010).

While most cross-sectional studies suggest that high consumption of soy products is associated with increased bone mass or decreased bone resorption or fractures, and many animal models have been supportive of the correlation as discussed above, intervention trials that examined the effect of soy isoflavones on BMD or markers of bone turnover are less conclusive than the epidemiologic studies (Kenny *et al.*, 2009). A possible explanation for the striking discrepancy between the promising findings in animal research and subsequent lack of confirmation in human trials, especially for BMD, may be found in species differences in the metabolism of isoflavones (Kreijkamp-Kaspers *et al.*, 2004). Again, metabolites like equol may play an important role. Nearly all rodents produce equol; however, only about one third of people produce equol when exposed to high amounts of daidzein due to the differences in intestinal microflora of individuals. The same four factors, as pointed out by Weaver and Cheong, also apply to the variations observed in human clinical trials on soy isoflavones and bone health (Weaver and Cheong, 2005).

## **16.5 Summary**

Soy and soy-based foods may play important roles in disease prevention. One of the most unique and bioactive groups of compounds in soy is isoflavones. Three major isoflavone aglycones genistein, daidzein, and glycitein, and their respective glucosides, malonylglucosides, and acetylglucosides are found in soy and soy-foods. Like other flavonoids, isoflavones are best analyzed using HPLC/UPLC coupled with different

detection techniques for food and biological samples. Isoflavones are weak estrogens in that they bind to estrogen receptors, however, their bioactivities go beyond the estrogenicity they possess; they are selective estrogen receptor modulators (SERMs) that can exert health benefits without having a negative effect on bone health. There is ample *in vitro* and *in vivo* animal evidence that points to the positive roles of isoflavones in improving bone health, however, human clinical trials have not conclusively verified such effect. There are many factors affecting the outcome of dietary isoflavones in bone health, life-stage of the subjects, metabolism of isoflavones in individuals, bioavailability, and ratio of different forms of isoflavones in the diet may all contribute to the discrepancy observed between the *in vitro* animal studies and the human clinical trials. Isoflavone metabolites such as equol may play more important biological roles than isoflavones themselves. These are, therefore, directions of future research.

## References

- Alekel, D.L., Van Loan, M.D., Koehler, K.J., Hanson, L.N., Stewart, J.W., Hanson, K.B., Kurzer, M.S., and Peterson, C.T. (2010) The soy isoflavones for reducing bone loss (SIRBL) study: a 3-y randomized controlled trial in postmenopausal women. *American Journal of Clinical Nutrition*, **91**: 218–230.
- Anderson, J.J., Anthony, M.S., Cline, J.M., Washburn, S.A., and Garner, S.C. (1999) Health potential of soy isoflavones for menopausal women. *Public Health Nutrition*, **2**: 489–504.
- Anderson, J.J.B., Ambrose, W.W., and Garner, S.C. (1998) Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. *Proceedings of the Society for Experimental Biology and Medicine*, **217**: 345–350.
- AOAC. (2005) *Method 2001. 10*, Official Methods of Analysis, 18th ed., AOAC International, Gaithersburg, MD, USA.
- Arjmandi, B.H., Alekel, L., Hollis, B.W., Amin, D., Stacewicz-Sapuntzakis, M., Guo, P., and Kukreja, S.C. (1996) Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *Journal of Nutrition*, **126**: 161–167.
- Arjmandi, B.H., Birnbaum, R., Goyal, N.V., Getlinger, M.J., Juma, S., Alekel, L., Hasler, C.M., Drum, M.L., Hollis, B.W., and Kukreja, S.C. (1998) Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. *American Journal of Clinical Nutrition*, **68**: 1364S–1368S.
- Atteritano, M., Mazzaferro, S., Frisina, A., Cannata, M.L., Bitto, A., D'Anna, R., Squadrito, F., Macri, I., Frisina, N., and Buemi, M. (2009) Genistein effects on quantitative ultrasound parameters and bone mineral density in osteopenic postmenopausal women. *Osteoporosis International*, **20**: 1947–1954.
- Bitto, A., Polito, F., Squadrito, F., Marini, H., D'Anna, R., Irrera, N., Minutoli, L., Granese, R., and Altavilla, D. (2010) Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation. *Current Medicinal Chemistry*, **17**: 3007–3018.
- Blair, H.C., Jordan, S.E., Peterson, T.G., and Barnes, S. (1996) Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *Journal of Cellular Biochemistry*, **61**: 629–637.
- Brink, E., Coxam, V., Robins, S., Wahala, K., Cassidy, A., and Branca, F. (2008) Long term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. *American Journal of Clinical Nutrition*, **87**: 761–770.
- Brynin, R. (2002) Soy and its isoflavones: a review of their effects on bone density. *Alternative Medicine Review*, **7**: 317–327.
- Chen, W.F. and Wong, M.S. (2006) Genistein modulates the effects of parathyroid hormone in human osteoblastic SaOS-2 cells. *British Journal of Nutrition*, **95**: 1039–1047.
- Chen, X., Garner, S.C., Quarles, L.D., and Anderson, J.J. (2003) Effects of genistein on expression of bone markers during MC3T3-E1 osteoblastic cell differentiation. *Journal of Nutritional Biochemistry*, **14**: 342–349.
- Churchwell, M.I., Twaddle, N.C., Meeker, L.R., and Doerge, D.R. (2005) Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS. *J Chromatography B*, **825**: 134–143.

- Collison, M.W. (2008) Determination of total soy isoflavones in dietary supplements, supplement ingredients, and soy foods by high-performance liquid chromatography with ultraviolet detection: collaborative study. *Journal of AOAC International*, **91**: 489–500.
- de Rijke, E., Out, P., Niessen, W.M.A., Ariese, F., Gooijer, C., and Brinkman, U.A.Th. (2006) Analytical separation and detection methods for flavonoids. *Journal of Chromatography A*, **1112**: 31–63.
- Delmonte, P., Perry, J., and Rader, J.I. (2006) Determination of isoflavones in dietary supplements containing soy, Red Clover and kudzu: Extraction followed by basic or acid hydrolysis. *Journal of Chromatography A*, **1107**: 59–69.
- Delmonte, P. and Rader, J.I. (2006) Analysis of isoflavones in foods and dietary supplements. *Journal of AOAC International*, **89**: 1138–1146.
- Fanti, P., Monier-Faugere, M.C., Geng, Z., Schmidt, J., Morris, P.E., Cohen, D., and Malluche, H.H. (1998) The phytoestrogen genistein reduces bone loss in short-term ovariectomized rats. *Osteoporosis International*, **8**: 274–281.
- Fatland, B.L., Ke, J., Anderson, M.D., Mentzen, W.I., Cui, L.W., Allred, C.C., Johnston, J.L., Nikolau, B.J., and Wurtele, E.S. (2004) Molecular characterization of a heteromeric ATP-citrate lyase that generates cytosolic acetyl-Coenzyme A in Arabidopsis. *Plant Physiology*, **130**: 740–756.
- Franke, A.A., Custer, L.J., Wang, W., and Shi, C.Y. (1998). HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. *Proceedings of the Society for Experimental Biology and Medicine*, **217**: 263–273.
- Harborne, J.B. and Williams, C.A. (2000) Advances in flavonoid research since 1992. *Phytochemistry*, **55**: 481–504.
- Harkness, L.S., Fiedler, K., Sehgal, A.R., Oravec, D., Lerner, E. (2004) Decreased bone resorption with soy isoflavone supplementation in postmenopausal women. *Journal of Women's Health (Larchmt)*, **13**: 1000–1007.
- Hsieh, H.C., Kao, T.H., and Chen, B.H. (2004) A fast HPLC method for analysis of isoflavones in soybean. *Journal of Liquid Chromatography and Related Technologies*, **27**: 315–324.
- Huang, H.Y., Yang, H.P., Yang, H.T., Yang, T.C., Shieh, M.J., and Huang, S.Y. (2006) One year soy isoflavone supplementation prevents early postmenopausal bone loss but without a dose-dependent effect. *Journal of Nutritional Biochemistry*, **17**: 509–517.
- Hwang, C.S., Kwak, H.S., Lim, H.J., Lee, S.H., Kang, Y.S., Choe, T.B., Hur, H.G., and Han, K.O. (2006). Isoflavone metabolites and their in vitro dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *Journal of Steroid Biochemistry and Molecular Biology*, **101**: 246–253.
- Ishimi, Y., Miyaura, C., Ohmura, M., Onoe, Y., Sato, T., Uchiyama, Y., Ito, M., Wang, X., Suda, T., and Ikegami, S. (1999) Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. *Endocrinology*, **140**: 1893–1900.
- Jenkins, D.J., Mirrahimi, A., Srichaikul, K., Berryman, C.E., Wang, L., Carleton, A., Abdunour, S., Sevenpiper, J.L., Kendall, C.W., and Kris-Etherton, P.M. (2010) Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. *Journal of Nutrition*, **140**: 2302S–2311S.
- Kanno, S., Hirano, S., and Kayama, F. (2004) Effects of the phytoestrogen coumestrol on RANK-ligand-induced differentiation of osteoclasts. *Toxicology*, **203**: 211–220.
- Kenny, A.M., Mangano, K.M., Abourizk, R.H., Bruno, R.S., Anamani, D.E., Kleppinger, A., Walsh, S.J., Prestwood, K.M., and Kerstetter, J.E. (2009) Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial. *American Journal of Clinical Nutrition*, **90**: 234–42.
- Kolios, L., Sehmisch, S., Daub, F., Rack, T., Tezval, M., Stuermer, K.M., and Stuermer, E.K. (2009) Equol but not genistein improves early metaphyseal fracture healing in osteoporotic rats. *Planta Medica*, **75**: 459–465.
- Kreijkamp-Kaspers, S., Kok, L., Grobbee, D.E., De Haan, E.H.F., Aleman, A., and Van der Schouw, Y.T. (2004) Soy protein with isoflavones and cognitive function in postmenopausal women; A randomised placebo-controlled double-blind trial. *Journal of the American Medical Association*, **292**: 65–74.
- Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., and Gustafsson, J.A. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors  $\alpha$  and  $\beta$ . *Endocrinology*, **138**: 863–870.
- Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B., and Gustafsson, J.A. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*, **139**: 4252–4263.
- Lampe, J.W. (2010) Emerging research on equol and cancer. *Journal of Nutrition*, **140**: 1369S–72S.

- Liu, J., Ho, S.C., Su, Y.X., Chen, W.Q., Zhang, C.X., and Chen, Y.M. (2009) Effect of long term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized control trials. *Bone*, **44**: 948–953.
- Luthria, D.L., Biswas, R., and Natarajan, S. (2007) Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chemistry*, **105**: 325–333.
- Lydeking-Olsen, E., Beck-Jensen, J.E., Setchell, K.D., and Holm-Jensen, T. (2004) Soy milk or progesterone for prevention of bone loss—a 2 year randomized, placebo-controlled trial. *European Journal of Nutrition*, **43**: 246–257.
- Ma, D.F., Qin, L.Q., Wang, P.Y., and Katoh, R. (2008) Soy isoflavone intake increases bone mineral density in the spine of menopausal women: meta-analysis of randomized controlled trials. *Clinical Nutrition*, **27**: 57–64.
- Mann, G.E., Rowlands, D.J., Li, F.Y., de Winter, P., Siow, R.C. (2007) Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression. *Cardiovascular Research*, **75**: 261–274.
- Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Atteritano, M., Gaudio, A., Mazzaferro, S., Frisina, A., Frisina, N., Lubrano, C., Bonaiuto, M., D'Anna, R., Cannata, M.L., Corrado, F., Adamo, E.B., Wilson, S., and Squadrito, F. (2007) Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Annals of Internal Medicine*, **146**: 839–847.
- McNeil, C. (1998) In search of the perfect SERM: beyond tamoxifen and raloxifene. *Journal of the National Cancer Institute*, **90**: 956–957.
- Mei, J., Yeung, S.S.C., and Kung, A.W.C. (2001) High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. *Journal of Clinical Endocrinology and Metabolism*, **86**: 5217–5221.
- Messina, M. and Messina, V. (2000) Soyfoods, soybean isoflavones, and bone health: a brief overview. *Journal of Renal Nutrition*, **10**: 63–68.
- Messina, M. (2010) A brief historical overview of the past two decades of soy and isoflavone research. *Journal of Nutrition*, **140**: 1350S–1354S.
- Messina, M.J., Ho, S., and Alekel, D.L. (2004) Skeletal benefits of soy isoflavones: a review of the clinical trial and epidemiologic data. *Current Opinion in Clinical Nutrition and Metabolic Care*, **7**: 649–658.
- Messina, M.J. (1999) Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*, **70**: 439S–450S.
- Messina, M.J. (1999) Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*, **70**: 439S–450S.
- Morris, C., Thorpe, J., Ambrosio, L., and Santin, M. (2006) The soybean isoflavone genistein induces differentiation of MG63 human osteosarcoma osteoblasts. *Journal of Nutrition*, **136**: 1166–1170.
- Picherit, C., Bennetau-Pelissero, C., Chanteranne, B., Lebecque, P., Davicco, M.-J., Barlet, J.-P., and Coxam, V. (2001) Soybean isoflavones dosedependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats. *Journal of Nutrition*, **131**: 723–728.
- Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., and Erdman, J.W. Jr. (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *American Journal of Clinical Nutrition*, **68**: 1375S–1379S.
- Register, T.C., Jayo, M.J., and Anthony, M.S. (2003) Soy phytoestrogens do not prevent bone loss in postmenopausal monkeys. *Journal of Clinical Endocrinology and Metabolism*, **88**: 4362–4370.
- Reinwald, S. and Weaver, C.M. (2006) Soy isoflavones and bone health: a double-edged sword? *Journal of Natural Products*, **69**: 450–459.
- Roudsari, A.H., Tahbaz, F., Hossein-Nezhad, A., Arjmandi, B., Larijani, B., and Kimiagar, S.M. (2005) Assessment of soy phytoestrogens' effects on bone turnover indicators in menopausal women with osteopenia in Iran: a before and after clinical trial. *Nutrition Journal*, **4**: 30.
- Saracino, M.A., and Raggi, M.A. (2010) Analysis of soy isoflavone plasma levels using HPLC with coulometric detection in postmenopausal women. *Journal of Pharmaceutical and Biomedical Analysis*, **53**: 682–687.
- Scheiber, M.D. and Rebar, R.W. (1999) Isoflavones and postmenopausal bone health: a viable alternative to estrogen therapy? *Menopause*, **6**: 233–241.
- Setchell, K.D.R. (2001) Soy Isoflavones—Benefits and Risks from Nature's Selective Estrogen Receptor Modulators (SERMs). *Journal of the American College of Nutrition*, **20**: 354S–362S.

- Shao, S., Duncan, A.M., Yang, R., Marcone, M.F., Rajcan, I., and Tsao, R. (2009) Tracking isoflavones: From soybean to soy flour, soy protein isolates to functional soy bread. *Journal of Functional Foods*, **1**: 119–127.
- Shao, S. and Tsao, R. (2011) Systematic evaluation of pre-HPLC sample processing methods on total and individual isoflavones in soybeans and soy products. *Food Research International* (in press).
- Song, T., Barua, K., Buseman, G., and Murphy, P.A. (1998) Soy isoflavone analysis: quality control and a new internal standard. *American Journal of Clinical Nutrition*, **68**: 1474S–1479S.
- Sugimoto, E. and Yamaguchi, M. (2000) Stimulatory effect of daidzein in osteoblastic MC3T3-E1 cells. *Biochemical Pharmacology*, **59**(5): 471–475.
- Tang, C.H., Chang, C.S., Tan, T.W., Liu, S.C., and Liu, J.F. (2010b). The novel isoflavone derivatives inhibit RANKL-induced osteoclast formation. *European Journal of Pharmacology*, **648**: 59–66.
- Tang, X., Zhu, X., Liu, S., Wang, S., and Ni, X. (2010a). Isoflavones suppress cyclic adenosine 3',5'-monophosphate regulatory element-mediated transcription in osteoblastic cell line. *Journal of Nutritional Biochemistry*, [Epub ahead of print].
- Torres, N., Torre-Villalvazo, I., and Tovar, A.R. (2006) Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. *Journal of Nutritional Biochemistry*, **17**: 365–373.
- Tsai, C.Y., Chen, Y.H., Chien, Y.W., Huang, W.H., and Lin, S.H. (2010) Effect of soy saponin on the growth of human colon cancer cells. *World Journal of Gastroenterology*, **16**: 3371–3376.
- Tsao, R. and McCallum, J. (2009) Chemistry of Flavonoids. In *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability*, Laura A. de la Rosa, Emilio Alvarez-Parrilla, and Gustavo Gonzalez-Aguilar (eds). Blackwell Publishing, Ames, Iowa. Chapter 5, pp. 131–153.
- Tsao, R., Papadopoulos, Y., Yang, R., Young, J.C., and McRae, K. (2006) Isoflavone profiles of red clovers and their distribution in different parts harvested at different growing stages. *Journal of Agricultural Food Chemistry*, **54**: 5797–5805.
- Uesugi, T., Fukui, Y., and Yamori, Y. (2002) Beneficial effects of soybean isoflavone supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: a four-week study. *Journal of the American College of Nutrition*, **21**: 97–102.
- Viereck, V., Gründker, C., Blaschke, S., Siggelkow, H., Emons, G., and Hofbauer, L.C. (2002) Phytoestrogen genistein stimulates the production of osteoprotegerin by human trabecular osteoblasts. *Journal of Cellular Biochemistry*, **84**: 725–735.
- Wang, C.C., Prasain, J.K., and Barnes, S. (2002) Review of the methods used in the determination of phytoestrogens. *Journal of Chromatography, B* **777**: 3–28.
- Weaver, C.M. and Cheong, J.M.K. (2005) Soy isoflavones and bone health: the relationship is still unclear. *Journal of Nutrition*, **135**: 1243–1247.
- Wong, W.W., Lewis, R.D., Steinberg, F.M., Murray, M.J., Cramer, M.A., Amato, P., Young, R.L., Barnes, S., Ellis, K.J., Shypailo, R.J., Fraley, J.K., Konzelmann, K.L., Fischer, J.G., and Smith, E.O. (2009) Soy isoflavone supplementation and bone mineral density in menopausal women: a 2-y multicenter clinical trial. *American Journal of Clinical Nutrition*, **90**: 1433–1439.
- Wu, A.H., Yu, M.C., Tseng, C.C., and Pike, M.C. (2008) Epidemiology of soy exposures and breast cancer risk. *British Journal of Cancer*, **98**: 9–14.
- Yamaguchi, M. and Sugimoto, E. (2000) Stimulatory effect of genistein and daidzein on protein synthesis in osteoblastic MC3T3-E1 cells: activation of aminoacyl-t-RNA synthetase. *Molecular and Cellular Biochemistry*, **214**: 97–102.
- Zhang, B., Hettiarachchy, N., Chen, P., Horax, R., Cornelious, B., and Zhu, D. (2006) Influence of the application of three different elicitors on soybean plants on the concentrations of several isoflavones in soybean seeds. *Journal of Agricultural Food Chemistry*, **54**: 5548–5554.
- Zhang, Y., Li, Q., Wan, H.Y., Helferich, W.G., and Wong, M.S. (2009) Genistein and a soy extract differentially affect three-dimensional bone parameters and bone-specific gene expression in ovariectomized mice. *Journal of Nutrition*, **139**: 2230–2236.

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# 17 Effects of dietary soy on the prevention of cardiovascular disease

Monica Whent and Margaret Slavin

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## 17.1 Introduction

Soybeans are one of the most widely consumed oilseeds both in the United States and worldwide (Ash *et al.*, 2006). According to the USDA Economic Research Service, the value of US soybean production was over \$16 billion as of 2005 (Ash *et al.*, 2006). Greater than 40% of US soybean production is exported to foreign countries. Most soybean meal is grown for animal food; however the human demand for soy foods is increasing. In 2004 alone, soymilk sales grew 16% in the US. The increasing popularity may be attributed in part to increased public awareness of potential health benefits from soy consumption (Ash *et al.*, 2006).

There is a noted difference in the incidence of cardiovascular disease (CVD) between many Asian cultures and Western cultures (Campbell *et al.*, 1998; Khoo *et al.*, 2003; Egusa and Yamane, 2004). While many lifestyle and genetic factors may be involved, the high intake of soy foods in Asian cultures is considered a potential protective factor (Nagata *et al.*, 1998; Ho *et al.*, 2000; Zhang *et al.*, 2003). Observational studies have shown a reduced incidence of CVD correlated with soy protein intake (Vega-Lopez and Lichtenstein, 2005). Much of the early research on soy and CVD was focused on the reduction of low-density lipoprotein (LDL) cholesterol (Anderson *et al.*, 1995). However, the mechanisms by which soy may improve CVD risk also include inhibition of LDL oxidation, anti-inflammatory properties, and improvement of hypertension (Messina and Lane, 2007).

## 17.2 Soy foods and serum cholesterol

Elevated total and LDL cholesterol are known risks for CVD, and reduction of serum cholesterol is a target of preventative programs against CVD (Gould *et al.*, 2007). Several components of the soybean may affect serum cholesterol levels. Soy isoflavones, soy protein, sterols, and fiber are all potential hypocholesterolemic compounds (Lo *et al.*, 1987; Jackman *et al.*, 2007; Xiao, 2008; AbuMweiss and Jones, 2008). There have been numerous studies in animals and humans on the effects of soy protein and isoflavones on serum cholesterol.



## 17.2.1 Soy protein

### 17.2.1.1 Animal studies

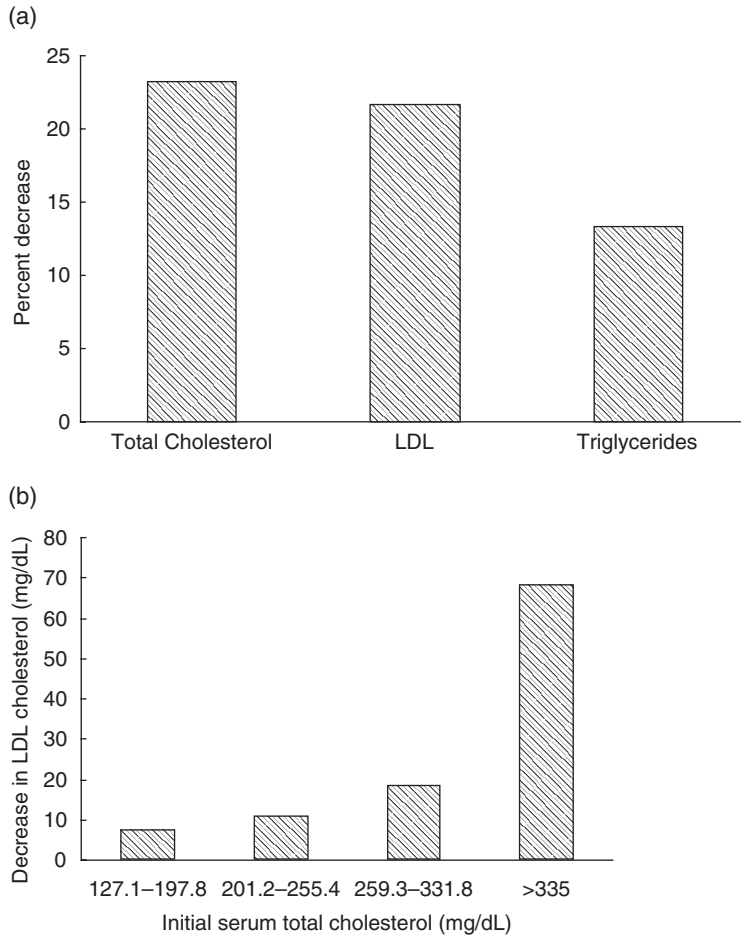
The relationship between soy protein and serum cholesterol was noted first in animal feeding studies. In rabbit feeding studies in the 1970s, it was noted that the type of dietary protein had an effect on serum cholesterol. Protein from animal sources resulted in higher cholesterol levels than plant-derived protein (Hamilton and Carroll, 1976). Further animal studies into the 1990s supported the finding of the hypocholesterolemic effect of soy protein (Carroll and Kurowska, 1995). More recent studies have continued to confirm the results. For example, Tovar *et al.* (2002, 2005) reported that, when compared with a casein diet, rats consuming soy protein have reduced cholesterol in the serum and liver (Tovar *et al.*, 2002, 2005). Yang *et al.* (2007) reported that rats fed soy protein had increased excretion of cholesterol and reduced plasma and liver lipids compared to rats fed casein. The findings of the earlier animal studies led to an interest in dietary soy for humans.

### 17.2.1.2 Human studies

Studies of human populations have demonstrated reduced serum cholesterol related to an increase in soy intake (Nagata *et al.*, 1998; Ho *et al.*, 2000). In 1995, a well-publicized meta-analysis of randomized controlled trials by Anderson *et al.* (1995) showed a significant reduction in total cholesterol, LDL-cholesterol (LDL) and triglycerides from soy protein compared with animal protein (Figure 17.1a). Following a surge in soy research in the late 1990s, the US Food and Drug Administration approved a health claim for soy protein and risk of coronary disease in 1999 (Stein, 2000). Since the approval of the health claim, a number of clinical trials have been conducted on dietary intake of soy protein (Balk *et al.*, 2005; Sirtori and Johnson, 2006; Xiao, 2008; Zhan and Ho, 2005). There have been conflicting results regarding the quantity and type of soy protein required for cholesterol reduction (Messina and Lane, 2007). This may be related in part to varying study design and initial cholesterol levels of subjects. For example, several studies have found a larger LDL reduction in hypercholesterolemic subjects as opposed to those with normal cholesterol levels (Zhan and Ho, 2005). In a review by Setchell and Cassidy (1999), 6–8 g of soy protein daily was estimated as the minimum to show cardiovascular benefit in the long-term. This is lower than the 25 g per day recommended by the US FDA to reduce risk of cardiovascular disease (Stein, 2000).

Many studies have examined the effect of feeding soy protein isolate compared with casein on serum cholesterol levels. The meta-analysis by Zhan and Ho (2005) studied the effects of soy protein containing isoflavones, and found an overall reduction of 3.77% in total cholesterol levels and 5.25% in LDL cholesterol. The total effect was dependent on the demographics of the population and also on initial cholesterol levels. The meta-analysis by Anderson *et al.* (1995) also reported that the total effect from soy protein was related to the subjects' initial cholesterol levels (Figure 17.1b).

A 2006 analysis by the American Heart Association (AHA) nutrition committee found that the effect of soy protein on cholesterol levels was not as significant as previously thought (Sacks *et al.*, 2006). The authors found that in well-controlled studies, LDL cholesterol reduced only 2–7 percentage points with intake of soy protein compared with animal protein. The announcement of the results cast doubt upon the previous FDA health claim. The AHA nutrition committee concluded that soy remains a heart-healthy food due to its low saturated fat content. However, the cholesterol-reducing effects may not be sufficient to warrant the previous promotion of soy.



**Figure 17.1** Summary of serum lipid changes reported from a meta-analysis of clinical trials (difference between a soy protein diet and a control diet): a) percent decrease in total serum cholesterol, LDL, and triglycerides; b) decrease in serum LDL cholesterol (mg/dL) as related to initial total serum cholesterol level.

Source: Based on data presented in Anderson *et al.*, 1995.

There are several possible reasons for the disparity between the previous studies that indicated strong hypocholesterolemic effects of soy and the later studies that are more equivocal. According to a review by Messina and Lane (2007), texturized soy protein was used frequently in earlier studies, and later studies often used soy protein isolate. The processing of soy protein may alter its effective properties. Gianazza *et al.* (2003) demonstrated that different soy protein products contained varying amounts of the 7S globulin and 11S globulin fractions. Soy protein isolate (used in many American studies) had less 7S globulin components than a soy protein product used in European studies that had shown significant cholesterol reduction. 7S globulin  $\alpha'$  is known for reduction of serum lipids and reduced atherosclerosis in animal models (Duranti *et al.*, 2004; Adams *et al.*, 2004). The study by Gianazza *et al.* (2003) suggests that soy protein isolate may be less

effective due to elimination of the 7S globulin  $\alpha'$  during processing. However, in a 2008 meta-analysis of randomized controlled trials, Hooper *et al.* (2008) indicated that soy protein isolate reduced LDL cholesterol but whole soy foods did not. A review by Lichtenstein *et al.* (2002) states that many studies have examined the intake of soy protein compared with casein, and that the peptide differences between the proteins may also have an unrealized effect on lipid metabolism. The isoflavone (phytochemical) content of soy protein may also potentially affect lipid levels, and will be discussed later in the chapter.

### 17.2.1.3 Mechanisms of soy protein effect

The mechanism of soy protein on LDL reduction has been attributed to reduced absorption of intestinal cholesterol, reduced cholesterol synthesis, or stimulation of LDL receptor transcription (Cho *et al.*, 2007). Rabbits have shown increased intestinal cholesterol absorption when consuming a casein diet compared with a soy protein diet (Huff *et al.*, 1982; Huff and Carroll, 1980). Baum *et al.* (1998) reported an increase in mRNA concentration of LDL receptors in women after 24 weeks of intake of soy protein, whereas this measurement was decreased on a casein diet. An *in vitro* study of soy protein hydrolysate on human liver cell lines showed significant increase of LDL receptor transcription (Cho *et al.*, 2007). This may then increase uptake of LDL-cholesterol in the liver and reduce serum levels. The same study failed to find a high bile acid-binding capability in soy protein hydrolysate, and found that at high levels the soy protein hydrolysate stimulated cholesterol synthesis. Based on these findings, the relationship between soy protein and serum cholesterol is complex, and further research may be necessary.

## 17.2.2 Soy isoflavones

Isoflavones are bioactive phytochemicals in the soybean that have similar structure to the hormone estrogen (Mortensen *et al.*, 2009). When examining the effect of soy on serum cholesterol, it is not clear whether it is the protein itself or the isoflavones that are responsible (Messina and Lane, 2007). Soy protein contains varying amounts of isoflavones, depending on the source and processing methods (Franke *et al.*, 1999). In studies of isoflavones separated from soy protein, the isoflavones generally do not have significant effect on reducing serum LDL (Sirtori and Johnson, 2006). However, soy protein without isoflavones retains its hypocholesterolemic properties (Sirtori and Johnson, 2006). Some evidence shows a synergistic effect of soy isoflavones and soy protein on reduction of LDL cholesterol (Sirtori and Johnson, 2006).

To demonstrate the effect of plant protein alone, Sirtori *et al.* (2004) examined the effect of white lupin seed protein (a legume that is naturally low in isoflavones) on plasma cholesterol levels of rats. They found a 21% decrease in total cholesterol and 30% decrease in combined VLDL and LDL levels in rats fed the lupin extract compared with controls. These results demonstrate that plant protein may have a cholesterol-lowering effect, and that the isoflavones in soy may not be the most important factor in its effect on serum cholesterol.

### 17.2.2.1 Animal studies

Although soy protein alone may reduce cholesterol, the combination of soy protein and isoflavones has demonstrated positive effects. In a study by Anthony *et al.* (1997), monkeys

were fed casein, soy protein depleted of isoflavones, or soy protein with intact isoflavones. After 14 months, monkeys that were fed soy protein with isoflavones had significantly reduced LDL levels and increased HDL levels compared to the other groups. Additionally, the monkeys fed isoflavone-rich protein had 90% fewer coronary atherosclerotic lesions than those fed casein. This may suggest that beneficial effects of isoflavones may go beyond reduction of LDL cholesterol.

#### 17.2.2.2 Human studies

Some human studies have also found that soy protein has a greater effect on cholesterol when isoflavones are increased (Wangen *et al.*, 2001; Merz-Demlow *et al.*, 2000). Merz-Demlow *et al.* (2000) conducted a triple crossover study in which normocholesterolemic women consumed soy protein with a high isoflavone level (~2 mg/kg body weight/day), soy protein with a low isoflavone level (~1 mg/kg body weight/day), and soy protein without isoflavones for three months each. They reported that the high level of isoflavones combined with soy protein lowered LDL cholesterol by as much as 10% more than the soy protein depleted of isoflavones. A 2007 study by Clerici *et al.* found that pasta enriched with isoflavones but containing no soy protein reduced LDL cholesterol by 8.6% from baseline, while no significant change occurred with non-enriched pasta. A meta-analysis performed by Weggemans and Trautwein (2003) on isoflavones and cholesterol found that average consumption of 36 g per day of soy protein with 52 mg per day of isoflavones reduced LDL cholesterol by 4% and increased HDL by 3%. However, there was not a change in cholesterol associated with changes in isoflavone intake. The Agency for Healthcare and Research Quality (AHRQ) performed an extensive review in 2005 of soy and isoflavone studies conducted on human subjects. From 68 studies they concluded that soy products (including protein and/or isoflavones) resulted in 3% reduction in LDL-cholesterol in persons with elevated cholesterol levels (Balk *et al.*, 2005). The meta-analysis of flavonoid trials by Hooper *et al.* (2008) found no effect of isoflavones on LDL cholesterol levels. Another 2008 meta-analysis of randomized-controlled trials on soy isoflavones separated from soy protein reported that an average of 70 mg per day did not affect total or LDL cholesterol in post-menopausal women (Taku *et al.*, 2008). Based on these findings, the authors proposed that extraction processes may reduce the effectiveness of isoflavones, or that they are most effective in combination with soy protein (Taku *et al.*, 2008).

It is also known that people metabolize soy isoflavones differently. More than 30% of human subjects do not produce the daidzein metabolite equol, which has high affinity for estrogen receptors (Setchell *et al.*, 2002). According to Setchell *et al.* (2002), it is possible that some studies did not control for equol production and that this may explain some of the contradicting outcomes in soy research. In a relatively small study, Meyer *et al.* (2004) found that equol producers had significantly lowered total cholesterol, LDL cholesterol, and triglycerides, while non-equol producers had no significant lipid changes when following the same regimen. However, Thorp *et al.* (2008) reported a triple crossover diet intervention study where there was no significant difference in lipids between equol producers and non-producers when following a diet supplemented with soy protein.

*In vitro* and *in vivo* studies have demonstrated the potential for soy protein and isoflavones to reduce serum cholesterol. However, even after a great number of studies have been conducted, there are still questions regarding the amount and type of soy food that is most effective. Although the overall benefit of soy protein on serum cholesterol levels remains controversial, there are other soybean components that have demonstrated positive effects.

### 17.2.3 Soy phytosterols

Sterols are lipid compounds that contain three 6-carbon rings, one 5-carbon ring, and an aliphatic tail (McClements and Dekker, 2008). Cholesterol in animals is one of the most well known sterols, and helps to stabilize membranes. Phytosterols maintain membrane fluidity in plants, as cholesterol does in animals (Brufau, Canelas, and Rafelas, 2008). Soybean oil is a rich source of phytosterols (Brufau *et al.*, 2008). The structure of phytosterols is similar to cholesterol. Consumption of phytosterols can reduce LDL cholesterol in humans, in part because they reduce absorption of cholesterol. It is believed that, within the intestinal lumen, phytosterols replace cholesterol in mixed micelles (Sanclemente *et al.*, 2009). There is evidence that the phytosterols do not need to be consumed at the same time as cholesterol to have a lipid-lowering effect (Plat *et al.*, 2000; Sanclemente *et al.*, 2009).

In a natural form, plant sterols have low bioavailability, but they may be esterified to improve absorption (Brufau *et al.*, 2008). Plant sterols can be saturated to form stanols, which are also more bioavailable (AbuMweis and Jones, 2008). Recently sterol esters and stanols have been added to food products with the aim of helping to reduce serum cholesterol. These food products are typically fat-soluble, such as margarine spreads, but also include yogurt, baked goods, juice, and chocolate (AbuMweis and Jones, 2008). A relatively low amount of sterol esters, such as 2–3 g/day, has shown to be effective in reducing LDL cholesterol (Brufau *et al.*, 2008).

A meta-analysis of randomized control trials by AbuMweis, Barake, and Jones (2008) showed that reduction in LDL cholesterol by dietary sterols/stanols was greater in subjects with hypercholesterolemia than those with normal cholesterol levels. The carrier food was important in the bioavailability of the sterols and stanols, with fat-containing spreads having the largest effect on LDL reduction. Consumers are usually directed to take sterol-containing products two to three times daily to maximize the benefit. However, there has been one study that determined once-daily consumption of phytosterols produced similar cholesterol-lowering effects as multiple servings per day (Plat *et al.*, 2000).

### 17.2.4 Soy fiber

Dietary fiber can be defined as an indigestible component of foods of vegetable origin (American Association of Cereal Chemists, 2001). Soybeans contain approximately 5% fiber by weight (Maier *et al.*, 1998). The soy cell wall has been found to contain 20% cellulose, 50% hemicellulose, and 30% pectin (Kikuchi *et al.*, 1971). Hemicellulose and cellulose are considered insoluble fibers, which are important in digestive health (Riaz, 2001). Pectin is a soluble fiber that can improve serum cholesterol and glucose levels (Riaz, 2001). The soybean hull, or seed coat, and the cotyledon provide the majority of its fiber (Riaz, 2001). The hull contains 32% of the soybean's iron, so soy fiber can be added to increase iron content in cereal-based foods (Riaz, 2001).

Soy fiber that is left as a residue from soymilk and tofu production is called okara. Okara contains 20% of the protein and 11% of the oil from the original soybean (Wang and Cavins, 1989). Because okara is a byproduct of food processing, its possible uses have been studied. It has demonstrated water-holding capacity and fat-holding capacity (Kuan and Liong, 2008). Thus, the soy fiber can improve emulsion stability and act as a fat replacement in baked goods. It has been used in a wide variety of reduced-calorie products, including puddings and beverages (Riaz, 2001).

Okara contains the anti-nutritional factor phytic acid; however, fermented okara has shown potential for nutraceutical value (O'Toole, 1999). Fermented okara contains antioxidants such as isoflavones and  $\gamma$ - and  $\delta$ -tocopherol. Jimenez-Escrig *et al.* (2008) reported the effect of a 10% okara diet on nutritional parameters in rats. Following four weeks of the diet, the rats had reduced total cholesterol, reduced weight gain, and higher antioxidant levels in the cecum than those on a control diet. This provides preliminary evidence that there may be cardiovascular benefits when soy fiber is added to foods.

## 17.3 Soy and inhibition of LDL oxidation

In addition to the research on dietary soy and serum cholesterol, there is increasing evidence that other mechanisms may explain the relationship between soy and cardiovascular disease. A part of the proposed inflammatory mechanism of the development of atherosclerotic plaque involves the oxidation of low density lipoprotein (ox-LDL), though the precise details of how inflammation and oxidation work together to form atherosclerotic plaques are not well understood. The interested reader is referred to reviews by Stocker and Keane (2004) and Lapointe *et al.* (2006) for more details on the proposed steps of the oxidation and deposition of LDL. For the purposes of this chapter, let it be sufficient to say that ox-LDL has been used as a marker of cardiovascular disease risk.

Soy has been investigated on many levels for its ability to inhibit the oxidation of LDL, including *in vitro*, animal, and human *in vivo* studies. In particular, isoflavones have been studied most heavily in this regard, though soy protein diets (with isoflavones present or removed) have also been studied. Though *in vitro* and *in vivo* experiments have focused on isoflavones, they are not the only bioactive component of soy proposed to inhibit LDL oxidation. Small bioactive peptides,  $\alpha$ -tocopherol, lignans, and unsaturated fatty acids have all been suggested to reduce LDL oxidation through various mechanisms (van Ee, 2009).

### 17.3.1 In vitro studies

LDL oxidation experiments performed *in vitro* are used in attempts to predict activity *in vivo* or to elucidate mechanisms involved behind active components. Protocols of this nature typically isolate LDL from healthy human subjects, expose it to soy components in the presence of an oxidizing agent (most often  $\text{Cu}^{2+}$ ), and measure the amount of time before LDL oxidation occurs (lag time) via spectrophotometric monitoring of conjugated diene formation at  $\sim 234$  nm. Rates of reaction and levels of thiobarbituric acid reactive substances (TBARS) have also been utilized as indicators of extent of oxidation.

Genistein and daidzein are the aglycone forms of the predominant isoflavones in soy and have been most researched of the soy isoflavones in this area. Kapiotis *et al.* (1997) reported that genistein is capable of inhibiting LDL oxidation under initiation by two separate oxidation systems *in vitro*: copper ( $2+$ ) and a superoxide/nitric oxide radical system ( $\text{O}_2^-/\text{NO}^\bullet$ ). Additionally, genistein was able to prevent oxidation of LDL in endothelial cells, as well as prevent damage to the cells by ox-LDL. Later, Exner and others (2001) incubated LDL with glucose to initiate oxidation, in order to model the increased oxidation seen in diabetic patients. When genistein was added to this system, it successfully reduced the oxidation of LDL, as measured by TBARS, although daidzein did not prove effective.

Recently, scrutiny has fallen on research involving the aglycones genistein and daidzein, because they exist only in minute quantities in the blood. Rather, after ingestion they are metabolized in intestinal and hepatic cells prior to circulation. Studies have also sought to assess the activity of these metabolites against LDL oxidation *in vitro*. The O- $\beta$ -D glucuronic acids of genistein and daidzein significantly increased the lag time of LDL oxidation, but not to the same extent as the aglycones (Kgomotso *et al.*, 2008). Similarly, the monosulphated conjugates of genistein and daidzein were less effective than the aglycones, and the disulphated conjugates displayed essentially no activity *in vitro* (Turner *et al.*, 2004). Meanwhile, equol was a more effective oxidation inhibitor than daidzein, though the sulphated conjugates of equol showed a similar trend of decreased inhibition (Turner *et al.*, 2004).

Interestingly, Patel and others (2001) report that isoflavones were not consumed in reaction mixtures where they effectively protected LDL against Cu<sup>2+</sup>-initiated and myoglobin-initiated oxidation, suggesting that some sort of regeneration of the phenolics occurs. Additionally, a synergistic effect in the presence of ascorbate was noticed. Hwang *et al.* (2000) also reported that the inhibition of LDL oxidation by genistein, daidzein, and the daidzein metabolite equol was enhanced synergistically by the presence of ascorbic acid.

Finally, oxidation of high density lipoprotein (HDL) has also been studied. Upon the indication that the anti-atherogenic properties of HDL are impaired by oxidation, Ferretti *et al.* (2004) showed that genistein also protects HDL from copper-induced oxidation, as measured by conjugated diene formation.

### 17.3.2 Animal studies

A variety of animal models have been used to study the effect of soy diets on LDL oxidation. LDL oxidation in these studies has most frequently been assessed by isolation of LDL from diet-treated animals, followed by induction of oxidation (most often by Cu<sup>2+</sup>) monitored by conjugated diene formation, oxidation lag time, or TBARS. Less frequently, direct analysis of lipid peroxides in blood or specific tissues is used.

Yamakoshi *et al.* (2000) fed New Zealand rabbits a cholesterol-containing diet supplemented with two doses of isoflavone-rich extracts of fermented soy extracts (0.33 and 1 g isoflavone aglycones/100 g food). After eight weeks, an increase in resistance to Cu-induced LDL oxidation was seen with the isoflavone-supplemented diets, as measured by HPLC analysis of cholesteryl ester hydroperoxides – the high dose produced a significant reduction in hydroperoxides (–94%), while the low dose produced a reduction that was not significant (–37%). Additionally, atherosclerotic lesions in the aortic arch were significantly decreased by both isoflavone treatment levels. No difference in cholesterol levels was seen. A similar saponin-enhanced diet in the same study showed no effect. Another study with New Zealand rabbits fed low doses of isolated isoflavones (2.5 and 5 mg/kg body weight administered every other day) – both doses significantly reduced plasma TBARS after 13 weeks (Yousef *et al.*, 2004).

A third study using New Zealand rabbits tested the effect of protein source in a high-cholesterol diet on LDL-oxidation (Damasceno *et al.*, 2000). Rabbits fed a diet with soy protein isolate (SPI) as the protein source had lower levels of highly oxidized LDL, more minimally oxidized LDL, and less area of atherosclerotic lesions at various time points throughout the study than rabbits fed a diet with casein as the protein source. However,

fluctuations in some of these values at different time points (0, 15, 30, 45, and 60 days) prompt the need for further study of protein source and ox-LDL. Tsai and Huang (1999) performed a similar study in hamsters, comparing the effects of SPI diets (with and without isoflavones extracted) with fish protein on Cu<sup>2+</sup>-induced LDL oxidation. The LDL isolated from hamsters fed the SPI diet containing isoflavones was most effective at prolonging oxidation lag time and inhibiting TBARS formation.

In another hamster study, Fang and others (2004) fed males a vitamin E deficient diet with 0, 50, or 200 mg genistein/kg chow. After five weeks, the high dose of genistein was shown to significantly increase the lag time for LDL oxidation, reduce the propagation rate of conjugated diene formation during LDL oxidation, and decrease TBARS formation.

Through soy protein feeding studies (with and without isoflavones) in LDL-receptor deficient C57BL/6J mice and their normal counterpart, the work of Kirk *et al.* (1998) suggests that the LDL-receptors play a role in isoflavone's ability to prevent atherosclerosis. However, the isoflavones-containing soy protein-based diet did not significantly change the lag time of LDL oxidation in this study.

Finally, a model for type 2 diabetes – the GK rat – has also been used to investigate the significant risk diabetics have of developing heart disease (Quan *et al.*, 2009). When compared to control rats fed standard chow, rats fed a diet supplemented with soybean hypocotyl extract experienced reduced levels of plasma lipid peroxides, reduced HDL lipid peroxides, lower TBARS in the liver, and increased lag time of LDL oxidation.

### 17.3.3 Human studies

Human studies use similar methods for analyzing LDL oxidization as animal studies, including primarily Cu<sup>2+</sup> mediated oxidation of LDL isolated from treated subjects and/or direct quantification of lipid peroxides in the blood. Also, antibodies against LDL-ox have been assessed, in addition to measurement of secondary lipid oxidation products excreted in urine, and F2 isoprostane measurement.

Human feeding studies are inevitably less controlled than animal studies and more often involve supplementation of the diet with soy products, rather than complete substitution of the protein source. Results of soy food supplementation on human LDL oxidation have been mixed, but tend to favor some positive effect.

A number of studies have shown that supplementation of the diet with a soy food resulted in a significantly prolonged lag phase of LDL oxidation as compared to control diets (Tikkanen *et al.*, 1998; Wiseman *et al.*, 2000; Ashton *et al.*, 2000; Scheiber *et al.*, 2001), decreased conjugated dienes in the LDL fraction (Jenkins *et al.*, 2000, 2002), and decreased the presence of secondary lipid oxidation products in the urine (Fritz *et al.*, 2003). There was significant variation in these research protocols, with effective doses ranging from 15 g soy protein/50 mg isoflavones per day (Wiseman *et al.*, 2000) to 33 g soy protein/86 mg isoflavones per day (Jenkins *et al.*, 2000). Length of dietary interventions ranged from 2 to 12 weeks. Additionally, the work of Scheiber and others (2001) showed a positive correlation between lag time of LDL oxidation and serum phytoestrogens.

However, a well-designed clinical crossover trial testing for both protein source and isoflavone presence in the diet of 42 hypercholesterolemic adults saw no difference in LDL oxidation during 42 day treatments (Vega-Lopez *et al.*, 2005). Engelman *et al.* (2005) also did not see a significant impact of soy supplementation (40 g/day + 85 mg isoflavones) on



ox-LDL levels. Despite noting a correlation between LDL concentration and ox-LDL levels, their study did not explicitly control for initial lipoprotein values.

Soy extracts and isolated isoflavones have also been assessed *in vivo* for their ability to mitigate LDL oxidation after consumption and digestion, though human data of isolated components are more limited. Hsu *et al.* (2007) showed consumption of 6 g soy germ extract containing 120 mg isoflavones/day  $\times$  4 weeks significantly increased lag time of isolated LDL by 10% and decreased TBARS by 6%.

Soy studies trend toward positive outcomes in LDL oxidation, but it is pertinent to note the short-term nature of the studies. More long-term trials are necessary to gain a more complete picture of soy's impact on ox-LDL formation.

## 17.4 Soy and inflammation

A newly appreciated link in the development of CVD involves inflammatory pathways – inflammation appears to be an integral part of the development of atherosclerosis, playing roles in the attraction and activation of leukocytes in the arterial intima, and the progression from fatty streak to a complicated lesion (Libby, 2002). Biomarkers used to study inflammation include levels of the C-reactive protein, pro-inflammatory cytokines (interleukins, tumor necrosis factors and monocyte chemoattractant proteins, among others), along with measures of expression of genes encoding for them.

The beneficial effects of isoflavones or isoflavone-containing foods on pro-inflammatory cytokine levels have been demonstrated *in vitro* and in animals (Blay *et al.*, 2010; Sato *et al.*, 2007; Paradkar *et al.*, 2004; Gottstein *et al.*, 2003; Kao *et al.*, 2007). Where comparisons were made, genistein appeared to have a stronger effect than daidzein (Blay *et al.*, 2010; Gottstein *et al.*, 2003). However, a recent review of human clinical trials suggests that soy consumption has no effect on *in vivo* expression of the cytokines interleukin-6 and tumor necrosis factor  $\alpha$  (Beavers *et al.*, 2009). Additionally, the clinical trials provided mixed results as to whether soy consumption was able to mediate the production of cellular adhesion molecules, which play a role in allowing leukocytes to adhere to arterial endothelial cells – a critical step in lesion formation.

C-reactive protein (CRP) is an acute-phase protein secreted in response to pro-inflammatory cytokines; thus, it is also used as a biomarker of inflammation, and serum levels are predictive of cardiovascular disease risk. A review of National Health and Nutrition Education Survey (NHANES) data from 1999–2002 showed that genistein and daidzein consumption were individually correlated to serum CRP concentrations in US adults (Chun *et al.*, 2008). However, a review of clinical human trials found limited evidence to suggest that soy protein or isoflavone consumption affect levels of C-reactive protein (Messina and Lane, 2007).

## 17.5 Soy and hypertension

Hypertension is a major modifiable risk factor for cardiovascular disease (Mancia, 2007). Although incidence varies by demographic groups, in 2002 approximately 26% of the US population had hypertension (Hajjar *et al.*, 2006). It has been estimated that an increase in

systolic blood pressure of 20 mm Hg or in diastolic blood pressure of 10 mm Hg will double cardiovascular disease risk in the population aged 40–70 years (Lawes *et al.*, 2002).

There has been some research showing an effect of soy consumption on hypertension in humans. Nagata *et al.* (2003) reported an inverse correlation between blood pressure and soy intake in Japanese men, when food intake was surveyed by questionnaire. However, the same study did not find a significant effect in women. Similarly, using a randomized crossover design, Jenkins *et al.* (2002) described a reduction of systolic blood pressure in men when following a diet enriched with soy protein isolate compared to a low-fat dairy protein diet. Rivas *et al.* (2002) demonstrated a significant decrease in systolic and diastolic blood pressure with consumption of soy milk versus cow's milk over a three month period. They also noted a correlation between decrease in blood pressure and urinary excretion of genistein. In 2007, Welty *et al.* reported that 25 g soy protein per day provided through soy nuts lowered systolic and diastolic blood pressure significantly in both hypertensive and normotensive women. The meta-analysis of soy protein intake studies by the Agency for Healthcare Research and Quality in 2005 found no significant effects of soy protein on hypertension (Balk *et al.*, 2005). As indicated by Messina and Lane (2007) there may be different effects on hypertension when consuming whole soy foods versus soy protein isolate, as was the case in some of the studies.

## **17.6 Soy and endothelial function**

Arterial stiffness is a marker of cardiovascular disease. It is known that hormone replacement therapy can improve arterial flow and endothelial function in post-menopausal women (Shaw *et al.*, 2009). The phytoestrogens have been investigated for possible effects on endothelial function as a mechanism for reduced CVD risk. Some studies have demonstrated that isoflavones may act similarly to estrogen on arterial endothelial function (Altavilla *et al.*, 2004). Mahn *et al.* (2005) found improved vascular reactivity and increased endothelial nitric oxide synthase in aortic tissues of rats fed a soy protein diet compared to those fed a non-soy diet. Steinberg *et al.* (2003) reported improvement in peak flow velocity of the brachial artery in human subjects following six weeks of dietary supplementation of soy protein containing isoflavones. Conversely, Teede *et al.* (2001) reported a decline in brachial artery flow-mediated vasodilation in men with supplementation of 40g soy protein (containing isoflavones) per day over three months. The above results indicate that to characterize the impact of soy intake on endothelial function, further research is needed.

## **17.7 Conclusions**

The collective research summarized here demonstrates the potential relationship between intake of soy foods and prevention of cardiovascular disease. As discussed, there are several possible mechanisms by which soy may have beneficial effects. The reduction of serum cholesterol levels is one area where extensive research has been conducted, but has not reached a definitive conclusion. Inhibition of LDL-oxidation by dietary soy shows an overall favorable effect. Soy foods or individual soy components have potential benefits for reduction of inflammation, hypertension, and improvement in endothelial function. Due to the diversity of soy foods and soy protein products, there may be variation in the outcome and interpretation of the research (Messina and Lane, 2007). It is known that soybean

chemical properties may vary widely by genotype and are influenced by growing environment (Riedl *et al.*, 2007; Lee *et al.*, 2003). It is of interest to determine the components of the soybean that may prevent the development of CVD, and subsequently it may be beneficial to develop cultivars that are rich in these specific biochemical components.

## References

- AbuMweis, S.S., Barake, R., Jones, P.J.H. (2008) Plants sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. *Food Nutrition Research*, **52** doi: 10.3402/fnr.v52i0.1811. Epub 2008 Aug 18.
- AbuMweiss, S.S., and Jones, P.J.H. (2008) Cholesterol-lowering effect of plant sterols. *Current Atherosclerosis Reports*, **10**: 467–472.
- Adams, M.R., Golden, D.L., Franke, A.A., Potter, S.M., Smith, H.S., and Anthony, M.S. (2004) Dietary soy  $\beta$ -conglycinin (7S Globulin) inhibits atherosclerosis in mice. *Journal of Nutrition*, **134**: 511–516.
- Altavilla, D., Crisafulli, A., Marini, H., Esposito, M., D'Anna, R., Corrado, F., Bitto, A., and Squadrito, F. (2004) Cardiovascular effects of the phytoestrogen genistein. *Current Medical Chemistry Cardiovascular Hematological Agents*, **2**: 179–186.
- American Association of Cereal Chemists (2001) The definition of dietary fiber. *Cereal Foods World*, **46**: 112–126.
- Anderson, J.W., Johnstone, B.M., and Cook-Newell, M.E. (1995) Meta-analysis of the effects of soy protein intake on serum lipids. *New England Journal of Medicine*, **333**(5): 276–282.
- Anthony, M.S., Clarkson, T.B., Bullock, B.C., and Wagner, J.D. (1997) Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **17**: 2524–2531.
- Ash, M., Livezey, J., and Dohlman, E. (2006) *Soybean Backgrounder*. Electronic outlook report from the Economic Research Service. USDA. 2–42.
- Ashton, E.L., Dalais, F.S., and Ball, M.J. (2000) Effect of meat replacement by tofu on CHD risk factors including copper induced LDL oxidation. *Journal of the American College of Nutrition*, **19**(6): 761–767.
- Balk, E., Chung, M., Chew, P., Ip, S., Raman, G., Kupelnick, B., Tatsioni, A., Sun, Y., Wolk, B., DeVine, D., and Lau, J. (2005) *Effects of Soy on Health Outcomes*. Evidence Report/Technology Assessment No. 126. (Prepared by Tufts-New England Medical Center Evidence-based Practice Center, under Contract No. 290-02-0022.) AHRQ Publication No. 05-E024-2. Rockville, MD: Agency for Healthcare Research and Quality.
- Baum, J.A., Teng, H., Erdman, J.W., Weigel, R.M., and Kein, B.P. *et al.* (1998) Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, post-menopausal women. *American Journal of Clinical Nutrition*, **68**: 545–551.
- Beavers, K.M., Jonnalagadda, S.S., and Messina, M.J. (2009) Soy consumption, adhesion molecules, and pro-inflammatory cytokines: A brief review of the literature. *Nutrition Reviews*, **67**(4): 213–221.
- Blay, M., Espinel, A.E., Delgado, M.A., Gaiges, I., Blade, C., Arola, L., and Salvado, J. (2010) Isoflavone effect on gene expression profile and biomarkers of inflammation. *Journal of Pharmaceutical and Biomedical Analysis*, **51**: 382–390.
- Brufau, G., Canela, M.A., and Rafecas, M. (2008) Phytosterols: Physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutrition Research*, **28**: 217–225.
- Campbell, T.C., Parpia, B., and Chen, J. (1998) Diet, lifestyle, and the etiology of coronary artery disease: The Cornell China Study. *American Journal of Cardiology*, **82**: 18T–21T.
- Carroll, K.K., and Kurowska, E.M. (1995) Soy consumption and cholesterol reduction: Review of animal and human studies. *American Journal of Clinical Nutrition*, **125**: 594S–597S.
- Cho, S.J., Juillerat, M.A., and Lee, C.H. (2007) Cholesterol lowering mechanism of soybean protein hydrolysate. *Journal of Agricultural and Food Chemistry*, **55**: 10599–10604.
- Chun, O.K., Chung, S.J., Claycombe, K.J., and Song, W.O. (2008) Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. *Journal of Nutrition*, **138**: 753–760.

- Clerici, C., Setchell, K.D.R., Battezzati, P.M., Pirro, M., and Giuliano, V., *et al.* (2007) Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. *Journal of Nutrition*, **137**: 2270–2278.
- Damasceno, N.R.T., Goto, H., Rodrigues, F.M.D., Dias, C.T.S., Okawabata, F.S., Abdalla, D.S.P., Gidlund, M. (2000) Soy protein isolate reduces the oxidizability of LDL and the generation of oxidized LDL autoantibodies in rabbits with diet-induced atherosclerosis. *Journal of Nutrition*, **130**: 2641–2647.
- Duranti, M., Lovati, M.R., Dani, V., Barbiroli, A., Scarafoni, A., Castiglioni, S., Ponzzone, C., and Morazzoni, P. (2004) The  $\alpha'$  subunit from soybean 7S globulin lowers plasma lipids and upregulates liver  $\beta$ -VLDL receptors in rats fed a hypercholesterolemic diet. *Journal of Nutrition*, **134**: 1334–1339.
- Egusa, G., and Yamane, K. (2004) Lifestyle, serum lipids, and coronary artery disease: Comparison of Japan with the United States. *Journal of Atherosclerosis and Thrombosis*, **11**(6): 304–312.
- Engelman, H.M., Alekel, D.L., Hanson, L.N., Kanthasamy, A.G., and Reddy, M.B. (2005) Blood lipid and oxidative stress responses to soy protein with isoflavones and phytic acid in postmenopausal women. *American Journal of Clinical Nutrition*, **81**: 590–596.
- Exner, M., Hermann, M., Hofbauer, R., Kapiotis, S., Quehenberger, P., Speiser, W., Held, I., and Gmeiner, B.M.K. (2001) Genistein prevents the glucose autoxidation mediated atherogenic modification of low density lipoprotein. *Free Radical Research*, **134I**: 101–112.
- Fang, Y.C., Chen, B.H., Huang, R.F., and Lu, Y.F. (2004) Effect of genistein supplementation on tissue genistein and lipid peroxidation of serum, liver and low-density lipoprotein in hamsters. *Journal of Nutritional Biochemistry*, **15**: 142–148.
- Ferretti, G., Bacchetti, T., Menanno, F., and Curatola, G. (2004) Effect of genistein against copper-induced lipid peroxidation of human high density lipoproteins (HDL). *Atherosclerosis*, **172**: 55–61.
- Franke, A., Hankin, J.H., Yu, M.C., Maskarinec, G., Low, S., and Custer, L.J. (1999) Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *Journal of Agriculture and Food Chemistry*, **47**: 977–986.
- Fritz, K.L., Seppanen, C.M., Kurzer, M.S., and Csallany, A.S. (2003) The in vivo antioxidant activity of soybean isoflavones in human subjects. *Nutrition Research*, **23**: 479–487.
- Gianazza, E., Eberini, I., Arnoldi, A., Wait, R., and Sirtori, C. (2003) A proteomic investigation of isolated soy proteins with variable effects in experimental and clinical studies. *Journal of Nutrition*, **133**: 9–14.
- Gottstein, N., Ewins, B.A., Eccleston, C., Hubbard, G.P., Kavanagh, I.C., Minihane, A.M., Weinberg, P.D., and Rimbach, G. (2003) Effect of genistein and dadizein on platelet aggregation and monocyte and endothelial function. *British Journal of Nutrition*, **89**: 607–615.
- Gould, A.L., Davies, G.M., Alemao, E., Yin, D.D., and Cook, J.R. (2007) Cholesterol reduction yields clinical benefits: Meta-analysis including recent trials. *Clinical Therapeutics*, **29**(5): 778–794.
- Hajjar, I., Kotchen, J.M., and Kotchen, T.A. (2006) Hypertension: Trends in prevalence, incidence, and control. *Annual Review of Public Health*, **27**: 465–490.
- Hamilton, R.M., and Carroll, K.K. (1976) Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets: Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis*, **24**: 47–62.
- Ho, S.C., Woo, J.L.F., Leung, S.S.F., Sham, A.L.K., and Janus E.D. (2000) Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. *Journal of Nutrition*, **130**: 2590–2593.
- Hooper, L., Kroon, P.A., Rimm, E.B., Cohn, J.S., Harvey, I., Le Cornu, K.A., Ryder, J.J., Hall, W.L., and Cassidy, A. (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition*, **88**: 38–50.
- Hsu, I.P., Jou, H.J., Huang, C.W., Wang, T.A., and Wu, W.H. (2007) The effects of soygerm extracts on blood lipoproteins, antioxidative capacity and urinary estrogen metabolites in postmenopausal women on hormone therapy. *International Journal of Gynecology and Obstetrics*, **98**: 29–33.
- Huff, M.W., Roberts, D.C., and Carroll, K.K. (1982) Long-term effects of semipurified diets containing casein or soy protein isolate on atherosclerosis and plasma lipoproteins in rabbits. *Atherosclerosis*, **41**: 326–327.
- Huff, M.W., and Carroll, K.K. (1980) Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *Journal of Lipid Research*, **21**: 546–558.
- Hwang, J., Sevanian, A., Hodis, H.N., and Ursini, F. (2000) Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. *Free Radical Biology and Medicine*, **29**(1): 79–89.

- Jackman, K.A., Woodman, O.L., and Sobey, C.G. (2007) Isoflavones, equol, and cardiovascular disease: Pharmacological and therapeutic insights. *Current Medicinal Chemistry*, **14**: 2824–2830.
- Jenkins, D.J.A., Kendall, C.W.C., Garsetti, M., Rosenberg-Zand, R.S., Jackson, C.J., Agarwal, S., Rao, A.V., Diamandis, E.P., Parker, T., Faulkner, D., Vuksan, V., and Vidgen, E. (2000) Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity – a controlled crossover trial. *Metabolism*, **49**(4): 537–543.
- Jenkins, D.J.A., Kendall, C.W.C., Jackson, C.J.C., Connelly, P.W., Parker, T., Faulkner, D., Vidgen, E., Cunnane, S.C., Leiter, L.A., and Josse, R.G. (2002) Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *American Journal of Clinical Nutrition*, **76**: 365–362.
- Jimenez-Escrig, A., Tenorio, M.D., Espinosa-Martos, I., and Ruperez, P. (2008) Health-promoting effects of concentrate of soybean fiber okara in rats. *Journal of Agricultural and Food Chemistry*, **56**: 7495–7501.
- Kao, T.H., Wu, W.M., Hung, C.F., Wu, W.B., and Chen, B.H. (2007) Anti-inflammatory effects of isoflavone powder produced from soybean cake. *Journal of Agricultural Food Chem*, **55**: 11068–11079.
- Kapiotis, S., Hermann, M., Held, I., Seelos, C., Ehringer, H., and Gmeiner, B.M.K. (1997) Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. *Arterioscler, Thrombosis, and Vascular Biology*, **17**: 2868–2874.
- Kgomotso, T., Chiu, F., and Ng, K. (2008) Genistein- and daidzein 7-O- $\beta$ -D-glucuronic acid retain the ability to inhibit copper-mediated lipid oxidation of low density lipoprotein. *Molecular Nutrition and Food Research*, **52**: 1457–1466.
- Khoo, K.L., Tan, H., Liew, Y.M., Deslypere, J.P., and Janus, E. (2003) Lipids and coronary heart disease in Asia. *Atherosclerosis*, **169**: 1–10.
- Kikuchi, T., Ishii, S., Fukushima, D., and Yokotsuka, T. (1971) Food chemical studies on soybean polysaccharides: Chemical and physical properties of soybean cell wall polysaccharides and their changing during cooking. *Nihon Nogeikagaku Kaishi*, **45**: 228–234.
- Kirk, E.A., Sutherland, P., Wang, S.A., Chait, A., and LeBoeuf, R.C. (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *Journal of Nutrition*, **128**: 954–959.
- Kuan, Y., and Liong, M. (2008) Chemical and physicochemical characterization of agrowaste fibrous materials and residues. *Journal of Agricultural Food Chemistry*, **56**: 9252–9257.
- Lapointe, A., Couillard, C., and Lemieux, S. (2006) Effects of dietary factors on oxidation of low-density lipoprotein particles. *Journal of Nutritional Biochemistry*, **17**: 645–658.
- Lawes, C.M., Bennett, D.A., Lewington, S., and Rodgers, A. (2002) Blood pressure and coronary heart disease: A review of the evidence. *Seminars in Vascular Medicine*, **2**: 355–368.
- Lee, S.J., Yan, W., Ahn, J.K., and Chung, I.L. (2003) Effects of year, site, genotype and their interactions on various soybean isoflavones. *Field Crops Research*, **81**: 182–192.
- Libby, P. (2002) Inflammation in atherosclerosis. *Nature*, **420**: 868–874.
- Lichtenstein, A.H., Jalbert, S.M., Adlercreutz, H., Goldin, B.R., and Rasmussen, H. *et al.* (2002) Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. *Arteriosclerosis, Thrombosis, and Vascular Biology* **22**: 1852–1858.
- Lo, G.S., Evans, R.H., Phillips, K.S., Dahlgren, R.R., and Steinke, F.H. (1987) Effect of soy fiber and soy protein on cholesterol metabolism and atherosclerosis in rabbits. *Atherosclerosis*, **64**: 47–54.
- Mahn, K., Borrás, C., Knock, G.A., Taylor, P., and Khan, I.Y. *et al.* (2005) Dietary soy isoflavone-induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. FASEB Journal express article 10.1096/fj.05–4008fje. Published online Aug 17, 2005.
- Maier, D.E., Reising, J., Briggs, J.L., Day, K.M., and Christmas, E.P. (1998) High value soybean composition. Grain Quality Task Force Fact Sheet No. 39. Purdue University, obtained April 12, 2009 from [www.ces.purdue.edu/extmedia/GQ/GQ-39.html](http://www.ces.purdue.edu/extmedia/GQ/GQ-39.html).
- Mancia, G. (2007) Blood pressure and cardiovascular outcomes: Past, present, and future. *American Journal of Cardiology*, **100**(3A): 3J–9J.
- McClements, J.D., and Dekker, E.A. (2008) Lipids, in Srinivasan, D., Parkin, K.L., Fennema, O.R. (eds) *Fennema's Food Chemistry*. Boca Raton, FL: CRC Press, pp. 155–216.
- Merz-Demlow, B.E., Duncan, A.M., Wangen, K.E., Xu, X., Carr, T.P., Phipps, W.R., and Kurzer, M.S. (2000) Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *American Journal of Clinical Nutrition*, **71**: 1462–1469.
- Messina, M., and Lane, B. (2007) Soy protein, soybean isoflavones and coronary heart disease risk: Where do we stand? *Future Lipidology*, **2**: 55–74.

- Meyer, B.J., Larkin, T.A., Owen, A.J., Astheimer, L.B., Tapsell, L.C., and Howe, P.R.C. (2004) Limited lipid-lowering effects of regular consumption of whole soybean foods. *Annals of Nutrition and Metabolism*, **48**: 67–78.
- Mortensen, A., Kulling, S.E., Schwartz, H., Rowland, I., Ruefer, C.E., Rimbach, G., Cassidy, A., Magee, P., Millar, J., Hall, W.L., Kramer Birkved, F., Sorensen, I.K., and Sontag, G. (2009) Analytical and compositional aspects of isoflavones in food and their biological effects. *Molecular Nutrition and Food Research*, **53**: S266–S309.
- Nagata, C., Shimizu, H., Takami, R., Hayashi, M., Takeda, N., and Yasuda, K. (2003) Association of blood pressure with intake of soy products and other food groups in Japanese men and women. *Preventive Medicine*, **36**: 692–697.
- Nagata, C., Takatsuka, N., Kurisu, Y., and Shimizu, H. (1998) Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. *Journal of Nutrition*, **128**: 209–213.
- O'Toole D. (1999) Characteristics and use of okara, the soybean residue from soy milk production – a review. *Journal of Agricultural Food Chemistry*, **47**: 363–371.
- Paradkar, P.N., Blum, P.S., Berhow, M.A., Baumann, H., and Kuo, S.M. (2004) Dietary isoflavones suppress endotoxin-induced inflammatory reaction in liver and intestine. *Cancer Letters*, **215**: 21–28.
- Patel, R.P., Boersma, B.J., Crawford, J.H., Hogg, N., Kirk, M., Kalyanaraman, B., Parks, D.A., Barnes, S., and Darley-Usmar, V. (2001) Antioxidant mechanisms of isoflavones in lipid systems: Paradoxical effects of peroxyl radical scavenging. *Free Radical Biology and Medicine*, **31**(12): 1570–1581.
- Plat, J., Kerckhoffs, D.A., and Mensink, R.P. (2000) Therapeutic potential of plant sterols and stanols. *Current Opinion in Lipidology*, **11**: 571–576.
- Quan, J., Yin, X., and Kanazawa, T. (2009) Effect of Soybean Hypocotyl Extract on Lipid Peroxidation in GK Rats. *Journal of Clinical Biochemistry and Nutrition*, **55**: 212–217.
- Riedl, K.M., Lee, J.H., Renita, M., St. Martin, S.K., Schwartz, S.J., and Vodovitz, Y. (2007) Isoflavone profiles, phenol content, and antioxidant activity of soybean seeds as influenced by cultivar and growing location in Ohio. *Journal of the Science of Food and Agriculture*, **87**: 1197–1206.
- Riaz, M.N. (2001) Uses and benefits of soy fiber. *Cereal Foods World*, **46**: 98–100.
- Rivas, M., Garay, R.P., Escanero, J.F., Cia, P., and Alda, J.O. (2002) Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. *Journal of Nutrition*, **132**: 1999–1902.
- Sacks, F.M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., and Winston, M. (2006) Soy protein, isoflavones, and cardiovascular health: An American Heart Association science advisory for professionals from the nutrition committee. *Circulation*, **113**: 1034–1044.
- Sancllemente, T., Marques-Lopes, I., Puzo, J., and Garcia-Otin, A.L. (2009) Role of naturally-occurring plant sterols on intestinal cholesterol absorption and plasmatic levels. *Journal of Physiology and Biochemistry*, **65**: 87–98.
- Sato, M., Sato, H., Ogawa, A., Nomura, R., Takashima, S., Bang, H.J., Matsuoka, H., and Imaizumi, K. (2007) Antiatherogenic Effect of Isoflavones in Ovariectomized Apolipoprotein E-Deficient Mice. *Journal of Agricultural and Food Chemistry*, **55**: 8967–8971.
- Scheiber, M.D., Liu, J.H., Subbiah, M.T.R., Rebar, R.W., and Setchell, K.D.R. (2001) Dietary inclusion of whole soy foods results in significant reductions in clinical risk factors for osteoporosis and cardiovascular disease in normal postmenopausal women. *Menopause: The Journal of the North American Menopause Society*, **8**(5): 384–392.
- Setchell, K.D.R., Brown, N.M., and Lydeking-Olsen, E. (2002) The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition*, **132**: 3577–3584.
- Setchell, K.D.R., and Cassidy, A. (1999) Dietary isoflavones: Biological effects and relevance to human health. *Journal of Nutrition*, **129**: 758S–767S.
- Shaw, L.J., Bugiardini, R., and Merz, C.N. (2009) Women and ischemic heart disease: Evolving knowledge. *Journal of the American College of Cardiology*, **54**: 1561–1575.
- Sirtori, C.R., and Johnson, S.K. (2006) Soy proteins, cholesterolemia, and atherosclerosis, In: Sugano, M. (ed.) *Soy in Health and Disease Prevention*. Boca Raton, FL: Taylor & Francis, pp. 17–41.
- Sirtori, C.R., Lovati, M.R., Manzoni, C., Castiglioni, S., and Duranti, M., et al. (2004) Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. *Journal of Nutrition*, **134**: 18–23.
- Stein, K. (2000). FDA approves health claim labeling for foods containing soy protein. *Journal of the American Dietetic Association*, **100**: 292.

- Steinberg, F.M., Guthrie, N.L., Villablanca, A.C., Kumar, K., and Murray, M.J. (2003) Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. *American Journal of Clinical Nutrition*, **78**: 123–130.
- Stocker, R., and Keaney, J.F. Jr. (2004) Role of oxidative modifications in atherosclerosis. *Physiological Reviews*, **84**: 1381–1478.
- Taku, K., Umegaki, K., Ishimi, Y., and Watanabe, S. (2008) Effects of extracted soy isoflavones alone on blood total and LDL cholesterol: Meta-analysis of randomized controlled trials. *Therapeutics and Clinical Risk Management*, **4**: 1097–1103.
- Teede, H.J., Fabien, S.D., Kotsopoulos, D., Liang, Y.L., Davis, S., and McGrath, B.P. (2001) Dietary soy has both beneficial and potentially adverse cardiovascular effects: A placebo-controlled study in men and postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, **86**: 3053–3059.
- Thorp, A.A., Hower, P.R.C., Mori, T.A., Coates, A.M., Buckley, J.D., Hodgson, J., Mansour, J., and Meyer, B.J. (2008) *American Journal of Clinical Nutrition*, **88**: 298–304.
- Tikkanen, M.J., Wahala, K., Ojala, S., Vihma, V., and Adlercreutz, H. (1998) Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proceedings of the National Academy of Sciences of the United States of America*, **95**: 3106–3110.
- Tovar, A.R., Murguía, F., Cruz, C., Hernández-Pando, R., Aguilar-Salinas, C.A., Pedraza-Chaverri, J., Correa-Rotter, R., and Torres, N. (2002) A soy protein diet alters hepatic lipid metabolism gene expression and reduces serum lipids and renal fibrogenic cytokines in rats with chronic nephrotic syndrome. *Journal of Nutrition*, **132**: 2562–2569.
- Tovar, A.R., Torre-Villalvazo, I., Ochoa, M., Elias, A.L., Ortiz, V., Aguilar-Salinas, C.A., and Torres, N. (2005) Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker *fafa* rats. *Journal of Lipid Research*, **46**: 1823–1832.
- Tsai, P.J., and Huang, P.C. (1999) Effects of isoflavones containing soy protein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids to in vitro oxidation in hamsters. *Journal of Nutritional Biochemistry*, **10**: 631–637.
- Turner, R., Baron, T., Wolfram, S., Minihane, A.M., Cassidy, A., Rimbach, G., and Weinberg, P. (2004) Effect of circulating forms of soy isoflavones on the oxidation of low density lipoprotein. *Free Radical Research*, **38**(2): 209–216.
- van Ee, J.H. (2009) Soy constituents: Modes of action in low-density lipoprotein management. *Nutrition Reviews*, **67**(4): 222–234.
- Vega-Lopez, S., and Lichtenstein, A.H. (2005) Dietary protein type and cardiovascular disease risk factors. *Preventive Cardiology*, Winter: 31–40.
- Vega-Lopez, S., Yeum, K.J., Lecker, J.L., Ausman, L.M., Johnson, E.J., Devaraj, S., Jialal, I., and Lichtenstein, A.H. (2005) Plasma antioxidant capacity in response to diets high in soy or animal protein with or without isoflavones. *American Journal of Clinical Nutrition*, **81**: 43–49.
- Wang, H.L., and Cavins, J.F. (1989) Yield and amino acid composition of fractions obtained during tofu production. *Cereal Chemistry*, **66**(5): 359–361.
- Wangen, K.E., Duncan, A.M., Xu, X., and Kurzer, M.S. (2001) Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *American Journal of Clinical Nutrition*, **73**: 225–231.
- Weggemans, R.M., and Trautwein, E.A. (2003) Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: A meta-analysis. *European Journal of Clinical Nutrition*, **57**: 940–946.
- Welty, F.K., Lee, K.S., Lew, N.S., and Zhou, J.R. (2007) Effect of soy nuts on blood pressure and lipid levels in hypertensive, prehypertensive, and normotensive postmenopausal women. *Archives of Internal Medicine*, **167**: 1060–1067.
- Wiseman, H., O'Reilly, J.D., Adlercreutz, H., Mallet, A.I., Bowey, E.A., Rowland, I.R., and Sanders, A.B. (2000) Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition*, **72**: 395–400.
- Xiao, C.W. (2008) Health effects of soy protein and isoflavones in humans. *Journal of Nutrition*, **138**: 1244S–1249S.
- Yamakoshi, J., Piskula, M.K., Izumi, T., Tobe, K., Saito, M., Kataoka, S., Obata, A., and Kikuchi, M. (2000) Isoflavone aglycone-rich extract without soy protein attenuates atherosclerosis development in cholesterol-fed rabbits. *Journal of Nutrition*, **130**: 1887–1893.

- Yang, S.C., Liu, S.M., Yang, H.Y., Lin, Y.H., and Chen, J.R. (2007) Soybean protein hydrolysate improves plasma and liver lipid profiles in rats fed high cholesterol diet. *Journal of the American College of Nutrition*, **26**(5): 416–423.
- Yousef, M.I., Kamel, K.I., Esmail, A.M., and Baghdadi, H.H. (2004) Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. *Food and Chemical Toxicology*, **42**: 1497–1503.
- Zhan, S., and Ho, S.C. (2005) Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *American Journal of Clinical Nutrition*, **81**: 397–408.
- Zhang, X., Shu, X.O., Gao, Y.T., Yang, G., Li, Q., Li, H., Jin, F., and Zheng, W. (2003) Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. *Journal of Nutrition*, **133**: 2874–2878.



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# 18 Dietary fiber and human health

Yanling Gao and Jin Yue\*

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## 18.1 Introduction

Dietary fiber is a group of non-digestible polysaccharides. It is generally classified into water-insoluble and water-soluble dietary fibers. Insoluble fibers may include cellulose, lignin, and hemicellulose, and can be found mainly in wheat, whole grains, and vegetables. They bind water and render fecal softer and bulkier. Soluble fibers are found inside cells of fruits, vegetables, beans, and oat bran, and consist of pectins, gums, and mucilages. Soluble fiber may enhance immune function and lower serum cholesterol and glucose levels.

The dietary fiber hypothesis was first proposed by Dennis Burkitt and Hugh Trowell (Burkitt and Trowell, 1977) in the 1970s, suggesting that intake of high-fiber food would reduce the risk of diabetes, cardiovascular disease, and cancer. Since then, more and more attention has been paid to dietary fiber and strong evidence has suggested its health effects. According to the experimental intake level to prevent coronary heart disease, the Dietary Reference Intake Committee established a sufficient intake of total fiber as 25 g/d for women and 38 g/d for men. However, in the United States the daily intake is only 15 g (Howarth *et al.*, 2001). It is believed that increased consumption of dietary fiber may reduce the risk of many chronic diseases, including obesity, diabetes, hypertension, coronary heart disease, stroke, and some gastrointestinal disorders such as gastroesophageal reflux disease, diverticulities, duodenal ulcer, hemorrhoids, and constipation.

## 18.2 Dietary fiber and metabolic syndrome

Metabolic syndrome (MS) is a combination of a group of metabolic abnormalities and includes central obesity, insulin resistance, low concentrations of plasma high-density lipoprotein (HDL) cholesterol, hypertension and hyperglycemia, and high triglycerides. Among them, obesity, insulin resistance, hypertension, and dyslipidemia are the primary components. People with MS have increased risk of coronary heart disease and type 2 diabetes. MS was first recognized in the 1920s, and it has now become the most important public health problem worldwide, particularly in the developed countries. It has been estimated that more than 20% of adults might develop MS in the United States.

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The most critical approach for prevention of MS is a healthy lifestyle, which consists of controlling weight, exercise, non smoking, and, more importantly, a healthy diet. Apart from low-fat and reduced-calorie intake, increased consumption of fiber rich food such as whole grains, cereals, fruits, vegetables, and legumes are highly recommended.

### 18.2.1 Dietary fiber and obesity

Obesity has become a large health problem in both developed and developing countries due to modern life patterns. About 59% of adults in the United States are thought to be obese (body mass index [BMI, kg/m<sup>2</sup>] >30) or overweight (BMI 25–29.9) (Must *et al.*, 1999). A healthy dietary style with increased fiber intake is considered to be an effective strategy for reducing the risk of obesity.

Epidemiologic data indicated that populations who consumed a low-fiber diet had higher risk of obesity than those consuming a high-fiber diet (Howarth *et al.*, 2001). A case–control study suggested that obese individuals usually consumed less fiber than normal-weight ones, and individuals consuming higher fiber were inclined to be leaner than those with lower fiber intake (Nelson and Tucker, 1996). To better show the effect of fiber on body weight control, statistical methodology was applied in some other epidemiologic studies. Ludwig and others (1999) studied more than 2000 young adults over a ten-year period and reported that individuals consuming the least fiber gained more weight than those consuming the most fiber at any level of fat intake.

Dietary fiber may act as an obstacle to energy intake and benefit patients with obesity through three generally accepted mechanisms, which were first proposed by Heaton (Heaton, 1973). Dietary fibers are not enzymatically degraded into absorbable subunits in the stomach and small intestine. Only about 40% of fiber is fermented in the colon into short chain fatty acids, which can be absorbed and used as energy, and hence, fiber-rich foods contain lower energy density. In addition, the water-binding function of both soluble and insoluble fiber further reduces the energy-to-weight ratio in foods (Rolls *et al.*, 1999). Consumption of same weight but less density food may lead to a promotion of satiety and a decrease in energy intake. An intervention study indicated that an additional 14 g/d of fiber lead to a 10% decrease of energy consumption and more than 1.9 kg loss of body weight within 3.8 months (Howarth *et al.*, 2001).

Dietary fiber affects not only energy consumption but also energy digestion. The bulking and viscous fiber trapping nutrients in the stomach and small intestine may result in the delay of digestion and absorption of energy. Wisker and others (1988) studied the difference in energy absorption between a low-fiber intake (20 g/day) group and a fiber-supplemented (48 g/day) group, and found that the individuals consuming high fiber had on average 8% lower energy absorption than those eating the low-fiber diet.

Food rich in dietary fiber requires longer chewing, and hence may reduce the rate of ingestion and promote satiety. The increased chewing also results in secretion of more saliva and gastric juice, which may cause the expansion of stomach. Furthermore, soluble fiber binds a large amount of water and forms gel matrix, which causes an additional stomach distention. Stomach distention may trigger afferent vagal signals of fullness, contributing to satiety during meals and satiation after meals (Aleixandre and Miguel, 2008).

Dietary fiber might stimulate the secretion of gut hormones or peptides, such as the glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), peptide YY, and neurotensin, and consequently affect energy intake and body weight. GLP-1 has been shown to delay the

gastric emptying and facilitate weight loss, and to be secreted less in obese individuals (Gutzwiller *et al.*, 1999). The nutrients such as glucose and fat, and the short chain fatty acids fermented from the fibers might stimulate the production of GLP-1. CCK, another peptide hormone, is secreted in the upper part of the small intestine. It regulates gut motility, gallbladder contraction, and pancreatic enzyme secretion. It was reported that fiber intake might stimulate the secretion of CCK, which would mediate the effect of viscous fiber to postprandial glycemic and insulinemic response (Bourdon *et al.*, 2001).

The wide acceptance that fibers from fruit, vegetables, legumes, nuts, and whole grains might prevent obesity, remains controversial if fibers from supplements might benefit body weight regulation. Several studies observed a decrease of weight gain in either humans or animals through consumption of fiber supplements rich in soluble fibers, such as guar gum, psyllium, or glucomannan (Backhed *et al.*, 2007). In a more recent study (Babio *et al.*, 2010), fiber supplement or a placebo was fed randomly to obese patients in the context of a calorie restricted diet, and the differences in weight loss between the two groups within 16 weeks were not statistically significant. Because of this conflicting evidence and lack of long-term study of fiber supplements in adults, fiber supplement may not be systematically recommended for preventing obesity.

## **18.2.2 Dietary fiber and diabetes**

There are two major types of diabetes: type 1 diabetes, which results from the incapability to produce insulin; and type 2 diabetes, resulting from low cellular sensitivity to insulin, particularly fat and muscle cells. The population with diabetes is increasing at an alarming rate because of population growth, aging, urbanization, and growing prevalence of obesity and physical inactivity. In the United States, the estimated population with diabetes for all ages is: diabetes, 23.6 million or 8%; prediabetes, 70.3 million or 23%; and the metabolic syndrome, 62 million or 20.3% (Anderson *et al.*, 2009). Among the diabetic patients, around 80% were obese and 90% were diagnosed with type 2 diabetes in 2008 (Anderson *et al.*, 2009). Compared to the general population, diabetic patients have higher chance of developing cardiovascular problems.

Glycemic control is of most importance for management of diabetes, especially type 2 diabetes. Considering that three quarters of a day was defined as a postprandial period, it is obvious that decreasing of postprandial glycemia and insulinemia, and enhancing insulin sensitivity are the most effective therapeutic interventions for reduction of vascular complications in diabetic patients.

Epidemiological studies showed that high amounts of dietary fiber intake play a significant role in the reduction of the prevalence of diabetes: and a 29% reduction was observed in individuals with high intake of whole grain or cereal fiber (Anderson, 2008). A meta-analysis of six prospective studies revealed that a two-serving-per-day increase in whole grain consumption might decrease the risk of diabetes by 21% (de Munter *et al.*, 2007). For the individuals with type 1 or type 2 diabetes, increasing consumption of fiber without varying the energy intake from proteins, carbohydrates, or fats substantially might benefit glycemic control and reduce the use of oral medication and insulin. A meta-analysis indicated that, based on the observation of eight randomized controlled trials (RCTs) including 136 subjects with diabetes (types 1 and 2), the moderate-carbohydrate, high fiber diets, compared to moderate-carbohydrate, low fiber diets, produced the following obvious reduction: postprandial plasma glucose: 21%; LDL-cholesterol 7.9%; and triglycerides, 8.3% (Anderson *et al.*, 2004).

Soluble and insoluble fibers may affect the glucose level and insulin sensitivity through different mechanisms. Soluble fibers are viscous and form gels in the stomach and small intestine that modulate postprandial glycemic responses. Several possible mechanisms may be involved. First, soluble fibers delay the gastric emptying, which could result in around 35% of the variance in peak glucose concentrations after drinking oral glucose (Jones *et al.*, 1996). Second, it might modify the gastrointestinal myoelectrical activity and delay small bowel transit. Third, it may limit glucose transportation through the unstirred water layer. Johnson and others (1983) studied the effect of guar gum and Nacarbonylmethyl-cellulose (CMC) on glucose diffusion using everted sacs of rat jejunum. The polysaccharide gum in the fluid film surrounding the villi increases its viscosity, and thickens the rate-limiting unstirred layer. In addition, the accessibility of  $\alpha$ -amylase to its substrates could be reduced because of the increased viscosity of gut content. When rats were fed a diet containing 10% guar gum, lipase, amylase, and total proteolytic activity were significantly greater in the intestine (Cherbut *et al.*, 1990). This enhanced enzyme activity in the intestine could lead to a decreased rate of enzyme degradation or to the elevation of enzyme secretion. The ability of guar gum to increase the volume of intestinal contents might also play an important role in its inhibition of absorption.

Insoluble fibers are mainly nonviscous and have limited influence on postprandial glucose content. More importantly insoluble fiber may increase insulin sensitivity and reduce serum insulin concentration to reduce the risk of diabetes. In a randomized controlled single-blind crossover study, obese or overweight subjects with normal glucose metabolism were fed with cereal for three days. After that their whole body insulin sensitivity was significantly improved (Marlett *et al.*, 2002). This may have been due to alterations in gut microbiota, since reduced gram-negative bacterial content was observed with high intake of dietary fiber compared to high-fat diet (Backhed *et al.*, 2007). The exact mechanism is still unclear.

Controversial viewpoints exist on the effect of fiber supplement on postprandial glycemia and insulin response. In an experiment on type 2 diabetes patients, diet with additional natural guar gum supplement (total fiber=15 g) did not exert any effects on postprandial glucose, insulin, or triglyceride response (Giacco *et al.*, 1998). While in almost all other studies on fiber supplement, including psyllium, glucomannan, pectin, and the same guar gum as used in the experiment mentioned above, a better postprandial metabolic response was observed with fiber supplementation (Williams *et al.*, 1980).

The beneficial effects of dietary fiber on blood glucose control in the long term used to be questioned because of limited available evidence. Lindstrom and colleagues' study published in 2006 strongly overthrew the above question (Bantle, 2008). They reported that over a 4.1-year period, individuals with the highest level of fiber intake had a 62% reduction in the risk of prediabetes to diabetes as opposed to those with the lowest fiber intake. Recently, the American Diabetes Association recommends that persons with diabetes consume 25–50 g/day (15–25 g/1000 Kcal) of fiber to improve glycemic control. Diabetic patients should be recommended an overall increased intake of dietary fiber, especially fiber from natural foods.

### 18.3 Dietary fiber and cancer

Cancer accounted for 13% of all human deaths globally in 2007 (7.6 million). The predicted number of new cases will increase to 15 million annually by 2020. It is estimated that some 35% of cancer deaths are probably related to unadvisable diet habits. More than one-third

of new cases could be avoided annually worldwide. Growing scientific investigation and epidemiological data have suggested the adverse association between diet habits and the risk of cancers such as cancers of the colon, breast, prostate, stomach, ovarian cancer, and others (Bravi *et al.*, 2009; Cade *et al.*, 2007).

### 18.3.1 Colon cancer

Colon cancer causes more than 655 000 deaths annually across the world. Environmental impact and lifestyle factors, including diet habit, may play an influential role both in the incidence and in the development of colon cancer. Ecological international comparisons and case-control studies have for a long time suggested that fiber can possibly reduce the risk of colon cancer (Bingham *et al.*, 2003). The possible mechanisms may include directly absorbing the carcinogen and excretion into feces, the production of short chain fatty acids (SCFA), enhancing the stool bulk and reducing intestine transition time (Harris *et al.*, 1996). The water-insoluble fibers such as lignified and suberized cell walls have been shown to effectively absorb hydrophobic carcinogens *in vitro* (Harris *et al.*, 1996). The absorption reduces the exposure of colonocytes to dietary carcinogens and protects against colon cancer. In addition, fiber may act as a substrate for bacterial fermentation, which may increase bacterial mass and the production of SCFA, predominantly acetic, propionic, and butyrate. SCFA, particularly butyrate, is a major source of energy for the distal colon, which has been shown to have anticarcinogenic effects *in vitro*. In cell lines butyrate has been proved to reduce cell proliferation and induce apoptosis, thus protecting against colon cancer by inhibiting the transformation of colonic epithelium to carcinoma. SCFA also may lower gut pH, resulting in a lower production rate of secondary bile acids via pH-dependent bacterial enzymes. Another potential mechanism of the reduced risk of colon cancer might be related to the increased stool weight and the reduced transit time by high intake of fiber. High-fiber diet (100–170 g/d) usually had a transit time of 30 hours and fecal weight of 500 g. In contrast, low-fiber diet (20 g/d) had a transit time of greater than 48 hours and a fecal weight of only 100 g (Lunn and Buttriss, 2007). Insoluble fiber might alter stool weight and transit time through several mechanisms: 1) water-insoluble fibers such as cellulose increase stool weight and reduce transit time as a result of their water-holding properties; 2) viscous and well fermented fibers such as those prepared from psyllium or oat might reduce the concentration of bile acids, which could be plausibly involved as promoting or trophic agents, contact with the bowel wall, and increase stool bulk (a diluting effect) or reduce transit time (a duration of exposure effect). In addition, soluble fiber such as short-chain fructo-oligosaccharides, polydextrose, or  $\beta$ -Glucan prepared from oats or barley may reduce the incidence of colon tumors and concomitantly developed gut-associated lymphoid tissue possibly via stimulation of antitumoral immunity by modulation of the colonic ecosystem in the hereditary min mouse cancer model or production of immunoglobulin A (IgA) in the large intestine.

### 18.3.2 Esophageal cancer

Though esophageal cancer only accounts for about 1% of total diagnosed cancers in the US, the new attack of esophageal cancer has been increasing. Healthy dietary habit with high ratios of whole grains, fruits, and vegetables has been suggested to reduce the risk of

esophageal cancer. The relationship between various types of fiber and esophageal cancer was studied using a five-year case-control study (Soler *et al.*, 2001). The results indicated that dietary fibers intake including soluble and insoluble fibers, lignin, and the fibers prepared from vegetable, fruit or grain products had a strong protective role against esophageal cancer. Recently, a study of Ireland patient dietary information proved that high intake of fiber might reduce the attack of Barrett's esophagus and esophageal adenocarcinoma by 56% (Mulholland *et al.*, 2009).

There are many biologically plausible mechanisms linking high fiber intake with a reduced risk of esophageal cancer. First, fibers are known to slow down digestion and absorption of carbohydrates and the consequent glycemic response, thus reducing hyperinsulinemia and the formation of insulin-like growth factor (IGF) (Soler *et al.*, 2001). Second, high-fiber diets have been associated with lower plasma concentrations of systemic inflammation markers such as interleukin-6, which might play an important role in the incidence of carcinogenesis (Ma *et al.*, 2008). Third, other protective effects of fiber that might be of etiological importance may include a reduced risk of gastro-esophageal reflux symptoms, the mechanical removal or binding of damaged cells and/or carcinogens from the epithelial surface of the esophagus, or regulation of the glycemic response.

### 18.3.3 Stomach cancer

Stomach cancer is the fourth most frequent cancer and the second leading cause of cancer death globally. Epidemiologic data and substantial experimentation suggested that diet played an important role in the incidence of stomach cancer. A case-control study including 230 subjects with histologically confirmed stomach cancer and 547 controls with acute non-neoplastic diseases was conducted in Italy between 1997 and 2007 (Francesca *et al.*, 2009). The result showed inverse relationship between soluble, insoluble fiber, lignin, and fiber derived from vegetables or fruit and the risk of stomach cancer. This study also suggested that fiber rich grains couldn't provide any positive effect against the risk of stomach cancer at all. However, some authors provided opposite opinion and believed that high intake of refined grains attribute to the incidence of stomach cancer (Jansen *et al.*, 1999).

Although the mechanism is unclear, potential elimination of carcinogens, thereby preventing carcinogens from prolonged exposure to stomach tissue, might be the possible mechanism (Francesca *et al.*, 2009). Furthermore, fiber, particularly water-soluble fiber, may delay the digestion of starch and reduce the glycaemic load, which is related to the risk of stomach cancer.

### 18.3.4 Hormone-related cancer

The association between dietary fiber and the reduced risk of hormone-related cancer such as breast cancer, prostate cancer, endometrial cancer, and ovarian cancer has been reported. Dietary fiber intake was inversely associated with the risk of breast cancer ( $P < 0.05$ ) through estrogen independent pathways among postmenopausal women according to a study involving 185 598 postmenopausal women at an average age of 62 years old. Similar association between grain fiber and ovarian cancer has been reported (Pelucchi *et al.*, 2001). Some studies also found significant inverse association between the risk of breast cancer and total fiber

consumption in premenopausal women ( $P < 0.01$ ) (Cade *et al.*, 2007). Rye bran has been shown to decrease the size and number of palpable tumors in transplantable a prostate tumor model (Landstrom *et al.*, 1998). The relationship between different types of dietary fiber with endometrial cancer has been investigated (Jain *et al.*, 2000). The results have shown that high intake of fruit and cereal fiber is associated with a stronger reduced risk of endometrial cancer than vegetable fiber. Insoluble fiber was suggested to be unrelated to endometrial cancer risk, whereas cereals or vegetable fibers might reduce the risk of prostate cancer (Suzuki *et al.*, 2009). The possible mechanism of the reduced risk of hormone-related cancer by dietary fiber may include: 1) dietary fiber, especially insoluble fiber, could bind and reduce the availability of the sex hormones such as estrogen and testosterone, which are normally suggested to stimulate tumor growth; 2) fiber might decrease the GI transit time and reduce the concentration of bile acids, similar to the mechanism for colon cancer protection; 3) high fiber intake exerts positive effects on the metabolism of glucose and insulin and insulin resistance, which have been demonstrated to be involved in the etiology of some cancer, such as endometrial cancer (Kaaks *et al.*, 2005); and 4) high fiber intake may increase protective effect of lignans or other phyto-estrogens that might decrease the bioavailability of steroid hormones.

## **18.4 Dietary fiber and cardiovascular diseases**

Cardiovascular diseases (CVD) are the most common killers worldwide, taking 17.1 million lives annually. According to the results from the American Heart Association, CVD accounted for 34.3% of all deaths in the US in 2006. It is estimated that the total monetary cost for CVD in 2010 was \$503.2 billion (Donald *et al.*, 2010). Technically, CVD refers to any disease which affects the cardiovascular system; however, we usually use CVD to describe arterial disease. CVD could be caused by functional disturbance of the heart and blood vessels. CVD can be treated with lifestyle changes and medications. It is important to reduce the risk of heart disease through healthy diet, exercise, and less smoking.

Hundreds of scientific studies have focused on the etiologies, protection, and/or treatment of CVD. Dietary fiber might play an important role in the prevention of CVD and has received considerable attention in recent years. High fiber foods were encouraged in the American Heart Association's 2006 dietary recommendations and the addition of purified dietary fibers to foods has been recommended by the Food and Drug Administration (FDA). The addition of psyllium has been proved to be protective against heart diseases in postmenopausal women (Vijay and Jennifer, 2008). Substantial scientific data worldwide have long supported that increased intake of whole grain and higher fiber food might provide protection against CVD (He *et al.*, 2004).

### **18.4.1 Reducing blood cholesterol**

Generally, it has been proved that a 1% reduction in serum level of LDL-cholesterol contributes to a 1–2% reduction in the incidence of CVD, indicating that LDL-cholesterol is a generally accepted promising biomarker for assessment of CVD risk. The decrease in blood cholesterol may significantly reduce the risk of CVD and stroke. Soluble fiber found in beans, oats, flax seed, and oat bran has been proved to reduce both total blood cholesterol and LDL-cholesterol. Researchers found in clinical trials that total and LDL-cholesterol

levels decreased 7% with the increased consumption of soluble fiber-rich dry beans (Anderson and Major, 2002). Eating three cups (six servings) of legumes weekly is recommended for people who consume about 2000 calories per day. A serving of legumes is equal to half a cup of cooked beans, peas, lentils, or tofu. Increasing GI fluid viscosity is a common character of all cholesterol-lowering fibers. It has been proved that fermentable dietary fiber, with normally high viscosity, contributes to the reduction of blood cholesterol (Anderson *et al.*, 2000). Beta-glucan is a linear soluble polysaccharide, which can be normally prepared from cereals, in particular oats and barley. FDA declared that daily intake of  $\beta$ -glucan at the amount of 3 g may exert clinically relevant serum cholesterol-lowering effect. Properties such as molecular weight and water-solubility of fiber may also affect the cholesterol-lowering effect of dietary fiber.

The potential mechanisms of dietary fiber's cholesterol-lowering effect may involve four different routes: 1) viscous and well fermentable fibers binding bile acids to increase their fecal excretion and increase hepatic cholesterol catabolism (Anderson *et al.*, 2000); 2) the production of SCFA, which is similar to the mechanism of colon cancer protection; and 3) the water-soluble fiber, such as glucomannans and galactomannans interfering with the lipid and/or bile acid metabolism, trapping the cholesterol and bile acids and at the same time hindering their further digestion in the enterocyte.

### 18.4.2 Other beneficial effects

Blood pressure was identified as being reduced by high fiber intake according to cross-sectional and prospective cohort studies (He *et al.*, 2004). The results suggested that a diet rich in fiber may lower blood pressure through trapping and eliminating sodium and glucose. High intake of fiber stabilized blood glucose and insulin levels and reduced the physical activity of insulin after several hours of fiber feeding in sows (de Leeuw *et al.*, 2004). Another study was performed by the Insulin Resistance and Atherosclerosis Study (IRAS) to evaluate the effect of usual dietary intake of foods containing whole grain on insulin sensitivity (Liese *et al.*, 2003). The result indicated that regular intake of whole grains was associated with increased insulin sensitivity. Great insulin sensitivity, or lower insulin resistance, may be one underlying factor leading to reduced risk of developing diabetes and, thus, may reduce the risk of CVD. C-reactive protein (CRP) is one of the markers of inflammation recently recognized as a strong predictor of CVD. Higher baseline concentrations of CRP were proved to be associated with increased risk of both myocardial infarction and stroke. Both soluble dietary fibers and insoluble fibers were proved to be protective against CVD by decreasing high levels of CRP (Ma *et al.*, 2008).

## 18.5 Potential undesirable effects

Dietary fibers may have some undesirable effects including a reduced assimilation and bioavailability of vitamins, minerals, cholesterol, and proteins from the gut and excessive gas production in the intestine with a large increase of fiber in diet over a short period of time. Besides the above negative effects, the interaction of dietary fiber with drugs is another concern with the increasing intake of fiber. The bioavailability of orally administered drugs can be affected by the presence of certain dietary components in the



gastrointestinal tract. When taken at the same time, guar gum was proved to slow down the absorption of digoxin, acetaminophen, and bumetanide. Pectin was reported to reduce the absorption of lovastatin. A study involving four healthy volunteers suggested that the reduced bioavailability of carbamazepine and ispaghula husk should be attributed to the fiber (Etman, 1995).

## 18.6 Summary

In summary, dietary fibers may reduce the risk of several aging-associated chronic human diseases including obesity, diabetes, cancer, and CVD. Insoluble and soluble fibers may differ in their effectiveness in reducing the risk of a selected chronic disease. Besides consumption of more fiber in general, intake of more whole grains and whole grain foods is recommended to receive the maximum benefit from each type of fiber present in foods, obtain necessary nutrients, and reduce the risks of chronic diseases. It needs to be pointed out that over-consumption of dietary fibers might cause undesirable side-effects such as decreased absorption of nutrients, bloating, diarrhea, gas, and general discomfort.

## References

- Aleixandre, A., and Miguel, M. (2008) Dietary fiber in the prevention and treatment of metabolic syndrome: A review. *Critical Reviews in Food Science and Nutrition*, **48**: 905–912.
- Anderson, J.W. (2008) Dietary fiber and associated phytochemicals in prevention and reversal of diabetes, in *Nutraceuticals, Glycemic Health and Type 2 Diabetes* (eds V.K. Pasupuleti and J.W. Anderson), Blackwell Publishing Professional, Ames, Iowa, pp. 111–142.
- Anderson, J.W., Allgood, L.D., Lawrence, A., Altringer, L.A., Jerdack, G.R., Gengehold, D.A., and Morel, J.G. (2000) Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: Meta-analysis of 8 controlled trials. *American Journal of Clinical Nutrition*, **71**: 472–479.
- Anderson, J.W. and Major, A.W. (2002) Pulses and lipaemia, short- and long-term effect: Potential in the prevention of cardiovascular disease. *British Journal of Nutrition*, **88**(Suppl 3): 263–271.
- Anderson, J.W., Baird, P., Davis, R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V., and Williams, C.L. (2009) Health benefits of dietary fiber. *Nutrition Reviews*, **67**: 188–205.
- Anderson, J.W., Kendall, C.W.C., and Jenkins, D.J.A. (2003) Importance of weight management in type 2 diabetes: Review with meta-analysis of clinical studies. *Journal of the American College of Nutrition*, **22**: 331–339.
- Anderson, J.W., Randles, K.M., Kendall, C.W.C., and Jenkins, D.J.A. (2004) Carbohydrate and fiber recommendations for individuals with diabetes: A quantitative assessment and meta-analysis of the evidence. *Journal of the American College of Nutrition*, **23**: 5–17.
- Babio, N., Balanza, R., Basulto, J., Bullo, M., and Salas-Salvado, J. (2010) Dietary fibre: Influence on body weight, glycemic control and plasma cholesterol profile. *Nutrition Hospitalaria*, **25**: 327–340.
- Backhed, F., Manchester, J.K., Semenkovich, C.F., and Gordon, J.I. (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, **104**: 979–984.
- Bantle, J.P., Wylie-Rosett, J., Albright, A.L., Apovian, C.M., Clark, N.G., Franz, M.J., Hoogwerf, B.J., Lichtenstein, A.H., Mayer-Davis, E., Mooradian, A.D., and Wheeler, M.L. (2008) Nutrition recommendations and interventions for diabetes: A position statement of the American Diabetes Association. *Diabetes Care*, **31**: S61–S78.
- Bingham, S.A., Day, N.E., Luben, R., Ferrari, P., Slimani, N., Norat, T., Coudray, C., Demigne, C., and Rayssiguier, Y. (2003) Effects of dietary fibers on magnesium absorption in animals and humans. *Journal of Nutrition*, **133**: 1–4.

- Bourdon, I., Olson, B., Backus, R., Richter, B.D., Davis, P.A., Schneeman, B.O. (2001) Beans, as a source of dietary fiber, increase cholecystokinin and apolipoprotein B48 response to test meals in men. *Journal of Nutrition*, **131**: 1485–1490.
- Bravi, F., Scotti, L., Bosetti, C., Bertuccio, P., Negri, E., and La Vecchia, C. (2009) Dietary fiber and stomach cancer risk: A case-control study from Italy. *Cancer Causes Control*, **20**: 847–853.
- Burkitt, D.P. and Trowell, H.C. (1977) Dietary fiber and western disease. *Irish Medical Journal*, **70**: 272–277.
- Cade, J.E., Burley, V.J., Greenwood, D.C., and the UK Women's Cohort Study Steering Group (2007) Dietary fibre and risk of breast cancer in the UK women's cohort study. *International Journal of Epidemiology*, **36**: 431–438.
- Cherbut, C., Albina, E., Champ, M., Doublier, J.L., and Lecannu, G. (1990) Action of guar gums on the viscosity of digestive contents and on the gastrointestinal motor function in pigs. *Digestion*, **46**: 205–213.
- de Leeuw, J.A., Jongbloed, A.W., and Verstegen, M.W.A. (2004) Dietary fiber stabilizes blood glucose and insulin levels and reduces physical activity in sows. *Journal of Nutrition*, **134**: 1481–1486.
- de Munter, J.S.L., Hu, F.B., Spiegelman, D., Franz, M., and van Dam, R.M. (2007) Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *Public Library of Science, Medicine*, **4**: 1385–1395.
- Donald, L.J., Robert, J.A., Brown, T.M., Mercedes, C., Dai, S., De Simone, G., Ferguson, T.B., Ford, E., Furie, K., Gillespie, C., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, M., Howard, V., Kissela B., Kittner, S., Lackland D., Lisabeth, L., Marelli, A., McDermott, M.M., Meigs, J., Mozaffarian, D., Mussolino, M., Nichol, G., Roger, V.L., Rosamond, W., Sacco, R., Stafford, R., Thom, T., Smoller, S.W., Wong, N.D., and Rosett, J.W. (2010) Executive summary: Heart disease and stroke statistics-2010 update. *Circulation*, **121**: 948–954.
- Etman, M.A. (1995) Effect of a bulk forming laxative on the bioavailability of carbamazepine in man. *Drug Development and Industrial Pharmacy*, **21**: 1901–1906.
- Francesca, B., Lorenza, S., Cristina, B., Paola, B., Eva, N., and La Carlo, V. (2009) Dietary fiber and stomach cancer risk: A case-control study from Italy. *Cancer Causes Control*, **20**: 847–853.
- Giacco, R., Clemente, G., Mancini, M., D'Avanzo, A., Mansueto, G., Brighenti, F., Lasorella, G., Riviaccio, A.M., Rivellese, A., and Riccardi, G. (1998) Mechanisms of the hypoglycemic effect of water soluble fiber in non insulin dependent diabetic patients. *Diabetologia*, **41**: 1025.
- Gutzwiller, J.P., Drewe, J., Goke, B., Schmidt, H., Rohrer, B., Lareida, J., and Beglinger, C. (1999) Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, **276**: R1541–R1544.
- Heaton, K.W. (1973) Food fibre as an obstacle to energy intake. *Lancet*, **302**: 1418–1421.
- Harris, P.J., Triggs, C.M., Robertson, A.M., Watson, M.E., and Ferguson, L.R. (1996) The adsorption of heterocyclic aromatic amines by model dietary fibres with contrasting compositions. *Chemico-Biological Interactions*, **100**: 13–25.
- He, J., Streiffer, R.H., Muntner, P., Krousel-Wood, M.A., and Whelton, P.K. (2004) Effect of dietary fiber intake on blood pressure: A randomized, double-blind, placebo-controlled trial. *Journal of Hypertension*, **22**: 73–80.
- Howarth, N.C., Saltzman, E., and Roberts, S.B. (2001) Dietary fiber and weight regulation. *Nutrition Reviews*, **59**: 129–139.
- Jain, M.G., Howe, G.R., and Rohan, T.E. (2000) Nutritional factors and endometrial cancer in Ontario, Canada. *Cancer Control*, **7**: 288–296.
- Jansen, M.C., Bueno-de-Mesquita, H.B., Räsänen, L., Fidanza, F., Menotti, A., Nissinen, A., Feskens, E.J., Kok, F.J., and Kromhout, D. (1999) Consumption of plant foods and stomach cancer mortality in the seven countries study. Is grain consumption a risk factor? Seven Countries Study Research Group. *Nutrition and Cancer*, **34**: 49–55.
- Johnson, I.T. and Gee, J.M. (1981) Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut*, **22**: 398–403.
- Jones, K.L., Horowitz, M., Carney, B.I., Wishart, J.M., Guha, S., and Green, L. (1996) Gastric emptying in early noninsulin-dependent diabetes mellitus. *Journal of Nuclear Medicine*, **37**: 1643–1648.
- Kaaks, R., Berrino, F., and Key, T. *et al.* (2005) Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *Journal of the National Cancer Institute*, **97**: 755–765.
- Landstrom, M., Zhang, J.M., Hallmans, G., Aman, P., Bergh, A., Damber, J.E., Mazur, W., Wahala, K., and Aldercreutz, H. (1998) Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. *Prostate*, **36**: 151–161.

- Liese, A., Roach, A., Sparks, K., Marquart, L., D'Agostino, Jr.R., and Davis-Mayer, E. (2003) Whole-grain intake and insulin sensitivity: The Insulin Resistance Atherosclerosis Study. *American Journal of Clinical Nutrition*, **78**: 965–971.
- Ludwig, D.S., Pereira, M.A., Kroenke, C.H., Hilner, J.E., Van Horn, L., Slattery, M.L., and Jacobs, D.R. (1999) Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA-J. American Medical Association*, **282**: 1539–1546.
- Lunn, J. and Buttriss, J.L. (2007) Carbohydrates and dietary fibre. *Nutrition Bulletin*, **32**: 21–64.
- Marlett, J.A., McBurney, M.I., and Slavin, J.L. (2002) Position of the American Dietetic Association: Health implications of dietary fiber. *Journal of the American Dietetic Association*, **102**: 993–1000.
- Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G., and Dietz, W. H. (1999) The disease burden associated with overweight and obesity. *JAMA-J. American Medical Association*, **282**: 1523–1529.
- Nelson, L.H. and Tucker, L.A. (1996) Diet composition related to body fat in a multivariate study of 203 men. *Journal of the American Dietetic Association*, **96**: 541–547.
- Ma, Y., Griffith, J.A., Chasan-Taber, L., Olendzki, B.C., Jackson, E., Stanek, E.J. 3rd, Li, W., Pagoto, S.L., Ma, Y., Hébert, J.R., Li, W., Bertone-Johnson, E.R., Olendzki, B., Pagoto, S.L., Tinker, L., Rosal, M.C., Ockene, I.S., Ockene, J.K., Griffith, J.A., and Liu, S. (2008) Association between dietary fiber and markers of systemic inflammation in the women's health initiative observational study. *Nutrition*, **24**: 941–949.
- Mulholland, H.G., Cantwell, M.M., Anderson, L.A., Johnston, B.T., Watson, R.G., Murphy, S.J., Ferguson, H.R., McGuigan, J., Reynolds, J.V., Comber, H., and Murray, L.J. (2009) Glycemic index, carbohydrate and fiber intakes and risk of reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. *Cancer Causes Control*, **20**: 279–288.
- Pelucchi, C., La Vecchia, C., Chatenoud, L., Negri, E., Conti, E., Montella, M., Calza, S., Dal Maso, L., Franceschi, S. (2001) Dietary fibres and ovarian cancer risk. *European Journal of Cancer*, **37**: 2235–2239.
- Rolls, B.J., Bell, E.A., Castellanos, V.H., Chow, M., Pelkman, C.L., and Thorwart, M.L. (1999) Energy density but not fat content of foods affected energy intake in lean and obese women. *Journal of the American College of Nutrition*, **69**: 863–871.
- Soler, M., Bosetti, C., Franceschi, S., Negri, E., Zambon, P., Talamini, R., Conti, E., Vecchia, C.L. (2001) Fiber intake and the risk of oral, pharyngeal and esophageal cancer. *International Journal of Cancer*, **91**: 283–287.
- Suzuki, R., Allen, N.E., Key, T.J., Appleby, P.N., Tjønneland, A., Johnsen, N.F., Jensen, M.K., Overvad, K., Boeing, H., Pischon, T., Kaaks, R., Rohrmann, S., Trichopoulou, A., Misirli, G., Trichopoulos, D., Bueno-de-Mesquita, H.B., van Duijnhoven, F., Sacerdote, C., Pala, V., Palli, D., Tumino, R., Ardanaz, E., Quirós, J.R., Larrañaga, N., Sánchez, M.J., Tormo, M.J., Jakszyn, P., Johansson, I., Stattin, P., Berglund, G., Manjer, J., Bingham, S., Khaw, K.T., Egevad, L., Ferrari, P., Jenab, M., and Riboli, E. (2009) A prospective analysis of the association between dietary fiber intake and prostate cancer risk in EPIC. *International Journal of Cancer*, **124**: 245–249.
- Vijay, G. and Jennifer, K. (2008) Serum lipid responses to psyllium fiber: differences between pre- and post-menopausal, hypercholesterolemic women. *Nutrition Journal*, **7**: 22.
- Williams, D.R., James, W.P., and Evans, I.E. (1980) Dietary fibre supplementation of a "normal breakfast administration to diabetics". *Diabetologia*, **18**: 379–383.
- Wisker, E., Maltz, A., and Feldheim, W. (1988) Metabolization energy of diets low or high in dietary fiber from cereals when eaten by humans. *Journal of Nutrition*, **118**: 945–952.

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# 19 Antioxidants and human health

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## 19.1 Introduction

Free radicals and other reactive species produced in the body under physiological and pathophysiological conditions play important roles in a number of regulatory activities and pathological processes. Oxidative stress arises from overproduction of reactive oxygen or nitrogen species (ROS/RNS; Table 19.1), which may attack, in principle, all cellular and extracellular components causing damage to cells and tissues. Oxidative stress accounts for many pathological alterations in the body and has been implicated in development of a number of diseases including cardiovascular disease, cancers, hypertension, diabetes, inflammation, and other autoimmune disorders as well as aging. ROS/RNS production *in vivo* is normally under the control of cellular defence systems by both endogenous and exogenous antioxidant substances. However, when antioxidant defences are not efficient, ROS/RNS escape elimination, posing a potential hazard and lead to oxidative cellular damage (Berger, 2005).

Antioxidants are able to attenuate oxidative stress via different modes of action, and some may act through a combination of several mechanisms such as scavenging ROS/RNS and prevent depletion of the body's endogenous antioxidant defence systems. The antioxidants may act as free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other ROS, metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes, among others (Shahidi and Zhong, 2007). In biological systems, the beneficial effects of antioxidants may also be rendered through mechanisms other than antioxidant potential, such as their binding capacity to various enzymes.

Antioxidants are naturally occurring and widely distributed in plant materials, animal tissues and microorganisms. Fruits, vegetables, cereals, grains, oilseeds, and teas are important sources of plant-derived antioxidants. A variety of antioxidant constituents present in such resources have been characterized and quantified, including vitamins E and C, polyphenols, carotenoids, antioxidant peptides and enzymes, as well as some antioxidant microelements such as selenium (Table 19.2). Consumption of whole grain cereals and legumes has been associated with the reduced risk of age-related diseases. Whole grain cereals are good sources of antioxidants, such as vitamin E, phenolic acids, flavonoids, lignins, lignans, carotenoids, phytic acid, folates,

**Table 19.1** Major reactive species in food and biological systems

Free radicals	Non-radicals
<i>Reactive oxygen species (ROS)</i>	Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )
Superoxide anion (O <sub>2</sub> <sup>•-</sup> )	Hydrochlorous acid (HOCl)
Hydroxyl (•OH)	Singlet oxygen ( <sup>1</sup> O <sub>2</sub> )
Alkyl (L•)	Ozone (O <sub>3</sub> )
Alkoxy (LO•)	Hydroxyalkenals
Peroxy (LOO•)	
Hydroperoxyl (HOO•)	
<i>Reactive nitrogen species (RNS)</i>	Peroxynitrite (ONOO <sup>-</sup> )
Nitric oxide (•NO)	Alkyl peroxynitrite (LOONO)
Nitrogen dioxide (NO <sub>2</sub> •)	Dinitrogen trioxide (N <sub>2</sub> O <sub>3</sub> )
	Nitrous acid (HNO <sub>2</sub> )

**Table 19.2** Major antioxidants from natural sources

Antioxidants	Examples	Sources
Tocopherols	α-, β-, γ-, and δ-tocopherols	Cereals, legumes, nuts, vegetable oils, rice bran, palm
Ascorbic acid	Ascorbic acid, ascorbate	Fruits, vegetables, etc.
Carotenoids	β-carotene, lycopene, canthaxanthin	Carrots, tomato, corn, beans, fish/shellfish, etc.
Phenolics	Ferulic acid, quercetin, catechin, resveratrol, cyanidin	Fruits, vegetables, cereals, legumes, etc.
Peptides	Ferritin, transferrin, lactoferrin	Milk, egg, beans, etc.
Enzymes	Superoxide dismutase, catalase, glutathione peroxidase	Plant and animal organisms
Microelements	Selenium, zinc, manganese	Cereals, legumes, nuts, meat

glutathione, melatonin, microelements (e.g. zinc, selenium), and alkylresorcinols (Zielinski, 2002; Fardet *et al.*, 2008). Dietary fiber and antioxidants in the outer layer and germ fractions of the grain are believed to act together to combat oxidative stress, inflammation, hyperglycaemia, and other pathophysiological conditions. Legumes such as beans provide a rich source of isoflavonoids with estrogenic activities, which may be effective in protection against hormone-related cancer and cardiovascular diseases (Lichtenstein, 1998). This chapter focuses on the effects of antioxidants on oxidative stress-mediated chronic diseases and health conditions, particularly inflammation, metabolic syndrome, cardiovascular disease and cancer.

## 19.2 Anti-inflammatory capacity of antioxidants

### 19.2.1 Oxidative stress and inflammation

Inflammation is characterized by the recruitment of immune cells (e.g. neutrophils, macrophages, and monocytes, etc.) and blood plasma from blood vessels to the inflamed tissue as a result of increased capillary permeability and production of adhesion

molecules from endothelial and immune cells (Aquilano *et al.*, 2008). It is a normal physiological response of the immune system to counteract pathological states such as irritation or infection caused by chemicals, microbial pathogens, and/or wounding. However, unbalanced or prolonged inflammation leads to progressive tissue damage and has been implicated in the development of many chronic diseases and health conditions. Chronic local inflammation is believed to contribute to the multistage carcinogenesis, particularly the promotion stage, in which the tissue suffers from persistent oxidative and inflammatory damage (Surh, 2002). In the vasculature, overproduction of ROS/RNS by inflammatory cells causes endothelial dysfunction and atherosclerosis (Ross, 1999). The microglia-mediated inflammation in neuronal tissue leads to decreased neuronal viability and has been associated with the development of neurodegenerative disorders such as Alzheimer's and Parkinson's (Aquilano *et al.*, 2008). Moreover, a causal relationship has recently been found between adipose tissue inflammation and obesity-induced complications, such as metabolic syndrome (Shahidi and Zhong, 2009).

Oxidative stress is considered to be one of the major fundamental tissue-destructive mechanisms in inflammation through continuous overproduction of ROS/RNS, which are capable of causing reversible and irreversible damages to biomolecules, including membrane phospholipids, cholesterol, proteins, enzymes, lipoproteins, and DNA. ROS/RNS released by activated phagocytes in turn promote inflammation by stimulating production of numerous pro-inflammatory cytokines (Shahidi and Zhong, 2009). Evidence has shown that ROS/RNS are involved in the activation of a variety of kinases and transcription factors, whose regulation is dependent on the redox changes. The transcription factor nuclear factor-kappa B (NF- $\kappa$ B), for example, is redox-sensitive and becomes activated under oxidative/nitrosative stress. Once activated, NF- $\kappa$ B is translocated from the cytoplasm to the nucleus, leading to up regulation of numerous inflammatory genes coding for many pro-inflammatory mediators. Some of the cytokine products are themselves activators of NF- $\kappa$ B (Janssen-Heininger *et al.*, 1999), and are also known to induce cellular ROS production (Adamson and Billings, 1992). Hence, ROS/RNS and pro-inflammatory cytokines work in a synergistic manner through a ROS/RNS-cytokine-transcription factor regulatory loop, thereby augmenting the inflammatory response and tissue damage (Fiocchi, 1998). In addition to cytokine regulation, ROS also appear to facilitate intensified tissue damage at the inflammation site by activating multiple tissue-destructive matrix metalloproteinases while inactivating their inhibitors (Frears *et al.*, 1996; Brenneisen *et al.*, 1997). Oxidative stress also promotes inflammation by down regulating the protective antioxidant genes, such as those coding for glutathione (Rahman and MacNee, 2000), and depleting antioxidant compounds *in vivo*.

Anti-inflammatory agents such as steroidal or non-steroidal chemical drugs can block the ROS/RNS and cytokine-involved inflammatory cascade. More recently, search for naturally derived substances with anti-inflammatory activity has emerged due to their limited side effects and intolerance as well as excellent availability at lower costs. Plant foods rich in antioxidants, such as fruits, vegetables, cereals, and legumes, have appeared as good candidates as natural sources of anti-inflammatory agents. Many antioxidants have been found to possess anti-inflammatory properties through various mechanisms related to or independent from their antioxidant activity.

### 19.2.2 Antioxidants as anti-inflammatory agents

Some oriental countries have a long history of using certain plants as traditional medicine for treating inflammation. Modern science and technology have revealed that the antioxidants in these plant materials are among the compounds responsible for their anti-inflammatory properties. There have been epidemiological data supporting the existence of an inverse relationship between consumption of fruits and vegetables rich in antioxidants and the risks for chronic inflammation and related diseases (Chen *et al.*, 2005; Zhang *et al.*, 2005). Whole grain intake has recently been proposed to be inversely related to the concentration of inflammatory proteins in human plasma (Qi and Hu, 2007). Masters *et al.* (2010) reported an inverse relationship between whole grain intake and concentrations of plasminogen activator inhibitor type 1 (PAI-1) and C-reactive protein (CRP) in populations of African American, non-Hispanic White, and Hispanic adults from the IRAS (Insulin Resistance Atherosclerosis Study), independent of demographic, lifestyle, and dietary variables. Anson *et al.* (2010) studied the bioaccessible compounds from aleurone, bran, and flour of wheat obtained from an *in vitro* upper gastrointestinal tract model for their antioxidant and anti-inflammatory activities and found that the bioaccessible compounds from aleurone with the highest antioxidant capacity provided a prolonged anti-inflammatory effect. Antioxidant compounds such as polyphenols present abundantly in plant foods including cereals and legumes have been demonstrated to possess anti-inflammatory activities through various mechanisms.

As noted earlier, antioxidants are effective scavengers of ROS/RNS, which are the major cellular and tissue destructive force and important promoters of inflammation. For instance, superoxide anion radical is involved in the infiltration and accumulation of neutrophils at the inflammation sites as well as mobilization of arachidonic acid, the precursor for many inflammatory mediators such as prostaglandins. Hydrogen peroxide not only acts as a neutrophil chemoattractant, but also plays an important role in leucocyte rolling, activation of T lymphocytes and induction of angiogenesis (Kruidenier and Verspaget, 2002). Antioxidants effectively eliminate ROS/RNS, thus attenuating the inflammatory damage. Moreover, oxidative stress is an important stimulus in regulating gene expression of pro-inflammatory cytokines through activation of a variety of kinases and transcription factors, such as NF- $\kappa$ B. Antioxidants, on the other hand, inhibit the oxidative stress-promoted cascade of the inflammation process through inhibiting activation of redox-sensitive transcription factors.

The NF- $\kappa$ B pathway has been recognized as an important target for anti-inflammatory agents and is the central coordinator of innate and adaptive immune responses, which also plays a critical role in cancer development and progression (Pan *et al.*, 2009). Activated NF- $\kappa$ B facilitates transcription of numerous inflammatory genes, including those coding for tumor necrosis factor (TNF)- $\alpha$ , interleukine (IL)-1, -6 and -8, vascular cell adhesion molecule (VCAM)-1, monocyte chemoattractant protein (MCP)-1, E-selectin, inducible nitric oxide synthase (iNOS), cyclooxygenase COX-2, 5-lipoxygenase (5-LOX), hypoxia inducible factor-1R (HIF-1R), and vascular endothelial growth factor (VEGF), among others, resulting in inflammation and tumorigenesis (Pan *et al.*, 2009). The NF- $\kappa$ B in the cytoplasm normally exists in a complex with the inhibitory protein inhibitor  $\kappa$ B (I $\kappa$ B) as a heterodimer, which is the inactive form of this transcription factor. Activation of NF- $\kappa$ B is induced by a cascade of events leading to phosphorylation, ubiquitination, and proteasomal degradation of I $\kappa$ B, leaving NF- $\kappa$ B free to translocate to the nucleus, where it induces gene transcription. The phosphorylation of I $\kappa$ B is catalyzed by I $\kappa$ B kinases (IKKs), which can be activated through

phosphorylation by upstream kinases, including NF- $\kappa$ B-inducing kinase, mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) (Pan *et al.*, 2009). Oxidative stress, UV and ionization radiation, mitogens and bacterial toxins cause rapid phosphorylation and subsequent degradation of I $\kappa$ B, which is critical for NF- $\kappa$ B activation (Surh, 2002). Antioxidants are able to block various components of this NF- $\kappa$ B-mediated signal transduction pathway and, therefore, interfere with the inflammatory genes activation. For example, flavonols have shown inhibition against PKC and MAPK, enzymes involved in activation of IKKs (Wadsworth *et al.*, 2001; Kempuraj *et al.*, 2005). In particular, genistein, a major isoflavone in soybean, can counteract the activity of IKKs, thus inhibiting activation of NF- $\kappa$ B (Liang *et al.*, 1999). Avenanthramides, the characteristic polyphenols in oats, are found to suppress NF- $\kappa$ B activation via inhibition of phosphorylation of IKK and I $\kappa$ B and proteasome activity in endothelial cells (Guo *et al.*, 2008).

In addition to NF- $\kappa$ B, activation of other redox-sensitive transcription factors involved in inflammation such as activator protein 1 (AP-1), cyclic adenosine monophosphate response element binding protein (CREB), hypoxia inducible factor (HIF), and NF-E2 related factor 2 (Nrf2) may be inhibited by antioxidants, which ultimately exert a suppressive effect against cytokine expression (Kundu and Surh, 2009). The lipophilic and membrane permeable antioxidants  $\alpha$ -lipoic acid and estradiol are able to scavenge AGE (advanced glycation end products)-induced oxygen free radicals, which have been proposed to act as second messengers in redox-sensitive signal transduction pathways (Wong *et al.*, 2001). Inhibition of transcription factors and down regulation of pro-inflammatory cytokines by antioxidants from dietary sources have been reported. Quercetin, wogonin, genistein, kaempferol, amentoflavone, resveratrol, curcumin, gingerol, capsaicin, and caffeic acid and its derivatives (Surh, 2002; Lorenz *et al.*, 2003; Kim *et al.*, 2004; Márquez *et al.*, 2004; Miles *et al.*, 2005; Kang *et al.*, 2007; Gonzales and Orlando, 2008) have been shown to counteract the activation of NF- $\kappa$ B and AP-1. Epicallocatchin gallate (EGCG) from green tea has been demonstrated to suppress the production of IL-1 and COX-2 induced by different means (Kim *et al.*, 2007). In cereals, the bioaccessible antioxidant extracts from different wheat fractions have shown TNF- $\alpha$  reduction in U937 macrophages (Anson *et al.*, 2010). Barley  $\beta$ -glucan treated macrophages reduced oxidation products accumulation and enhanced level and activity of antioxidant enzymes and inhibited iNOS expression (Yang *et al.*, 2008). Isoflavonoids reduce LPS-induced IL-6 cytokine production, while proanthocyanidins inhibit iNOS and COX-2 expression through blocking phosphorylation of IKK, degradation of I $\kappa$ B, nuclear translocation, and DNA binding of NF- $\kappa$ B (Hou *et al.*, 2007a, b).

In addition to the down regulation of cytokines at protein expression level, some antioxidants directly inhibit the activity of pro-inflammatory mediators such as iNOS and COX-2. NOS comprise a family of enzymes that catalyze the production of NO *in vivo*, and are commonly known to exist in two forms: cNOS that is constitutively present in several types of cells (e.g. neurons and endothelial cells), and iNOS whose expression is induced in response to pro-inflammatory cytokines and bacterial LPS (Pan *et al.*, 2009). iNOS has also been proposed to be a major factor involved in pathological vessel dilation and tumorigenesis (Biesalski, 2007). NO is produced by NOS under physiological and pathophysiological conditions as an important oxidative and inflammatory mediator. It is a lipid-soluble free radical with a considerably long life and is capable of diffusing several cell diameters from its synthesis site (Kruidenier and Verspaget, 2002). NO itself at nanomolar concentrations is not particularly harmful and in some occasions may even exert beneficial effects (Kruidenier and Verspaget, 2002). However, it is the precursor of a more damaging RNS peroxynitrite



anion (ONOO<sup>-</sup>), a stable and reactive oxidizing/nitrating agent that can damage a broad array of biomolecules in cells. Excessive and uncontrolled production of NO in activated immune cells during inflammation contributes to the major destructive force in tissue injury. COX-2 is an inducible enzyme catalyzing the conversion of arachidonic acid to prostaglandins. Arachidonic acid is the precursor of a number of pro-inflammatory mediators, which are critical to neutrophil activation and therefore inflammatory damage to cells and tissues (Benavente-Garcia and Castillo, 2008). Arachidonic acid-dependent pathways, including the actions of phospholipase A<sub>2</sub>, lipoxygenases and COX, are among the major targets for anti-inflammatory agents, such as many flavonoids (Manthey *et al.*, 2001). Bioactive lipids produced from arachidonic acid by COX-2 such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), have been identified as potent inflammatory mediators that can also promote tumor growth and metastasis (Mann *et al.*, 2005). Prostaglandins are a group of arachidonic acid-derived eicosanoids biosynthesized through COX pathway. They are lipid mediators that coordinate a wide variety of physiologic and pathologic processes (Fitzpatrick and Soberman, 2001). Prostaglandins under physiologic conditions play important roles in the cytoprotection of gastric mucosa, hemostasis, and renal hemodynamic. However, their stimulated biosynthesis is implicated in pathogenesis of various diseases including inflammation and cancer (Patrignani *et al.*, 2005). Enhanced prostaglandin production is observed in immune cells, due to the relatively high levels of arachidonic acid as the precursor in the immune cell membrane phospholipids as well as the induction of COX-2 in response to cellular stimulation. The production of NO and prostaglandins by iNOS and COX-2, respectively, is considered to be the most prominent molecular mechanism in the inflammatory processes (Moncada, 1999; Turini and DuBois, 2002). Suppression of induction and activities of iNOS and COX-2 provides an important approach to preventing inflammation. In addition to down regulating the biosynthesis of iNOS and COX-2 at transcriptional level through blocking the NF-κB-mediated signal transduction pathway, as discussed earlier, some polyphenols can directly inhibit their action at the enzyme level, which is independent from their antioxidant activities. For instance, the green tea polyphenol EGCG inhibits the catalytic activity of iNOS and COX-2 in human chondrocytes (Ahmed *et al.*, 2002). Chrysin, a natural flavonoid found in many plants, and its derivatives act as antagonist against iNOS and COX-2 in murine macrophage (Cho *et al.*, 2004). Instead of inhibition of NF-κB activation and cytokine induction, the authors proposed that these compounds reduce the activity of iNOS and COX-2 through inhibiting the enzyme dimerization and flavonoid-enzyme binding. The primary isoflavones and antioxidant components in soybeans genistein, daidzein, and glycitein exhibit significant direct inhibition against iNOS enzyme activity (Sheu *et al.*, 2001). Suppression of COX-2 activity by *p*-coumaric acid, a phenolic acid widely distributed in cereals, has also been reported (Luceri *et al.*, 2004).

## 19.3 Antioxidants and metabolic syndrome

### 19.3.1 Introductory remarks

Metabolic syndrome is a combination of abnormalities that increase the risk of developing diabetes and cardiovascular disease, and the symptoms generally include central or abdominal obesity, hyperglycemia or impaired glucose tolerance, dyslipidemia, and hypertension. It is the most common nutritional disorder and has become a growing medical problem in

industrialized countries. Obesity appears to play a causative role in the complex state of metabolic syndrome.

Obesity is generally considered to be a chronic disease recognized by over-accumulation of fat stores in adipocytes and has been linked to certain pathologies such as diabetes and atherosclerosis, presumably through oxidative stress and chronic inflammation. Being overweight, or obesity, which may arise from complex combinations of genetics and environment as well as social and personal reasons, is usually accompanied by increased low-level inflammation in the adipose tissue, and obesity-induced inflammation is believed to play a crucial role in the development of metabolic syndrome (Kang *et al.*, 2007). Adipose tissue, although once thought to be a simple and inert storage depot for excess fat in the form of triacylglycerols, has been found to participate in the physiological and metabolic control as a major endocrine source through several signaling mechanisms including autonomic nervous stimulation and secreting hormones, peptides and smaller bioactive molecules (Mohamed-Ali *et al.*, 1998). Under normal conditions, these substances can effectively control energy preservation through lipogenesis during the post-prandial period and energy mobilization through lipolysis in response to increased energy expenditure (Gonzales and Orlando, 2008). Adipose tissue is also capable of secreting a number of inflammation mediators, including cytokines, chemokines, and acute phase proteins. Higher oxidative stress, possibly by ROS from excess blood sugar and lipid, has been proposed to be the cause of elevated inflammation status in obesity (Ferroni *et al.*, 2004; Dandona *et al.*, 2005). It has been demonstrated that adipose tissue, particularly central or abdominal adipose tissue, produces pro-inflammatory cytokines leading to enhanced chronic inflammation, which has been associated with type 2 diabetes mellitus and cardiovascular disorders through insulin resistance and atherosclerotic lesions, respectively (Finley, 2004). Inflammatory cytokines produced in adipose tissue such as TNF- $\alpha$  and IL-6 have been shown to impair insulin signaling by promoting serine phosphorylation of insulin receptor substrate (IRS)-1 and suppressing the expression of insulin-sensitive glucose transporter 4 (Hotamisligil *et al.*, 1993; Feinstein *et al.*, 1993; Hotamisligil, 2003; Guri *et al.*, 2007). TNF- $\alpha$  may also increase fatty acid-induced systemic insulin resistance by promoting the release of fatty acids from adipose tissue into the bloodstream to act on peripheral tissues such as muscle and liver (Calabro and Yeh, 2007). Moreover, adiponectin, a regulatory molecule secreted exclusively by adipocytes, plays an important role in regulation of energy metabolism, insulin resistance, and vascular microenvironment (Kang *et al.*, 2007). Dysregulation of adiponectin production contributes to the onset or aggravation of chronic inflammation in obesity and in turn the development of obesity-related pathologies.

Strategies to attenuate inflammation and oxidative stress, such as use of antioxidants, are of great interest in reducing the risk of inflammation-mediated complications as in the case of metabolic syndrome. Antioxidants have been suggested to be effective in counteracting multiple factors causing the metabolic syndrome through a variety of mechanisms, some of which are independent from their antioxidant activity.

### **19.3.2 Antioxidants and obesity**

Obesity is a medical condition in which excessive accumulation of body fat poses an adverse effect on health, leading to reduced life expectancy and/or increased risk of health problems. It has been implicated in the development of heart disease, hypertension,

diabetes, and fatty liver, as well as certain cancers. Awareness by the general public of obesity in relation to compromised life quality has led to a high demand of pharmaceuticals and nutraceuticals for body weight control. Anti-obesity drugs in the current market account for approximately 2–6% of the total healthcare cost in the developed countries (Birari and Bhutani, 2007). However, the side-effects of many drugs remain a concern, thus necessitating the search for naturally derived anti-obesity agents. There has been convincing evidence for reduced risk of obesity by consumption of fruits and vegetables as well as whole grains (Christa and Soral-Smietana, 2008). Their antioxidants, especially the polyphenols, are found to be, at least partially, responsible for their effectiveness in body weight control. Antioxidants have been shown to suppress fat accumulation through modulating lipid metabolism and other mechanisms such as satiety and energy expenditure control.

Obesity is most commonly caused by imbalanced fat or energy intake, although genetic susceptibility is also known to play an important role as a non-environmental factor. Substances capable of regulating the energy homeostasis may serve as potential anti-obesity agents for treatment and prevention of obesity and associated comorbidities. Body weight control by antioxidants is generally achieved through suppressing food intake, lipid digestion/absorption, and lipogenic activity, which are important factors affecting fat accumulation in the body, while enhancing energy expenditure through thermogenesis, fat oxidation, and other forms of lipolysis. Antioxidants have been found to suppress appetite. Diet supplemented with antioxidants-rich blueberry extracts resulted in the reduction of food intake and decrease in body weight in a rat model, and the blueberry antioxidants were believed to act as a satiety inducer and weight management modulator (Molan *et al.*, 2008). The exact mechanism behind this has not yet been fully established, but it may be involved in releasing hormones that reduce gastric emptying and stimulate pancreatic juices, and/or triggering receptors that inform the brain centers of adequate energy or nutrient ingestion (Molan *et al.*, 2008).

Antioxidants may also suppress food intake through modulation on induction and activity of two appetite regulatory peptides leptin and adiponectin, whose secretion by adipocytes is affected by oxidative stress. Leptin is an adipocyte hormone that plays a regulatory role in food intake control through a direct effect on the hypothalamus (Calabro and Yeh, 2007). Adiponectin, a hormone secreted exclusively from adipose tissue into the bloodstream, is involved in regulation of adipose tissue, food intake, and body weight with an anorexigenic function via the brain (De Graaf *et al.*, 2004). Circulating levels of leptin and adiponectin in the blood are closely correlated with oxidative stress and the degree of obesity, although their cause-and-effect relationship remains unclear (Shen *et al.*, 2010). Elevated levels of leptin (leptin resistance) and decreased levels of adiponectin are observed in obese individuals. The antioxidant vitamin E supplementation was found to be effective in increasing serum adiponectin while decreasing the leptin levels (alleviate leptin resistance) in rats (Shen *et al.*, 2010).

Antioxidants reduce fat accumulation through regulating lipid digestion, absorption, metabolism, and excretion in the body. Lipid-lowering and lipid profile-modulatory effects have been reported for antioxidants from various sources. Caffeic, ferulic, and coumaric acids significantly lowered the plasma lipid and hepatic cholesterol levels in rats (Yeh *et al.*, 2009). Consumption of soy isoflavones significantly reduced plasma total lipids, triacylglycerols, total cholesterol, low density lipoprotein (LDL) cholesterol, and very low density lipoprotein (VLDL) cholesterol levels, while increasing the HDL cholesterol level

in male rabbits (Yousef *et al.*, 2004). Tocotrienols isolated from rice bran oil were found to decrease lipid parameters in rats in a dose-dependent manner (Minhajuddina *et al.*, 2005). Intake of selenium, an antioxidant present in many cereal grains, is associated with a lower plasma concentration of total, LDL and, VLDL cholesterol in hamsters (Poirier *et al.*, 2002). Flax lignan was able to alleviate lead acetate-induced oxidative damage and hyperlipidemia in both the serum and liver of rats (Newairy and Abdou, 2009). The hypolipidemic activity of antioxidants has been attributed to their ability to inhibit digestion, to delay triacylglycerol and cholesterol absorption, to reduce chylomicron secretion, and to enhance fecal lipid excretion. Many polyphenols exhibit an inhibitory effect against gastric, pancreatic, and carboxylester lipases, the important enzymes for digestion of dietary triacylglycerols, thus blocking the lipase-catalyzed hydrolysis and delaying their subsequent absorption (Raghavendra *et al.*, 2007; Sugiyama *et al.*, 2007). Polyphenols also interfere with cholesterol absorption through competition for bile-assisted micellar solubilization. A grapeseed extract capable of enhancing the erythrocyte antioxidant defense system significantly increased fecal excretion of triacylglycerols, suggesting its lipid-lowering potential (Cho *et al.*, 2007). Increased fecal lipid excretion was also found in rats receiving supplementary phytic acid, an antioxidant compound widely present in whole grain cereals (Lee *et al.*, 2007).

Antioxidants can inhibit lipogenesis and enhance lipolysis, thus reducing adipose and visceral fat deposition. Resveratrol reduced lipogenesis from glucose possibly due to disturbed mitochondrial metabolism of the sugar in rat adipocytes (Szkudelska *et al.*, 2009). Quercetin and EGCG have been shown to inhibit fatty acid and triacylglycerol synthesis by suppressing the expression and/or activity of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Gnoni *et al.*, 2009; Huang *et al.*, 2009). Park *et al.* (2008) studied the effect of antioxidative  $\alpha$ -lipoic acid on hepatic lipogenesis and demonstrated that it acted through both adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent pathways to suppress the expression of sterol regulatory binding protein-1c (SREBP-1c), which in turn suppresses the expression of FAS and ACC.

Another important mechanism for the anti-obesity effect of antioxidants, particularly polyphenols, is their ability to enhance energy expenditure, as reported for tea catechins that promote thermogenesis and fat oxidation (Dulloo *et al.*, 1999; Rumlper *et al.*, 2001; Komatsu *et al.*, 2003).

### 19.3.3 Antioxidants and diabetes

Diabetes mellitus is a chronic metabolic disorder and a major health problem worldwide. It is characterized by insufficient secretion and/or action of insulin associated with persistent hyperglycemia and alterations in carbohydrate, lipid, and protein metabolism (Dukworth, 2001). Development of diabetes is usually accompanied by dyslipidemia or hyperlipidemia, which is implicated in cardiovascular complications, a major cause of illness and death (Markku, 1995). Oxidative stress is believed to play an important role in the pathology of diabetes, being both the cause and consequence of this metabolic abnormality. An increased formation of ROS and a decreased antioxidant defense efficiency have been observed in diabetes. Oxidative stress is one of the major causes of loss and dysfunction of the ROS-sensitive pancreatic  $\beta$ -cells, where insulin is produced and released, resulting in impaired insulin

secretion. Oxidative stress also interferes with production of insulin by down regulating the transcription of the insulin gene and other relevant  $\beta$ -cell genes through inhibition of the transcription factor pancreatic and duodenal homeobox factor-1 (PDX-1) (Kaneto *et al.*, 2006). On the other hand, insulin deficiency-caused hyperglycemia in turn stimulates ROS generation and antioxidant depletion, eventually leading to oxidative damage of cellular components and disruption of  $\beta$ - and other cells. In type 2 diabetes, for example, increased plasma glucose levels, often associated with high concentrations of circulating free fatty acids, result in the enhanced mitochondrial superoxide production and increased exposure of cells to ROS (Novelli *et al.*, 2010). Hyperglycemia may cause glycation of tissue proteins, which generates hydrogen peroxides, hydroxyl radicals, protein reactive ketoaldehydes, and other AGE leading to oxidative damage of the pancreatic cells. Hyperglycemia can also induce oxidative stress by glucose autooxidation, abnormal arachidonic acid metabolism and its coupling to COX catalysis, protein kinase C activation, increase of the NOS activity, and activation of the aldose reductase pathway, among others (Grattagliano *et al.*, 2008). Therefore, strengthening the antioxidant defense system can not only prevent development of diabetes but also alleviate its symptoms and related complications.

Plants rich in antioxidants, including vitamins E and C, carotenoids, and polyphenols have been used as medicinal materials in treating diabetes in oriental countries. Antioxidants as effective scavengers of ROS can protect pancreatic  $\beta$ -cells from oxidative damage and, therefore, may serve as antidiabetic agents. It has been suggested that plasma concentration of vitamin E improves pancreatic compensation for insulin resistance (Costacou *et al.*, 2008). The antidiabetic effect of some antioxidants may also arise from actions other than scavenging of ROS. These include inhibition against sugar absorption, mostly through inhibition of the digestion enzymes such as  $\alpha$ -glucosidase and  $\alpha$ -amylase, inhibition of gluconeogenesis, stimulation of langerhans islets, and improvement of peripheral sensitivity to remnant insulin, among others (Sefi *et al.*, 2010; Dembinska-Kiec *et al.*, 2008).

A number of individual antioxidants have been shown to possess hypoglycaemic and hypolipidemic properties. Caffeic and isoferulic acids were found to reduce the fasting glycemia and attenuate the increase of plasma glucose in rats (Hsu *et al.*, 2000). Tea catechins, soy isoflavones, tannic acid, chlorogenic acid, and saponins inhibited glucose absorption by decreasing the S-Glut-1 mediated intestinal transport of glucose (Tiwari and Rao, 2002). Anthocyanins have been shown to affect absorption and insulin secretion/activity (Dembinska-Kiec *et al.*, 2008). Plant extracts with antioxidant activities have also shown effectiveness in lowering blood sugar via different mechanisms. The treatment of diabetic rats with a polyphenol rich leaf extract of *A. campestris* decreased significantly blood glucose, total cholesterol, total triacylglycerol, and LDL-cholesterol and increased serum insulin levels, possibly through its stimulatory effect on insulin secretion from pancreatic  $\beta$ -cells, glucose oxidation, and lipid synthesis pathway (Sefi *et al.*, 2010). Ranilla *et al.* (2009) studied five cereals from the Peruvian Andean region for their antidiabetes effect and found that purple corn exhibited the highest inhibition against  $\alpha$ -glucosidase, an enzyme managing early stages of type 2 diabetes, and that the inhibitory effect was correlated with total phenolic content and antioxidant capacity. Decreased blood glucose and increased plasma insulin levels as well as elevated hepatic glycogen synthesis and glucokinase activity were observed in diabetic mice fed a phenolic acid fraction of rice bran (Jung *et al.*, 2007). A dietary anthocyanin-rich bilberry extract ameliorated hyperglycemia and insulin sensitivity in diabetic mice via activation of AMPK, which was accompanied by an up regulation of glucose transporter-4 in white adipose

tissue and skeletal muscle and suppression of glucose production and lipid content in the liver (Takikawa *et al.*, 2010). A flavonoid-rich extract of *Eugenia jambolana* (EJ) significantly improved various biochemical parameters in diabetic mice, including glucose tolerance, lipid profile, glycogen biosynthesis, glucose uptake, and insulin release *in vivo* and *in vitro*, as well as differential regulation/expression of glucose homeostatic enzymes (e.g. glucose-6-phosphatase and hexokinase), suggesting its hypoglycemic and hypolipidemic effects (Sharma *et al.*, 2008).

Epidemiological evidence for antidiabetic effect of antioxidants or extracts, however, is limited and controversial. A health and nutrition survey carried out in the Attica region in Greece (the ATTIC study) concluded that dietary modification towards higher consumption of antioxidants is associated with better control of glycemic markers in individuals and reduced risk of diabetes (Psaltopoulou *et al.*, 2010). In a community-based study of adult men, serum concentrations and dietary intakes of  $\beta$ -carotene and vitamin E independently predicted insulin resistance and type 2 diabetes incidence during 27 years of follow-up (Arnlöv *et al.*, 2009). These results support a relationship between impaired antioxidant status and development of insulin resistance and type 2 diabetes. However, another randomized trial showed no significant overall effect of vitamin C, vitamin E, and  $\beta$ -carotene on the risk of developing type 2 diabetes in women at high risk of CVD (Song *et al.*, 2009). The experimental design and screening of other risk factors for diabetes such as genetic background are important in revealing the relationship between oxidative stress, antioxidants, and diabetes. More research is needed to translate the findings on antioxidants and prevention/treatment of diabetes from basic scientific research to nutritional and medical applications.

### **19.3.4 Antioxidants and hypertension**

Hypertension is defined as a systolic blood pressure of 140 mm Hg and higher, or a diastolic blood pressure of 90 mm Hg and higher, while morbidity may also occur in people with a blood pressure above 115/75 mm Hg, which is now considered one of the most important risk factors of death worldwide. Hypertension is associated with increased risk of stroke, myocardial infarction, heart failure, renal failure, and cognitive impairment (Briones and Touyz, 2010). Development of hypertension usually involves structural and functional changes of blood vessels, including endothelial dysfunction, altered contractility, and vascular remodeling, among others (Briones and Touyz, 2010). Endothelial dysfunction, arterial stiffness and increased oxidative stress, for example, have been identified in essential (or primary) hypertension, which is most commonly (90–95%) diagnosed (Plantinga *et al.*, 2007).

Oxidative stress is believed to play a major role in the pathogenesis of hypertension and target organ damage. ROS act as potent modulators of vascular, renal, and cardiac function and structure through their effects on cell growth, contraction/dilation, and inflammatory responses (Briones and Touyz, 2010). ROS are produced in all cell types (endothelial, smooth muscle, and adventitial cells) of the vascular wall by various enzyme systems, including NADPH oxidase, xanthine oxidase, and NOS (Harrison and Gongora, 2009). A number of mechanisms are involved in the modulation of vascular function/structure by ROS. These include quenching of the vasodilator NO by superoxide anion, depletion of tetrahydrobiopterin (a key cofactor for endothelial NOS), generation of vasoconstrictor

lipid oxidation products (e.g. F2-isoprostanes), activation of proinflammatory transcription factors (e.g. NF- $\kappa$ B and AP-1), stimulation of growth factor production, and induction of fibrosis through activation of matrix metalloproteinases, many of which can cause increased intracellular calcium, activation of growth and inflammatory signaling pathways, and increased extracellular matrix deposition, leading to endothelial dysfunction, increased vascular smooth muscle reactivity, and vascular remodeling (Briones and Touyz, 2010). The accumulated vascular changes may eventually lead to increased peripheral resistance and blood pressure elevation, which may stimulate generation of ROS and promote oxidative damage.

Angiotensin converting enzyme (ACE), is another key factor in the regulation of blood pressure and electrolyte homeostasis (the equilibrium of water and salt in the body). ACE catalyzes the conversion of angiotensin I into angiotensin II; the latter is a potent vasoconstrictor, and many of its pathophysiological effects have at least partially been attributed to the promotion of oxidative stress via activation of NADPH oxidase (Harrison and Gongora, 2009). Increased vascular, renal, cardiac, and neural production of superoxide anion and hydrogen peroxide was observed in angiotensin II-induced hypertension of rodents (Briones and Touyz, 2010). Moreover, ACE inactivates the vasodilator bradykinin, which also leads to increased blood pressure. ACE inhibitors can decrease systemic vascular resistance without increasing heart rate and promote natriuresis, thus exerting a vasorelaxing effect on blood vessels (Hanif *et al.*, 2010).

The causal relationship between oxidative stress and high blood pressure suggests that antioxidants may be used for preventing/treating hypertension. Antioxidant therapy has been found effective in increasing the body's antioxidant defenses and decreasing oxidative stress, and hence lowering the blood pressure. Small clinical studies have reported blood pressure-lowering effects of antioxidants. A randomized study demonstrated that combined oral antioxidant treatment with vitamins C and E for eight weeks improved arterial stiffness and endothelial function in untreated essential hypertensive patients (Plantinga *et al.*, 2007). Diet pattern has a great impact in the development of hypertension. Diets low in saturated fats and sodium, and high in fruits, vegetables, and unrefined whole grains have been associated with reduced risk of hypertension. Antioxidants are among the major active components responsible for antihypertensive properties of plant-based diets. A blueberry extract rich in antioxidants reduced the magnitude of hypertension in spontaneously hypertensive stroke-prone rats, possibly by protecting the kidneys from oxidative damage (Shaughnessy *et al.*, 2009). Administration of flavanol-rich dark chocolate reduced ambulatory blood pressure by 11.9 mmHg (24 h systolic) and 8.5 mmHg (24 h diastolic) in essential hypertensive patients, whereas flavanol-free white chocolate had no effect (Sirtori *et al.*, 2009). Many ACE inhibitors, angiotensin II type-1 receptor antagonists, and calcium channel blockers were found to display antioxidant activity (Tylicki *et al.*, 2003). Wheat, buckwheat, corn and oats, sprouts, and seedlings contain phenolics that may act as antioxidants and ACE inhibitors (Randhir *et al.*, 2008). Peptides and protein hydrolysates with antioxidant and ACE inhibitory activities have been identified. Hydrolysis of soybean protein with papain and bromelain yielded peptides with strong antioxidant and ACE inhibitory activities (Lee *et al.*, 2008). Hydrolytic products of potato and its by-products exhibited antioxidant activity and ACE inhibition *in vitro* (Pihlanto *et al.*, 2009). However, the antihypertensive property of ACE inhibitor peptides needs to be further investigated *in vivo* due to the digestive stability issue when administered orally.

## 19.4 Antioxidants and cancer

The cancer development, which is a complex process in humans, involves cellular and molecular changes caused by internal as well as external stimuli. Oxidative DNA damage is mainly responsible for the causation of cancer and associated with chromosomal defects and oncogene activation induced by free radicals (Surh, 2003; Valko *et al.*, 2004, 2006, 2007; Bahorun *et al.*, 2006). Free radicals as well as non-radical ROS are continuously formed in the body. Mitochondrial electron transfer process and products of enzymatic reactions of xanthine oxidase, lipoxygenases, and cyclooxygenases generate ROS within the cell (Szocs, 2004). ROS perform many potential actions on cells such as promoting cell-cycle stasis, senescence, apoptosis, necrosis or other types of cell death, and inhibiting angiogenesis or promoting proliferation, invasiveness, angiogenesis, metastasis, and suppressing apoptosis. Advances in cancer research support the evidence for the relationship between ROS and carcinogenesis. Therefore, the significant role of dietary antioxidants in inhibition of free radicals, and their formation in cancer risk reduction has become an area of primary interest. It has been postulated that antioxidants may reduce cancer risk by several mechanisms such as modulating redox status, preventing oxidation of biological molecules, and inhibiting the formation of carcinogens, among others.

Whole grains and their products are rich in compounds possessing antioxidant activity. It is known that whole grain cereals are a good source of phenolic acids, avenanthramides, tocopherols, folates, selenium, phytic acids, lignins, lignans, and alkylresorcinols, among others. In addition, insoluble antioxidants are present as cinnamic acid esters bound to arabinoxylans side chains of hemicellulose. In *in vitro* studies bound phenolics of cereal grains also have demonstrated notable antioxidant activity (Liyana-Pathirana and Shahidi, 2006; Madhujith and Shahidi, 2009; Chandrasekara and Shahidi, 2010). It has been shown that variable gastric pH conditions (Liyana-Pathirana and Shahidi, 2005) and colonic microbial fermentation (Kroon *et al.*, 1997) can release bound phenolic compounds, which may provide antioxidant protection reducing the risk of colon cancer.

Although many individualized studies on legumes regarding cancer research are not widely reported so far, legumes are potential candidates for disease risk reduction and health promotion. They contain a wide variety of phytochemicals including phenolic compounds such as phenolic acids, flavonoids, and lignins, among others (Sosulski and Dabrowski, 1984). The color of the seed coat is attributed to the presence of phenolic compounds, namely flavonol glycosides, condensed tannins and anthocyanins (Takeoka *et al.*, 1997; Beninger *et al.*, 1999; Choung *et al.*, 2003; Romani *et al.*, 2004; Salinas-Moreno *et al.*, 2005). Several authors have reported that legumes with a dark seed coat such as soybean, broad bean, faba bean, lentil, and peas are rich in phenolic compounds and, hence, antioxidant activity (Amarowicz *et al.*, 2000; Amarowicz *et al.*, 2000; Shahidi *et al.*, 2001; Takahata *et al.*, 2001; Troszynska and Ciska, 2002; Cardador-Martinez *et al.*, 2002; Amarowicz *et al.*, 2004; Madhujith and Shahidi, 2005). The antioxidant, antimutagenic, and anticarcinogenic activities as well as free radical scavenging properties of phenolic compounds are well established (Gonzalez de Mejia *et al.*, 1999; Cardador-Martinez *et al.*, 2002; Madhujith *et al.*, 2004). Duenas *et al.* (2006) reported that the seed coat of lentils (*Lens culinaris* L. Var. Pardina and Castellana) possessed a higher antioxidant capacity in the form of free radical scavenging activity than did the cotyledons. The seed coat showed free radical-scavenging capacity with EC<sub>50</sub> values between 0.05 and 0.07 (mg of sample), whereas for cotyledon



these values were between 21 and 29 (mg of sample). The same authors suggested that high content of flavonoids in the seed coat of lentils may be responsible for its high antioxidant activity compared to the corresponding cotyledon (Duenas *et al.*, 2006). Dong *et al.* (2007) showed that the extracts of black beans had potent antiproliferative activity toward MCF-7 human breast cancer cells, HepG<sub>2</sub> human liver cancer cells, and Caco-2 human colon cancer cells. In this study, triterpenoids and flavonoids were among the isolated and identified bioactive compounds in the seed coats of black beans with potent antiproliferative and antioxidant activities. In addition, several other compounds present in legumes may be responsible for cancer prevention potential (Table 19.3).

Several mechanisms such as modulation of carcinogen metabolism or inflammatory pathways and regulation of cell proliferation and apoptosis may be involved when flavonoids act in cancer prevention (Pierini *et al.*, 2008). One cellular defense mechanism involved with oxidative stress is the antioxidant response element (ARE). ARE is a *cis*-regulatory DNA sequences located in the enhancer region, that is upstream of many phase II detoxification and antioxidant enzymes such as heme oxygenase-I and glutathione-S-transferases (Friling *et al.*, 1990; Rushmore *et al.*, 1991; Rushmore and Pickett, 1990, 1993). Prochaska and Talalay (1988) have shown that substances interacting primarily with ARE, which selectively induce phase II enzymes without simultaneous inducing of phase I activity, are potent anticarcinogens. Flavanols such as EGCG exhibited this property (Chou *et al.*, 2000). Compounds that interact with anti-inflammatory factors may serve as chemopreventive agents because inflammation is a risk factor for many types of cancer. Several authors have shown that chronic use of anti-inflammatory drugs reduced the risk of colon and oesophageal cancers (Chan *et al.*, 2005; Ranka *et al.*, 2006; Vaughan *et al.*, 2005). Among others, one factor of interest is compounds that react with NF- $\kappa$ B. NF- $\kappa$ B is important as an up regulator of the inflammatory response and phenolic substances inhibit NF- $\kappa$ B activation at various stages. Natarajan *et al.* (1996) have reported that caffeic acid phenethyl ester specifically prevents binding of NF- $\kappa$ B to its target DNA sequence. In addition, flavonoids modulate cyclooxygenase (prostaglandin H synthase) enzyme system that comprises two distinct isoforms COX-1 and COX-2. The COX-2 may be induced by tumor promoters and endogenous cytokines, and produces prostaglandins involved in inflammation; flavonoids are potential COX-2 enzyme inhibitors (Baumann *et al.*, 1980). Flavonoids such as quercetin suppress colorectal crypt cell proliferation in the rat *in vivo* (Hara *et al.*, 1999) and inhibit the  $\beta$ -catenin pathway *in vitro* (Jaiswal *et al.*, 2002; Joe *et al.*, 2002). It is clear that the survival of cancer cells depends on their ability to divide continuously, and to evade apoptosis. Different flavonoids have been shown to inhibit proliferation and enhance apoptosis *in vitro* (Agullo *et al.*, 1996; Wenzel *et al.*, 2000; Kim *et al.*, 2005). Increasing epidemiological support for the protective effects of fruit and vegetable consumption against various cancers has drawn the attention of researchers to the possibility of flavonoids serving as chemopreventive agents. Furthermore, isoflavonoids generally occur in legumes and also possess estrogenic, antiangiogenic, antioxidant, and anticancer properties (Dixon, 1999; Dixon and Ferreira, 2002).

Selenium is an essential trace element for animals and humans. It exerts antioxidant function mainly in the form of selenocysteine residues as an integral constituent of ROS detoxifying selenoenzymes such as glutathione peroxidases (GPx) and thioredoxin reductases (Scalbert and Williamson, 2000). Epidemiological studies have supported that inadequate status of selenium increases the risk of prostate, lung, stomach, the pancreas, colon, rectum, esophagus, and breast cancers (Tavani *et al.*, 2004; Feng *et al.*, 2005; Zhang *et al.*, 2006).

**Table 19.3** Evidence of legumes on cancer prevention

<b>Study</b>	<b>Food/Compound</b>	<b>Outcome</b>	<b>Reference</b>
Prospective study in Japanese men ages 45–74 y	Isoflavone	Decreased risk of localized prostate cancer	Kurahashi <i>et al.</i> , 2007
Prospective study in Japanese female ages 40–59 y	Isoflavone	Reduced risk of breast cancer	Yamamoto <i>et al.</i> , 2003
Prospective study in Dutch females ages 40–70 y	Isoflavones/lignans	Not related to breast cancer risk	Keinan-Broker <i>et al.</i> , 2004
Case-control study in Chinese men ages 50–89 y	Soy foods/Isoflavones	Reduced risk of prostate cancer	Lee <i>et al.</i> , 2003
Case –control study in Japanese, Korean, and American residents	Isoflavone	Reduced risk in prostate cancer	Akaza <i>et al.</i> , 2004
<i>In vitro</i> in cancer cells PC-3 (prostate), MDA-MB-231 (breast), H460 (lung). BxPC3–3 (pancreas)	Genistein	Inhibition of cell growth and induction of apoptosis	Li <i>et al.</i> , 2005
<i>In vitro</i> in breast carcinoma cell lines MDA-468, MCF-7 MCF-7-D40	Isoflavones	Inhibit growth of cells	Peterson and Barnes, 1991
<i>In vitro</i> in prostate cancer cell lines LNCaP and PC-3	Genistein	Inhibition of proliferation dose dependently	Suzuki <i>et al.</i> , 2002
<i>In vitro</i> in stomach cancer cell lines HSC-41E6, HSC-45M2, SH101-P4	Biochanin A, Genistein	Inhibit the cell growth of cancer cell lines through activation of a signal transduction pathway for apoptosis	Yanagihara <i>et al.</i> , 1993
<i>In vitro</i> in LNCaP human prostate cancer cells	Genistein	Inhibit growth of LNCaP cancer cells	Onozawa <i>et al.</i> , 1998

On the other hand, some studies have reported the protective role of selenium against various types of cancer (Kono *et al.*, 1995; Feng *et al.*, 2005; Zhang *et al.*, 2006).

Phytic acid, an anti-nutrient in whole grain and legume seeds, possesses antioxidant activity (Graf and Eaton, 1990). Phytic acid chelates polyvalent cations such as  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$ . It has been established that iron-catalyzed oxidative reactions play a crucial role in the oxidative damage to different biological materials such as lipids, proteins, and nucleic acids. The antioxidant properties of phytic acid are likely derived from its relatively high binding affinity for iron: phytic acid preserves the solubility of iron, while making the metal less active.

Phytic acid may inhibit iron driven oxidative reactions by three mechanisms, namely making the iron catalytically inert, blocking the redox cycling of the metal ions, and preventing binding of iron to the host molecule (Graf and Eaton, 1990). It seems that by the same mechanisms phytic acid could suppress oxidant damage and lower the incidence of cancers particularly in the gastrointestinal tract. However, some earlier supplementation and prospective studies conducted with single antioxidants have reached controversial conclusions about the effect of antioxidants on cancer (Li *et al.*, 1993; Taylor *et al.*, 1994). The results of a six-year prospective intervention, placebo-controlled trial, conducted in Linxian China for 3318 persons with cytologic evidence of esophageal dysplasia, revealed that there was no substantial short-term beneficial effect on the incidence or mortality for esophageal cancer following daily supplementation with multiple vitamins and minerals (Li *et al.*, 1993). In another study with the general population of 29 584 adults, Taylor *et al.* (1994) reported that the prevalence of gastric cancer among participants receiving retinol and zinc was 62% lower than those not receiving these supplements ( $P=0.09$ ), while participants receiving beta-carotene, vitamin E, and selenium had a 42% reduction in esophageal cancer prevalence (0.34).

Serafini *et al.* (2002), in a case control population based study conducted in Sweden during 1989–1995, reported that total antioxidant potential of dietary plant food was inversely associated with the risk of gastric cancer. In a meta-analysis Jacobs *et al.* (1998) reported that whole grain intake was protective against a number of digestive tract and hormone-related cancers. Furthermore, Chatenoud *et al.* (1998), using a systemic review of case-control studies conducted in Northern Italy, indicated that a higher frequency of whole grain consumption was associated with reduced risk of cancer.

Ling *et al.* (2001) have reported that consumption of rice bran could produce an antioxidant activity and a hypocholesterolemic effect. According to Hu *et al.* (2003), the black rice anthocyanins reduced both reactive oxygen species and nitric oxide in chemical and biological model systems. Some other studies have demonstrated that cyanidin and malvidin isolated from black rice may cause significant growth inhibition through G2/M arrest and inhibition of DNA fragmentation, together with significant induction of apoptotic cell death on human monocytic leukemia cells (Hyun and Chung, 2004). In addition, Ichikawa *et al.* (2001) showed that cyanidin 3-glucoside contributes to the antioxidant activity of purple black rice through its strong scavenging activity for superoxide radical but not hydroxyl radical.

In a study designed to test whether vitamin E supplementation decreases risks of cardiovascular disease and cancer among healthy women, Lee *et al.* (2005) reported that 600 IU of vitamin E taken every other day provided no overall benefit for major cardiovascular events or cancer in healthy women. The effect of vitamin E and beta-carotene on the incidence of lung and other types of cancer in male smokers was studied; the vitamin E group had fewer incidences of prostate and colorectal cancers compared to the control group

without vitamin E supplementation (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994). Another study concluded that vitamin E supplements did not protect against cancer or cardiovascular disease (HOPE and HOPE-TOO, 2005). The Iowa women study conducted with 35 215 women aged 55–69 years showed that high intake of vitamin E may decrease the risk of colon cancer, especially in those under 65 years of age (Bostick *et al.*, 1993). Other large cohort prospective studies on the association between supplemental vitamin E and colon and rectal cancer, such as nurses' health study (NHS) and health professionals follow-up study (HPFS), did not provide consistent support for an inverse association between supplemental vitamin E and colon cancer risk (Wu *et al.*, 2002). The US adults cancer prevention study II (CPSII) supported the hypothesis that long-duration vitamin E supplementation may reduce the risk of bladder cancer mortality (Jacobs *et al.*, 2002). Taken together, vitamin E supplementation for the prevention of cancer cannot be recommended with certainty.

Epidemiological studies suggest that consumption of coffee, the most abundant source of chologenic acid, may protect against colon cancer (Brown *et al.*, 1990; Giovannucci, 1998; Tavani and LaVecchia, 2004; Michels *et al.*, 2005; Higdon and Frei, 2006; Lee *et al.*, 2007; LaVecchia and Tavani, 2007). Metabolic activation of azoxymethane (AOM) is known to occur through hepatic cytochrome P450-mediated oxidative process (Sohn *et al.*, 2001), which in turn may result in altered hepatic redox status. Recently, Parka *et al.* (2010) demonstrated that chronic dietary chologenic acid supplementation in a mouse model of carcinogen induced colon cancer but did not inhibit the tumorigenic process, although chologenic acid further reduced the capacity of hepatic thiol pool. Furthermore, they reported the contrasting effects of AOM and chologenic acid on thiol redox status in organs responsible for metabolic activation of AOM.

Determination of the effect of any one food on disease risk is ambiguous as a higher consumption of one particular food is generally associated with lower consumption of other foods that may also influence cancer risk. Furthermore, various nutrients have been shown to influence the bioavailability and absorption of other nutrients. Dietary intakes of individual nutrients, foods, food groups, and other dietary components are interrelated as free-living individuals eat a variety of foods.

Antioxidants such as carotenoids and phenolic compounds have been shown to effectively modulate photocarcinogenesis induced by UV light. The best known mechanisms by which carotenoids protect cells from oxidative stress include quenching triplet-state sensitizers, singlet oxygen, and peroxy radicals (Krinsky, 1989; Sies, 1993). The photoprotective activity of carotenoids was demonstrated by both delayed tumor appearance and reduced tumor growth in a number of studies in mice (Mathews-Roth, 1982).

In human studies, however, beta-carotene not only failed to render a protective effect, but also displayed a pro-carcinogenic effect (Black and Lambert, 2001). For instance, the findings of the ATBC study indicated that lung cancer incidence was increased among participants who received beta-carotene as a supplement (Albanes *et al.*, 1996). Furthermore, Greenberg *et al.* (1990) showed that oral beta-carotene supplementation of 50 mg per day for a median of 4.3 years did not decrease the incidence of nonmelanoma skin cancer cells in patients. However, in some studies phenolic compounds have shown protective effects against human skin cancer. The topical application of EGCG, the major polyphenolic constituent in green tea, inhibited UVB (280–320 nm)-induced infiltration of leukocytes, reduced myeloperoxidase activity, and decreased the UVB-induced erythema in human skin (Katiyar *et al.*, 1999; Afaq *et al.*, 2003).

In addition to disease risk reduction and cancer prevention some studies examined the effect of antioxidants on reducing the toxicity associated with anticancer agents. According to Conklin (2000), dietary antioxidants as supplements may provide a safe and effective means of enhancing the response to cancer chemotherapy. Furthermore, vitamin E may enhance antineoplastic activity of cancer cells because of its role in preventing lipid peroxidation, thus maintaining the rapid rate of proliferation of cancer cells. However, according to a review by Ladas *et al.* (2004) there was no evidence to support the positive effects of individual antioxidant vitamin supplements in reducing the side effects of anticancer agents. This could be due to the facts that these antioxidants did not reduce toxicity or more potent antioxidants and higher doses of individual antioxidants are required to minimize the side effects of anticancer agents.

Salganik (2001) discussed that oxidative status of cells may determine the efficacy of antioxidants in protecting against cancer or to promote cancer. Antioxidants can prevent cancers in healthy individuals by controlling increased levels of ROS and damage to DNA and other biomolecules. On the other hand, ROS in moderate concentrations act as crucial elements in cancer-protective apoptosis and phagocytosis, in individuals with a low ROS level, thus an excess of antioxidants may block these cancer-preventive mechanisms and promote cancer. In addition, an excess of antioxidants may interfere with apoptosis and promote cancer in those who are constantly exposed to environmental carcinogens such as tobacco smoking, and pollutants, which may lead to high accumulation of pre-cancerous and cancerous cells. Furthermore, in cancer patients, an excess of antioxidants can interfere with the therapeutic activity of anticancer drugs, which might kill cancer cells by ROS-dependent apoptosis.

## 19.5 Antioxidants and cardiovascular diseases

Cardiovascular diseases (CVD) include a range of pathological conditions, of the arteries supplying blood to heart muscles (Ischemic heart disease, IHD), the brain (cerebrovascular disease or stroke), and the extremities such as legs (peripheral vascular disease). The disease progression could be of atherosclerosis, the lesions of the arteries caused by fat deposition, arteriosclerosis, resulting from calcium deposition, or thrombosis occurring due to blood clotting. Overall CVD are the most common cause of death in Western countries. Increased cellular production of ROS could contribute to atherogenesis and CVD progression.

Pathogenesis of atherosclerotic CVD is multifaceted. Atherosclerotic lesions, which may be clinically silent, may exist in individuals for decades, before producing clinical events such as acute myocardial infarction, angina, or cardiac death. These events are often associated with acute rupture or erosion of a vulnerable plaque, which releases highly thrombogenic sub-endothelium to the circulation system. This may result in acute, platelet rich, mural thrombosis that leads to possible infarction and ischemia in the arterial lumen. The mechanisms for plaque vulnerability and rupture could be the local inflammation within the plaque, thinning of the fibrous cap, and accumulation of plaque lipid (Libby, 1995). Following the plaque rupture, thrombosis formation and changing of vascular tone may lead to the occurrence of ischemia/infarction. Among risk factors, diet affects atherogenesis by modulating at the cellular level, pro-inflammatory processes that initiate and perpetuate endothelial dysfunction, plaque formation, and plaque rupture.

ROS and RNS damage cells and biological molecules such as lipids, proteins and DNA, thus contributing to cellular dysfunction and CVD. It is known that oxidative damage to

LDL can produce modified particles containing both lipid oxidation products and damaged apoprotein oxidized particles that may enhance atherogenic effects (Witztum, 1991). The oxidized LDL may contribute to all stages of the atherosclerotic process, including activation of inflammatory events, endothelial damage, recruitment of macrophages, and unregulated uptake of oxidized LDL by these cells to form foam cells, the characteristic cells of early atherosclerotic lesions (Berliner and Heinecke, 1996).

Epidemiological studies revealed an increased risk of IHD and stroke at the low plasma concentration of antioxidants such as vitamins E, A, and C (Gey *et al.*, 1987; Gey and Puska, 1989). Taking into consideration cross cultural, case control and prospective studies by Gey *et al.* (1993) showed that suboptimal concentrations of essential antioxidants such as carotenes and vitamin C may increase the risk of CVD. Hence, it is important to maintain essential and synergistically linked antioxidants at appropriate levels in the body for CVD risk reduction.

Whole grains are known to contain a number of water-soluble and fat-soluble antioxidants, namely trace minerals, vitamins, phenolic acids, lignans, phytic acid, tocopherols, tocotrienols, and phytoestrogens. Cereal grains are also rich sources of carbohydrates including dietary fiber and protein. On the other hand, they also contribute to daily intake of vitamin B group, vitamin E, and a number of minerals such as iron, zinc, magnesium, and phosphorus. Non-nutrient phytochemicals together with vitamins and minerals in whole grains may provide protection against cancers and CVD. Slavin *et al.* (2003) discussed that phytochemicals in grains may act synergistically to exert the aforementioned protection in the human body.

Findings from these epidemiological studies support the hypothesis that a higher intake of whole grains may lead to a lower risk of CVD. A meta analysis of 12 population-based cohort studies showed that whole grain foods significantly reduced the risk of coronary heart disease (CHD) by 26% after adjusting for multiple risk factors (Anderson *et al.*, 2000). As reviewed by Kris-Etherton *et al.* (2002), several epidemiological studies have reported reductions in CVD risk ranging from 25 to 40% in individuals consuming one to three servings of whole grains per day. Consistent with other large prospective studies, Liu *et al.* (2003) also showed that both total and CVD-specific mortality were inversely related to whole grain, but not refined grain breakfast cereal intake in the US Physicians' Health Study (PHS).

In a prospective cohort study, Bazzano *et al.* (2001) showed a significant inverse relationship between legume intake and risk of CHD suggesting the contribution of legumes to the primary prevention of CHD in general population. They further showed that individuals with an intake of legumes at least 4 times a week had a 22% lower risk of CHD and an 11% lower risk of CVD compared with those consuming legumes less than once a week.

Vita and Keaney (2002) suggested that alterations in endothelial function contribute to the pathogenesis and clinical syndrome expression of cardiovascular diseases. Endothelial cells are major regulatory cells in the blood vessel wall and play a key role in the regulation of vascular homeostasis. Endothelial cells regulate vascular homeostasis by producing factors that act locally in the vessel wall and lumen. According to Ignarro *et al.* (1987) a key endothelial product is nitric oxide. Nitric oxide regulates important aspects of vascular homeostasis, namely prevention of adherence of leukocytes to the endothelial surface inhibiting expression of leukocyte adhesion molecules at the endothelial surface, preventing platelet adhesion and platelet aggregation, inhibiting vascular smooth muscle cell proliferation by altering expression of noncellular components that constitute the matrix of the vascular wall, making nitric oxide relevant to lesion formation, hypertrophy of the vessel

wall, and vascular compliance. Therefore, endothelium-derived nitric oxide plays a role in vasodilator, antiinflammatory, antithrombotic, and growth suppressions that are relevant to all stages of atherosclerosis (Vita and Keane, 2002). The loss of nitric oxide results in changes of other regulatory mechanisms in endothelium, leading to the development of a pathologic endothelial phenotype. Other endothelium-derived products that regulate vascular homeostasis include substances that influence vascular tone, as well as fibrinolytic, coagulation, and proinflammatory factors (Widlansky *et al.*, 2003). Several studies suggest that flavonoids may have beneficial effects on endothelial control of thrombosis, inflammation, and vascular tone (Hertog *et al.*, 1993; Knekt *et al.*, 1996; Keli *et al.*, 1996). In addition flavonoids may have beneficial effects on platelets, which appear in the arterial lumen with the setting of acute coronary syndromes.

Epidemiological study results for the relationship between flavonoid intake and cardiovascular disease risk are sometimes contradictory (Hertog *et al.*, 1993; Brown *et al.*, 1993; Knekt *et al.*, 1996; Keli *et al.*, 1996; Rimm *et al.*, 1996; Hertog *et al.*, 1997; Yochum *et al.*, 1999; Sesso *et al.*, 1999; Geleijnse *et al.*, 2002; Mukamal *et al.*, 2002; Sesso *et al.*, 2003, 2003). The overall evidences suggest that individuals with the highest flavonoid intake may modestly reduce the risk of cardiovascular diseases (Hertog *et al.*, 1993; Knekt *et al.*, 1996; Keli *et al.*, 1996; Yochum *et al.*, 1999; Sesso *et al.*, 1999; Geleijnse *et al.*, 2002). On the other hand, some studies have shown a strong positive apparent benefit of flavonoids. For example, Hertog *et al.* (1993) reported that the tertile of subjects consuming the highest amount of flavonoids had a 68% lower risk of CVD, after adjustment for known cardiovascular risk factors, compared to individuals in the lowest tertile. In a case-control study, Sesso *et al.* (1999) demonstrated that individuals who had more flavonoid consumption showed a 44% reduction in cardiovascular risk, though studies were conducted with tea and coffee consumption. In addition to the reported benefits of flavonoid intake in the primary prevention of CVD, Mukamal *et al.* (2002) suggested that flavonoid intake in the form of tea might have benefit amongst individuals already suffering from cardiovascular disease. However, some studies demonstrated no relationship between flavonoid intake and cardiovascular risk (Brown *et al.*, 1993; Rimm *et al.*, 1996; Hertog *et al.*, 1997; Sesso *et al.*, 2003). It is possible that confounding factors may mask benefits of flavonoid consumption in those studies. Duffy *et al.* (2001) also showed that water-soluble antioxidants, such as flavonoids, have beneficial effects on endothelial function. Both short-term and long-term tea consumption improved endothelial function. Although tea contains flavonoids, it was not clear whether the antioxidant effect explains the observed benefits of tea as this study did not demonstrate an effect of tea consumption on plasma antioxidant capacity or plasma concentrations of F2-isoprostanes, markers of systemic lipid peroxidation, or 8-hydroxydeoxyguanosine, a marker of DNA oxidation.

After reviewing several epidemiological and interventional studies, Vita (2005) concluded that polyphenols have beneficial effects in reducing the risk of cardiovascular disease. Furthermore, this review suggested that improved endothelial function, antiplatelet, and anti-inflammatory properties could be the important possible mechanisms for the reported benefits. Antioxidants may exert an effect on endothelial function as oxidative stress could be a cause of endothelial dysfunction. Endothelial dysfunction is considered an early step in the process of atherosclerosis. The normal healthy endothelium has many anti-atherogenic functions, such as regulation of blood flow, inhibition of blood clotting, and prevention of adhesion of inflammatory cells to blood cells lining, which are performed through mediators like NO and prostacyclin released by the endothelium. Endothelial dysfunction is characterized

by decreased bioactivity of NO and impaired flow-mediated vasodilation. According to Duffy *et al.* (1999) acute treatment with ascorbic acid reverses endothelial dysfunction in atherosclerosis, among others. Huang *et al.* (2000) suggested that the benefit of ascorbic acid could be in part due to increased activity of nitric oxide synthase resulting from stabilization of tetrahydrobiopterin, an essential cofactor for enzyme activity. However, the effects of lipid-soluble antioxidants on endothelium-dependent dilation among patients with atherosclerosis and cardiovascular risk factors have not been clearly understood (Vita, 2005). Platelet aggregation plays a critical role in the pathogenesis of acute coronary syndromes. Several studies have demonstrated that flavonoids inhibit platelet aggregation (Demrow *et al.*, 1995; Freedman *et al.*, 2001). Other phenolic compounds have also been reported to have beneficial effects on endothelial and platelet function. Squadrito *et al.* (2002) reported that soy products, which are rich sources of isoflavones, namely genistein and daidzein, improve endothelial function, possibly through an effect on the estrogen receptor.

Osganian *et al.* (2003) showed the results of a 12 years follow up study that indicated higher intakes of foods rich in  $\alpha$ -carotene or  $\beta$ -carotene were associated with a reduced risk of coronary artery disease (CAD). *In vitro* studies have shown that carotenoids effectively scavenge singlet oxygen (Stahl and Sies, 1993), and free radicals (Krinsky, 1991; Esterbauer *et al.*, 1991), as well as inhibit lipid peroxidation (Terao, 1989; Jialal *et al.*, 1991; Jorgenson, 1993). In addition, carotenoids may prevent the development of atherosclerosis by directly inhibiting LDL oxidation (Diaz *et al.*, 1997; Steinberg *et al.*, 1989; Berliner and Heinecke, 1996). Furthermore, carotenoids may protect vascular cells from oxidative injury and prevent vascular dysfunction through tissue-specific actions that are independent of the direct inhibition of LDL oxidation (Diaz *et al.*, Steinberg *et al.*, 1989).

Polyphenolic compounds are known for their cardioprotective properties. A proposed mechanism for the apparent benefit of various polyphenols is their positive effects on platelet aggregation (Ruf, 2004; Cordova *et al.*, 2005). It is known that antiplatelet therapy may decrease the risk of CVD (Youssef *et al.*, 2005). Polyphenols may inhibit platelet aggregation through a number of different mechanisms, such as inhibition of COX, lipoxygenase (Schubert *et al.*, 1999; Hong *et al.*, 2001), and phosphodiesterase activities (Dell'Agli *et al.*, 2005). In addition, other inhibitory effects of polyphenols on platelet aggregation include scavenging of ROS such as superoxide radical anion (Frei and Higdon, 2003), and inhibition of lipid peroxidation (Aviram *et al.*, 2002). Luceri *et al.* (2007), using both *ex vivo* and *in vitro* studies, demonstrated that *p*-coumaric acid is a potent antioxidant with the properties of ADP-induced platelet aggregation inhibition, increase in plasma antioxidant activity, and the reduction of thromboxane B2 production. Furthermore, it was demonstrated that *p*-coumaric acid commonly found in cereals and legumes as well as other plant foods, is an antioxidative compound with antiplatelet activity at doses that can be obtained through dietary intervention.

Manach *et al.* (2005) reviewed a number of intervention studies and showed that several biomarkers of cardiovascular risk were influenced by the consumption of polyphenols-rich foods. However, the effects on biomarkers of oxidative stress, lipidemia, and inflammation are inconclusive, but comparatively more consistent results have been obtained on endothelial function and haemostasis. One of the arguments raised in this review was that almost all clinical studies have used foods or beverages containing a mixture of different polyphenols, thus the nature of the active compounds in the extract remained unclear. Moreover, absorption, metabolism, and elimination vary widely among polyphenols and might be affected by many other factors.



The results of the Insulin Resistance Atherosclerosis Study (IRAS) revealed that whole grain intake is inversely associated with carotid artery atherosclerosis (CCA) and carotid intimal medial thickness (IMT) (Mellen *et al.*, 2007). IMT is a noninvasive marker of systemic atherosclerosis, and IMT progression has been associated with subsequent cardiovascular events (Crouse III, 2001). In addition, considering several prospective cohort studies, Mellen *et al.* (2008) reported that a consistent, inverse association exists between the consumption of dietary whole grains and incidence of cardiovascular disease in epidemiological studies.

Lignans such as matairesinol (MAT), secoisolariciresinol (SECO), pinoresinol (PINO), and lariciresinol (LARI) are another group of compounds that have drawn the attention of researchers with potential antioxidant functionality. The major sources of dietary lignans in humans are cereals, in addition to fruits such as berries. Among legumes, soybean is a major source of lignans. Within cereals there is also considerable variation in lignan precursors, and rye is particularly high in lignans such as matairesinol (MAT) and secoisolariciresinol (SECO) that are converted to the active mammalian lignans enterolactone and enterodiol by the colonic microflora. In addition, lignans are present in flaxseeds and sesame seeds. Lignans are potent antioxidant molecules and have been shown to be protective against hormone-related cancers (Pietinen *et al.*, 2001; Pietinen and Kilkkinen, 2002; Hallmans *et al.*, 2003; Qu *et al.*, 2005). According to Saarinen *et al.* (2000) lignans do not bind to the oestrogen receptor *in vitro*, but enterolactone may inhibit human oestrogen synthetase by binding to the active site of the P450 enzyme (Adlercreutz *et al.*, 1993). It is possible that lignans may possess similar cardioprotective effects to those reported for other polyphenolic compounds, including reducing LDL-cholesterol oxidation, changing cholesterol synthesis and re-cycling, and altering the metabolism of triacylglycerols (Zern and Fernandez, 2005).

Two main mechanisms may possibly work to delay the process of atherosclerosis in the vascular wall by lignan intake. Intestinal bacteria convert plant lignans into enterolignans such as enterolactone and enterodiol (Heinonen *et al.*, 2001); enterolignans exhibit a weak estrogen-like activity through binding to estrogen receptors, which are also expressed in the vascular endothelium (Mousavi, 1992). In addition, they promote the synthesis of sex hormone-binding globulin and inhibit the activity of several enzymes such as aromatase and 5 alpha-reductase (Basly, 2005). The second mechanism is linked to the antioxidant activity of both lignans and, to a lesser extent, enterolignans (Niemeyer, 2003).

Men from the Kuopio Ischaemic Heart Disease Risk Factor Study in the highest quartile of serum enterolactone concentrations were found to have a 65% lower risk of acute coronary events than men from the lowest quartile (Vanharanta *et al.*, 1999). However, serum total cholesterol and LDL-cholesterol concentrations were no different between the quartiles. Recently, Pellegrini *et al.* (2010) showed that higher intakes of matairesinol (MAT) were associated with a lower vascular inflammation and endothelial dysfunction, which may have implications for CVD prevention.

Dietary phytochemicals account for a number of paradoxical variations in the incidence of CVD. The most popular cases are the French (Artaud-Wild *et al.*, 1993; Criqui and Ringler, 1994), the Maasai (John, 1996; Day *et al.*, 1976), and the Mediterranean (de Lorgeril *et al.*, 1996) paradoxes, among others.

In the French paradox, the relatively low rate of CVD among the French population compared to other industrialized European countries with similar high intakes of saturated fatty acids and comparable serum cholesterol, blood pressure, and prevalence of smoking, has been attributed to a lower consumption of whole milk and greater consumption of plant

foods and red wine (Artaud-Wild *et al.*, 1993). The protective effect of red wine has been ascribed to the presence of polyphenolics (John, 1996).

The occurrence of atherosclerosis and CVD among the Maasai pastoralists of East Africa is low despite their high intake of fat and cholesterol and low consumption of carbohydrates (Bliss *et al.*, 1971; Mann *et al.*, 1972). While Maasai obtain up to 66% of their energy from fat and mainly eat diets high in milk, yogurt and meat (Mann *et al.*, 1972), they suffer from low incidences of CVD. The satisfactory explanation is given for the underlying situation as the Maasai ethnobotany factor (Day *et al.*, 1976), the fact that the Maasai generally consume wild plant materials as foods, as ingredients of milk and meat-based soups, as masticants, and as herbal medicines (Johns *et al.*, 1994).

The Mediterranean paradox is the lower incidence of coronary heart disease among the southern European populations inhabiting the Mediterranean region, compared to the rest of Europe, despite comparable blood lipid profiles, blood pressure, saturated fat consumption, and smoking. The paradox has been attributed to the protective effects of the traditional Mediterranean diet (de Lorgeril *et al.*, 1996), which typically uses olive oil as the major culinary fat. Unrefined olive oil is rich in monosaturated fatty acids and contains a considerable amount of polyphenolic compounds, such as hydroxytyrosol and oleuropein (Patrik and Uzick, 2001). These polyphenolic compounds are responsible for the peculiar, pungent taste and high stability of extra-virgin oil (Visioli *et al.*, 2001). There is evidence that olive oil polyphenols are powerful antioxidants both *in vitro* and *in vivo* and exert other biological activities that could partially account for the observed healthful effects of the Mediterranean diet (Visioli *et al.*, 2001; Visioli and Galli, 2002).

## 19.6 Summary and conclusions

Antioxidants have been widely recognized to provide potential beneficial effects on human health based on the large body of *in vitro*, *ex vivo*, *in vivo*, and epidemiological studies. Their effectiveness in attenuating the major degenerative diseases has been of great interest to researchers, health professionals, and the general public. Consumption of antioxidants from natural sources in day to day life as a part of the diet may help reduce disease risk and promote health. Whole grain cereals and legumes provide important sources of natural antioxidants and their intake has been associated with a decreased incidence of many chronic diseases. More research on the effective doses, bioavailability, and toxic effects of antioxidants are needed for better understanding of their health effects.

## References

- Adamson, G.M. and Billings, R.E. (1992) Tumor necrosis factor induced oxidative stress in isolated mouse hepatocytes. *Archives of Biochemistry and Biophysics*, **294**: 223–229.
- Adlercreutz, H., Bannwart, C., Wahala, K., Makela, T., Brunow, G., Hase, T., Arosemena, P.J., Kellis, J.J.T., and Vickery, L.E. (1993) Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *Journal of Steroid Biochemistry and Molecular Biology*, **44**: 147–153.
- Afaq, F., Adhami, V.M., Ahmad, N., and Mukhtar, H. (2003) Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea Constituent (–)-epigallocatechin-3-gallate. *Oncogene*, **22**: 1035–1044.

- Agullo, G., Gamet-Payraastre, L., Fernandez, Y., Anciaux, N., Demigne, C., and Remesy, C. (1996) Comparative effects of flavonoids on the growth, viability and metabolism of a colonic adenocarcinoma cell line (HT29 cells). *Cancer Letters*, **105**: 61–70.
- Ahmed, S., Rahman, A., Hasnain, A., Lalonde, M., Goldberg, V.M. and Haqqi, T.M. (2002) Green tea polyphenol epigallocatechin-3-gallate inhibits the IL-1 beta-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes. *Free Radical Biology and Medicine*, **33**: 1097–1105.
- Akaza, H., Miyana, N., Takashima, N., Naito, S., Hirao, Y., Tsukamoto, T., Tomoaki, F., Mori, M., Kim, W., Song, J.M., and Pantuck, A.J. (2004) Comparisons of percent equol producers between prostate cancer patients and controls: Case controlled studies of isoflavones in Japanese, Korean and American residents. *Japanese Journal of Clinical Oncology*, **34**: 86–89.
- Albanes, D., Heinonen, O.P., Taylor, P.R., Virtamo, J., Edwards, B.K., Rautalahti, M., Hartman, A.M., Palmgren, J., Freedman, L.S., Haapakoski, J., Barrett, M.J., Pietinen, P., Malila, N., Tala, E., Liippo, K., Salomaa, E.R., Tangrea, J.A., Teppo, L., Askin, F.B., Taskinen, E., Erozan, Y., Greenwald, P., and Huttunen, J.K. (1996) Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer prevention study: Effects of base-line characteristics and study compliance. *Journal of the National Cancer Institute*, **88**: 1560–1570.
- Amarowicz, R., Karamac, M., and Weidner, S. (2000a) Antioxidant activity of phenolic fraction of pea (*Pisum sativum*). *Czech Journal of Food Sciences*, **19**: 139–142.
- Amarowicz, R., Naczka, M., Zadernowski, R., and Shahidi, F. (2000b) Antioxidant activity of condensed tannins of beach pea, canola hulls, evening primrose, and faba bean. *Journal of Food Lipids*, **7**: 195–205.
- Amarowicz, R., Troszy, A., Ko-Pikielna, S.B., and Shahidi, F. (2004) Polyphenolics extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids*, **11**: 278–286.
- Anderson, J.W., Hanna, T.J., Peng, X., and Kryscio, R.J. (2000) Whole grain foods and heart disease risk. *Journal of the American College of Nutrition*, **19** (suppl): 291S–299S.
- Anson, N.M., Havenaar, R., Bast, A., and Haenan, R.M.M. (2010) Antioxidant and anti-inflammatory capacity of bioaccessible compounds from wheat fractions after gastrointestinal digestion. *Journal of Cereal Science*, **51**: 110–114.
- Aquilano, K., Baldelli, S., Rutilio, G. and Ciriolo, M.R. (2008) Role of nitric oxide synthases in Parkinson's disease: A review on the antioxidant and anti-inflammatory activity of polyphenols. *Neurochemical Research*, **33**: 2416–2426.
- Arnlov, J., Zethelius, B., Risérus, U., Basu, S., Berne, C., Vessby, B., Alftan, G., and Helmersson, J. (2009) Serum and dietary beta-carotene and alpha-tocopherol and incidence of type 2 diabetes mellitus in a community-based study of Swedish men: Report from the Uppsala Longitudinal Study of Adult Men (ULSAM) study. *Diabetologia*, **52**: 97–105.
- Artaud-Wild, S.M., Connor, S.L., Sexton, G., and Connor, W.E. (1993) Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. *Circulation*, **88**: 2771–2779.
- Aviram, M., Dornfeld, L., Kaplan, M., Coleman, R., Gaitini, D., Nitecki, S., Hofman, A., Rosenblat, M., Volkova, N., Presser, D., Attias, J., Hayek, T., and Fuhrman, B. (2002) Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: Studies in atherosclerotic mice and in humans. *Drugs under Experimental and Clinical Research*, **28**: 49–62.
- Bahorun, T., Soobrattee M.A., Luximon-Ramma, V., and Aruoma, O.I. (2006) Free Radicals and Antioxidants in Cardiovascular Health and Disease. *International Journal of Medical Sciences, Update*, **1**: 25–41.
- Basly, J.P. and Lavier, M.C. (2005) Dietary phytoestrogens: Potential selective estrogen enzyme modulators? *Planta Medica*, **71**: 287–94.
- Baumann, J., von Bruchhausen, F., and Wurm, G. (1980) Flavonoids and related compounds as inhibition of arachidonic acid peroxidation. *Prostaglandins*, **20**: 627–639.
- Bazzano, L.A., He, J., Ogden, L.G., Loria, C., Vupputuri, S., Myers, L., and Whelton, P.K. (2001) Legume consumption and risk of coronary heart disease in US men and women: NHANES I Epidemiologic Follow-up Study. *Archives of Internal Medicine*, **161**: 2573–2578.
- Benavente-Garcia, O. and Castillo, J. (2008) Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. *Journal of Agricultural and Food Chemistry*, **56**: 6185–6205.
- Berger, M.M. (2005) Can oxidative damage be treated nutritionally? *Clinical Nutrition*, **24**: 172–183.

- Berliner, J.A. and Heinecke, J.W. (1996) The role of oxidized lipoproteins in atherogenesis. *Free Radical Biology and Medicine*, **20**: 707–727.
- Biesalski, H.K. (2007) Polyphenols and inflammation: Basic interactions. *Current Opinion in Clinical Nutrition and Metabolic Care*, **10**: 724–728.
- Birari, R.B. and Bhutani, K.K. (2007) Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discovery Today*, **12**: 879–889.
- Black, H.S. and Lambert, C.R. (2001) Radical reactions of carotenoids and potential influence on UV carcinogenesis, in *Oxidants and Antioxidants in Cutaneous Biology* (eds J.J. Thiele and P. Elsner). Basel: Karger, pp. 140–156.
- Bostick, R.M., Potter, J.D., McKenzie, D.R., Sellers, T.A., Kushi, L.H., Steinmetz, K.A., and Folsom, A.R. (1993) Reduced risk of colon cancer with high intake of vitamin E: The Iowa Women's Health Study. *Cancer Research*, **53**: 4230–4237.
- Brenneisen, P., Briviba, K., Wlaschek, M., Wenk, J., and Scharffetter-Kochanek, K. (1997) Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) increases the steady-state mRNA levels of collagenase/MMP-1 in human dermal fibroblasts. *Free Radical Biology and Medicine*, **22**: 515–524.
- Briones, A.M. and Touyz, R.M. (2010) Oxidative stress and hypertension: Current concepts. *Current Hypertension Reports*, **12**: 135–142.
- Brown, C.A., Bolton-Smith, C., Woodward, M., and Tunstall-Pedoe, H. (1993) Coffee and tea consumption and the prevalence of coronary heart disease in men and women: Results from the Scottish Heart Health Study. *Journal of Epidemiol Community Health*, **47**: 171–175.
- Brown, S.R., Cann, P.A., and Read, N.W. (1990) Effect of coffee on distal colon function. *Gut* **31**: 450–453.
- Calabro, P. and Yeh, E.T.H. (2007) Obesity, inflammation and vascular disease, in *Inflammation in the Pathogenesis of Chronic Diseases* (ed. R.E. Harris), Springer, New York, NY, pp. 63–91.
- Cardador-Martinez, A., Loarca-Pina, G., and Oomah, B.D. (2002) Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricultural Food Chemistry*, **50**: 6975–6980.
- Chan, A.T., Giovannucci, E.L., Meyerhardt, J.A., Schernhammer, E.S., Curhan, G.C., and Fuchs, C.S. (2005) Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. *Journal of the American Medical Association*, **294**: 914–923.
- Chandrasekara, A. and Shahidi, F. (2010) The content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry*, **58**: 6706–6714.
- Chatenoud, L., Tavani, A., L.A., Vecchia, C., Jacobs, D.R., Negri, E., Levi, F., and Franceschi, S. (1998) Whole grain food intake and cancer risk. *International Journal of Cancer*, **77**: 24–28.
- Chen, C.C., Liu, L.K., Hsu, J.D., Huang, H.P., Yang, M.Y. and Wang, C.J. (2005) Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food Chemistry*, **91**: 601–607.
- Cho, H., Yun, C.W., Park, W.K., Kong, J.Y., Kim, K.S., Park, Y., Lee, S. and Kim, B.K. (2004) Modulation of the activity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. *Pharmacological Research*, **49**: 37–43.
- Cho, Y.S., Jang, E.M., Jang, S.M., Chun, M.S., Shon, M.Y., Kim, M.J. and Lee, M.K. (2007) Effect of grape seed water extract on lipid metabolism and erythrocyte antioxidant defense system in high-fat diet-induced obese C57BL/6 mice. *Journal of the Korean Society of Food Science and Nutrition*, **36**: 1537–1543.
- Chou, F.P., Chu, Y.C., Hsu, J.D., Chiang, H.C., and Wang, C.J. (2000) Specific induction of glutathione S-transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. *Biochemical Pharmacology*, **60**: 643–650.
- Choung, M.G., Choi, B.R., An, Y.N., Chu, Y.H., and Cho, Y.S. (2003) Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). *Journal of Agricultural Food Chemistry*, **51**: 7040–7043.
- Christa, K. and Soral-Smietana, M. (2008) Buckwheat grains and buckwheat products – nutritional and prophylactic value of their components – a review. *Czech Journal of Food Sciences*, **26**: 153–162.
- Conklin, K.A. (2000) Dietary Antioxidants During Cancer Chemotherapy: Impact on Chemotherapeutic Effectiveness and Development of Side Effects. *Nutrition and Cancer*, **37**: 1–18.
- Cordova, A.C., Jackson, L.S., Berke-Schlessel, D.W., and Sumpio, B.E. (2005) The cardiovascular protective effect of red wine. *Journal of the American College of Surgeons*, **200**: 428–439.
- Costacou, T., Ma, B., King, I.B. and Mayer-Davis, E.J. (2008) Plasma and dietary vitamin E in relation to insulin secretion and sensitivity. *Diabetes, Obesity and Metabolism*, **10**: 223–228.
- Criqui, M.H. and Ringler, B.L. (1994) Does diet or alcohol explain the French paradox? *Lancet*, **344**: 1719–1723.

- Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., and Garg, R. (2005) Metabolic syndrome: A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation*, **111**: 1448–1454.
- Day, J., Carruthers, M., Bailey, A., and Robinson, D. (1976) Anthropometric, physiological and biochemical differences between urban and rural Maasai. *Atherosclerosis*, **23**: 357–361.
- De Graaf, C., Blom, W.A., Smeets, P.A., Stafleu, A., and Hendriks, H.F. (2004) Biomarkers of satiation and satiety. *American Journal of Clinical Nutrition*, **79**: 946–961.
- De Lorgeril, M., Salen, P., Martin, J.L., Mamelle, N., Monjaud, I., Touboul, P., and Delaye, J. (1996) Effect of a Mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. *Insights into the cardioprotective effects of certain nutriments. Journal of the American College of Cardiology*, **28**: 1103–1108.
- Dell'Agli, M., Galli, G.V., Vrhovsek, U., Mattivi, F., and Bosisio, E. (2005) *In vitro* inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes. *Journal of Agricultural Food Chemistry*, **53**: 1960–1965.
- Dembinska-Kiec, A., Mykkänen, O., Kiec-Wilk, B., and Mykkänen, H. (2008) Antioxidant phytochemicals against type 2 diabetes. *British Journal of Nutrition*, **99**: ES109–117.
- Demrow, H.S., Slane, P.R., and Folts, J.D. (1995) Administration of wine and grape juice inhibits *in vivo* platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation*, **91**: 1182–1188.
- Diaz, M.N., Frei, B., Vita, J.A., and Keaney, J.F. Jr. (1997) Antioxidants and atherosclerotic heart disease. *New England Journal of Medicine*, **337**: 408–416.
- Dixon, R.A. (1999) Isoflavonoids: Biochemistry, molecular biology and biological functions, in *Comprehensive Natural Products Chemistry*, vol 1. (ed. U. Sankawa), Elsevier, Oxford, pp. 773–823.
- Dixon, R.A., and Ferreira, D. (2002) Molecules of interest: Genistein. *Phytochemistry*, **60**: 205–211.
- Dong, M., He, X., Liu, R.H. (2007) Phytochemicals of black bean seed coats: Isolation, structure elucidation and their antiproliferative and antioxidative activities. *Journal of Agricultural Food Chemistry*, **55**: 6044–6051.
- Duenas, M., Hernandez, T., and Estrella, I. (2006) Assessment of *in vitro* antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, **98**: 95–103.
- Duffy, S.J., Keaney, J.F. Jr, Holbrook, M., Gokce, N., Swerdloff, P.L., Frei, B., and Vita, J.A. (2001) Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation*, **104**: 151–156.
- Dukworth, W.C. (2001) Hyperglycemia and cardiovascular disease. *Current Atherosclerosis Reports*, **3**: 383–391.
- Dulloo, A.G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., Chantre, P., and Vandermander, J. (1999) Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *American Journal of Clinical Nutrition*, **70**: 1040–1045.
- Esterbauer, H., Puhl, H., Dieber-Rotheneder, M., Waeg, G., and Rabl, H. (1991) Effect of antioxidants on oxidative modification of LDL. *Annals of Medicine*, **23**: 573–581.
- Fardet, A., Rock, E., and Révész, C. (2008) Is the *in vitro* antioxidant potential of whole-grain cereals and cereal products well reflected *in vivo*? *Journal of Cereal Science*, **48**: 258–276.
- Feinstein, R., Kanety, H., Papa, M.Z., Lunenfeld, B., and Karasik, A. (1993) Tumor necrosis factor- $\alpha$  suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *Journal of Biological Chemistry*, **268**: 26055–26068.
- Feng, R., Lu, Y., Bowman, L.L., Qian, Y., Castranova, V., and Ding, M. (2005) Inhibition of activator protein-1, NF- $\kappa$ B, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. *Journal of Biological Chemistry*, **280**: 27888–27895.
- Ferroni, P., Basili, S., Falco, A., and Dari, G. (2004) Inflammation, insulin resistance, and obesity. *Current Atherosclerosis Reports*, **6**: 424–431.
- Finley, J.W. (2004) Phenolic antioxidants and prevention of chronic inflammation. *Food Technology*, **58**: 42–46.
- Fiocchi, C. (1998) Inflammatory bowel disease: Etiology and pathogenesis. *Gastroenterology*, **115**: 182–205.
- Fitzpatrick, F.A. and Soberman, R. (2001) Regulated formation of eicosanoids. *Journal of Clinical Investigation*, **107**: 1347–1351.
- Fraser, G.E. (1994) Diet and coronary heart disease: Beyond dietary fats and low-density lipoprotein cholesterol. *American Journal of Clinical Nutrition*, **59**: S1117–S1123.

- Frears, E.R., Zhang, Z., Blake, D.R., O'Connell, J.P., and Winyard, P.G. (1996) Inactivation of tissue inhibitor of metalloproteinase-1 by peroxynitrite. *FEBS Letters*, **381**: 21–24.
- Freedman, J.E., Parker, C., III, Li, L., Jacob, A., Frei, P.B., Ivanov, V., Deak, L.R., Iafrazi, M.D., and Folts, J.D., (2001) Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric. *Circulation*, **103**: 2792–2798.
- Frei, B. and Higdon, J.V. (2003) Antioxidant activity of tea polyphenols in vivo: Evidence from animal studies. *Journal of Nutrition*, **133**: 3275S–3284S.
- Friling, R.S., Bensimon, A., Tichauer, Y., and Daniel, V. (1990) Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. *Proceedings of the National Academy of Sciences of the United States of America*, **87**: 6258–6262.
- Geleijnse, J.M., Launer, L.J., Van der Kuip, D.A., Hofman, A., and Witteman, J.C. (2002) Inverse association of tea and flavonoid intakes with incident myocardial infarction: *The Rotterdam Study*. *American Journal of Clinical Nutrition*, **75**: 880–886.
- Gey, K.F., Moser, U.K., Jordan, P., Stahelin, H.B., Eichholzer, M., and Ludin, E. (1993) Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: An epidemiological update with special attention to carotene and vitamin C. *American Journal of Clinical Nutrition*, **57** (suppl): 787S–797S.
- Gey, K.F., and Puska, P. (1989) Plasma vitamins E and A inversely related to mortality from ischemic heart disease in cross-cultural epidemiology. *Annals of the New York Academy of Sciences*, **570**: 268–282.
- Gey, K.F., Stahelin, H.B., Puska, P., and Evans, A. (1987) Relationship of plasma vitamin C to mortality from ischemic heart disease. *Annals of the New York Academy of Sciences*, **498**: 110–123.
- Giovannucci, E. (1998) Meta-analysis of coffee consumption and risk of colorectal cancer. *American Journal of Epidemiology*, **147**: 1043–1052.
- Gnoni, G.V., Paglialonga, G., and Siculella, L. (2009) Quercetin inhibits fatty acid and triacylglycerol synthesis in rat-liver cells. *European Journal of Clinical Investigation*, **39**: 761–768.
- Gokce, N. and Vita, J.A. (2002) Clinical manifestations of endothelial dysfunction, in: *Thrombosis and Hemorrhage* (eds J. Loscalzo and A.I. Schafer), Lippincott Williams & Wilkins, Philadelphia, pp. 685–706.
- Gonzales, A.M. and Orlando, R.A. (2008) Curcumin and resveratrol inhibit nuclear factor-KappaB-mediated cytokine expression in adipocytes. *Nutrition and Metabolism*, **5**: 17–29.
- Gonzalez de Mejia, E., Castano-Tostado, E., and Loarca-Pina, G. (1999) Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis*, **441**: 1–9.
- Graf, E. and Eaton, J.W. (1990) Antioxidant functions of phytic acid. *Free Radical Biology and Medicine*, **8**: 61–69.
- Grattagliano, I., Palmieri, V.O., Portincasa, P., Moschetta, A., and Palasciano, G. (2008) Oxidative stress-induced risk factors associated with the metabolic syndrome: A unifying hypothesis. *Journal of Nutritional Biochemistry*, **19**: 491–504.
- Greenberg, E.R. and Sporn, M.B. (1996) Antioxidant vitamins, cancer and cardiovascular diseases. *The New England Journal of Medicine*, **330**: 1029–1035.
- Guo, W., Wise, M.L., Collins, F.W., and Meydani, M. (2008) Avenanthramides, polyphenols from oats, inhibit IL-1 beta-induced NF- $\kappa$ B activation in endothelial cells. *Free Radical Biology and Medicine*, **44**: 415–429.
- Guri, A.J., Hontecillas, R., Si, H., Liu, D., and Bassaganya-Riera, J. (2007) Dietary abscisic acid ameliorates glucose tolerance and obesity-related inflammation in db/db mice fed high-fat diets. *Clinical Nutrition*, **26**: 107–116.
- Hallmans, G., Zhang, J.X., Lundin, E., Stattin, P., Johansson, A., Johansson, I., Hulten, K., Winkvist, A., Lenner, P., Aman, P., and Adlercreutz, H. (2003) Rye, lignans and human health. *Proceedings of the Nutrition Society*, **62**: 193–199.
- Hanif, K., Bid, H.K., and Konwar, R. (2010) Reinventing the ACE inhibitors: Some old and new implications of ACE inhibition. *Hypertension Research*, **33**: 11–21.
- Hara, H., Gee, J.M., and Johnson, I.T. (1999) Antiproliferative effects of quercetin in the rat gastrointestinal tract, in *Antiproliferative Effects of Quercetin in the Rat Gastrointestinal Tract*, (eds A. Kroghdahl, S.D. Mathieson, and I.F. Pryme), European Commission, Brussels, pp. 49–53.
- Harrison, D.G. and Gongora, M.C. (2009) Oxidative stress and hypertension. *Medical Clinics of North America*, **93**: 621–635.

- Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Nishibe, S., and Adlcreutz, H. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine*, **334**: 1145–1149.
- Hertog, M.G., Sweetnam, P.M., Fehily, A.M., Elwood, P.C., and Kromhout, D. (1997) Antioxidant flavonols and ischemic heart disease in a Welsh population of men: The Caerphilly Study. *American Journal of Clinical Nutrition*, **65**: 1489–1494.
- Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Martijn, B., and Kromhout, D. (1993) Dietary antioxidant flavonols and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet*, **342**: 1007–1011.
- Higdon, J.V. and Frei, B. (2006) Coffee and health: A review of recent human research. *Critical Reviews in Food Science and Nutrition*, **46**: 101–123.
- Hong, J., Smith, T.J., Ho, C.T., August, D.A., and Yang, C.S. (2001) Effects of purified green and black tea polyphenols on cyclooxygenase and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues. *Biochemical Pharmacol*, **62**: 1175–1183.
- HOPE and HOPE-TOO trial investigators (2005) Effects of Long-term Vitamin E Supplementation on Cardiovascular Events and Cancer: A Randomized Controlled Trial. *Journal of the American Medical Association*, **293**: 1338–1347.
- Hotamisligil, G.S. (2003) Inflammatory pathways and insulin action. *International Journal of Obesity and Related Metabolic Disorders*, **27**(S3): S53–55.
- Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993) Adipose expression of tumor necrosis factor- $\alpha$ : Direct role in obesity-linked insulin resistance. *Science*, **259**: 87–91.
- Hou, D.X., Luo, D., Tanigawa, S., Hashimoto, F., Uto, T., Masuzaki, S., Fujii, M., and Sakata, Y. (2007a) Prodelphinidin B-4 3'-O-gallate, a tea polyphenol, is involved in the inhibition of COX-2 and iNOS via the downregulation of TAK1-NF- $\kappa$ B pathway. *Biochemical Pharmacology*, **74**: 742–751.
- Hou, D.X., Masuzaki, S., Hashimoto, F., Uto, T., Tanigawa, S., Fujii, M., and Sakata, Y. (2007b) Green tea proanthocyanidins inhibit cyclooxygenase-2 expression in LPS-activated mouse macrophages: Molecular mechanisms and structure – activity relationship. *Archives of Biochemistry and Biophysics*, **460**: 67–74.
- Hsu, F.L., Chen, Y.C., and Cheng, J.T. (2000) Caffeic acid as active principle from the fruit of xanthium strumarium to lower plasma glucose in diabetic rats. *Planta Medica*, **66**: 228–230.
- Hu, C., Zawistowski, J., Ling, W., and Kitts, D.D. (2003) Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *Journal of Agricultural and Food Chemistry*, **51**: 5271–5277.
- Huang, A., Vita, J.A., Venema, R.C., and Keaney, J.F. Jr. (2000) Ascorbic acid enhances endothelial nitric oxide synthase activity by increasing intracellular tetrahydrobiopterin. *Journal of Biological Chemistry*, **275**: 17399–17406.
- Huang, C.H., Tsai, S.J., Wang, Y.J., Pan, M.H., Kao, J.Y., and Way, T.D. (2009) EGCG inhibits protein synthesis, lipogenesis, and cell cycle progression through activation of AMPK in p53 positive and negative human hepatoma cells. *Molecular Nutrition and Food Research*, **53**: 1156–1165.
- Hyun, J.W. and Chung, H.S. (2004) Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. *Journal of Agricultural Food Chemistry*, **52**: 2213–2217.
- Ichikawa, H., Ichiyanagi, T., Xu, B., Yoshii, Y., and Nakajima, M. (2001) Antioxidant activity of anthocyanin extract from purple black rice. *Journal of Medicinal Food*, **4**: 211–218.
- Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E., Chaudhuri, G. (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America*, **84**: 9265–9269.
- Jacobs, E.J., Henion, A.K., Briggs, P.J., Connell, C.J., McCullough, M.L., Jonas, K.R., Rodriguez, C., E. Calle, E.E., and Thun, M.J. (2002) Vitamin C and vitamin E supplement use and bladder cancer mortality in a large cohort of US men and women. *American Journal of Epidemiology*, **156**: 1002–1010.
- Jacobs, Jr. D.J., Meyer, K.A., Kushi, L.H., and Folsom, A.R. (1998) Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: The Iowa Women's Health Study. *American Journal of Clinical Nutrition*, **68**: 248–257.
- Jaiswal, A.S., Marlow, B.P., Gupta, N., and Narayan, S. (2002)  $\beta$ -catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene*, **21**: 8414–8427.
- Janssen-Heininger, Y.M.W., Macara, I., and Mossman, B.T. (1999) Cooperativity between oxidants and tumor necrosis factor in the activation of new clear factor (NF)- $\kappa$ B. Requirement of RAS/mitogen-activated

- protein kinases in the activation of NF- $\kappa$ B by oxidants. *American Journal of Respiratory Cell and Molecular Biology*, **20**: 942–952.
- Jialal, I., Norkus, E.P., Cristol, L., Grundy, S.M. (1991) Beta-Carotene inhibits the oxidative modification of low-density lipoprotein. *Biochimica et Biophysica Acta*, **1086**: 134–138.
- Joe, A.K., Liu, H., Suzui, M., Vural, M.E., Xiao, D., and Weinstein, I.B. (2002) Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. *Clinical Cancer Research*, **8**: 893–903.
- Johns, T. (1996) Phytochemicals as evolutionary mediators of human nutritional physiology. *International Journal of Pharmacognosy*, **34**: 327–334.
- Johns, T., Mhoro, E.B., Sanaya, P., and Kimanani, E.K. (1994) Herbal remedies of the Batemi of Ngorongoro District, Tanzania: A quantitative appraisal. *Economic Botany*, **48**: 90–95.
- Jorgensen, K. and Skibsted, L.H. (1993) Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z Lebensm Unters Forsch*, **196**: 423–429.
- Jung, E.H., Kim, S.R., Hwang, I.K., and Ha, T.Y. (2007) Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. *Journal of Agricultural and Food Chemistry*, **55**: 9800–9804.
- Kaneto, H., Nakatani, Y., Kawamori, D., Miyasuka, T., Matsuoka, T.A., Matsuhisa, M., and Yamasaki, Y. (2006) Role of oxidative stress, endoplasmic reticulum stress, and c-Jun N-terminal kinase in pancreatic beta-cell dysfunction and insulin resistance. *International Journal of Biochemistry and Cell Biology*, **38**: 782–793.
- Kang, J.H., Kim, C.S., Han, I.S., Kawada, T., and Yu, R. (2007) Capsaicin, a spicy component of hot peppers, modulates adipokine gene expression and protein release from obese-mouse adipose tissues and isolated adipocytes, and suppresses the inflammatory responses of adipose tissue macrophages. *FEBS letters*, **581**: 4389–4396.
- Katiyar, S.K., Matsui, M.S., Elmets, C.A., and Mukhtar, H. (1999) Polyphenolic antioxidant (–)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. *Photochemistry and Photobiology*, **69**: 148–153.
- Keinana-Boker, L., van Der Schouw, Y.T., Grobbee, D.E., and Peeters, P.H.M. (2004) *American Journal of Clinical Nutrition*, **79**: 282–288.
- Keli, S.O., Hertog, M.G.L., Feskens, E.J.M., and Kromhout, D. (1996) Dietary flavanoids, antioxidant vitamins, and incidence of stroke: The Zutphen study. *Archives of Internal Medicine*, **156**: 637–42.
- Kempuraj, D., Madhappan, B., Christodoulou, S., Boucher, W., Cao, J., Papadopoulos, N., Cetrulo, C.L., and Theoharides, T.C. (2005) Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *British Journal of Pharmacology*, **145**: 934–944.
- Kim, H.P., Son, K.H., Chang, H.W., and Kang, S.S. (2004) Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Science*, **96**: 229–245.
- Kim, M.J., Kim, D.H., Na, H.K., Oh, T.Y., Shin, C.Y., Surh D.P.Y. J. (2005) Eupatilin, a pharmacologically active flavone derived from Artemisia plants, induces apoptosis in human gastric cancer (AGS) cells. *Journal of Environmental Pathology, Toxicology and Oncology*, **24**: 261–269.
- Kim, S.J., Jeong, H.J., Lee, K.M., Myung, N.Y., An, N.H., Mo Yang, W., Kyu Park, S., Lee, H.J., Hong, S.H., Kim, H.M., and Um, J.Y. (2007) Epigallocatechin-3-gallate suppresses NF- $\kappa$ B activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373 MG cells. *Journal of Nutritional Biochemistry*, **32**: 1720–1725.
- Knekt, P., Jarvinen, R., Reunanen, A., and Maatela, J. (1996) Flavonoid intake and coronary mortality in Finland: A cohort study. *British Medical Journal*, **312**: 478–81.
- Komatsu, T., Nakamori, M., Komatsu, K., Hosoda, K., Okamura, M., Toyama, K., Ishikura, Y., Sakai, T., Kunii, D., and Yamamoto, S. (2003) Oolong tea increases energy metabolism in Japanese females. *Journal of Medical Investigation*, **50**: 170–175.
- Kono, Y., Shibata, H., Kodama, Y., and Sawa, Y. (1995) The suppression of the nitrosating reaction by chlorogenic acid. *Biochemistry Journal*, **312**: 947–953.
- Krinsky, N.I. (1989) Antioxidant functions of carotenoids. *Free Radical Biology and Medicine*, **7**: 617–635.
- Krinsky, N.I. (1991) Effects of carotenoids in cellular and animal systems. *American Journal of Clinical Nutrition*, **53** (suppl): 238S–46S.
- Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Amy E. Griel, A.E., Terry D., and Etherton, T.D. (2002) Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. *American Journal of Medicine*, **113**: 71 s–88 s.



- Kroon, P.A., Faulds, C.B., Ryden, P., Robertson, J.A., and Williamson, G. (1997) Release of covalently bound ferulic acid from fiber in the human colon. *Journal of Agricultural and Food Chemistry*, **45**: 661–667.
- Kruidenier, L. and Verspaget, H.W. (2002) Review article: Oxidative stress as a pathogenic factor in inflammatory bowel disease – radicals or ridiculous? *Alimentary Pharmacology and Therapeutics*, **16**: 1997–2015.
- Kundu, J.K. and Surh, Y.J. (2009) Molecular basis of chemoprevention with dietary phytochemicals: Redox-regulated transcription factors as relevant targets. *Phytochemistry Reviews*, **8**: 333–347.
- Kurahashi, N., Iwasaki, M., Sasazuki, S., Otani, T., Inoue, M., Tsugane, S., and The Japan Public Health Center-based prospective study group. (2007) Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. *Cancer Epidemiology, Biomarkers and Prevention*, **16**: 538–544.
- Ladas, E.J., Jacobson, J.S., Kennedy, D.D., Teel, K., Fleischauer, A., and Kelly, M. (2004) Antioxidants and Cancer Therapy: A Systematic Review. *Journal of Clinical Oncology*, **22**: 517–528.
- LaVecchia, C. and Tavani, A. (2007) Coffee and cancer risk: An update. *European Journal of Cancer Prevention*, **16**: 385–389.
- Lee I.M., Cook N.R., and Manson, J.E. (1999) Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. *Journal of the National Cancer Institute*, **91**: 2102–2106.
- Lee S.H., Park H.J., Chun H.K., Cho S.Y., Jung H.J., Cho S.M., Kim, D.Y., Kang, M.S., and Lillehoj, H.S. (2007) Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet. *Nutrition Research*, **27**: 505–510.
- Lee, I., Cook, N.R., Gaziano, J.M., Gordon, D., Ridker, P.M., Manson, J.E., Hennekens, C.H., and Buring, J.E. (2005) Vitamin E in the primary prevention of cardiovascular disease and cancer. *Journal of the American Medical Association*, **294**: 56–65.
- Lee, J.S., Yoo, M.A., Koo, S.H., Baek, H.H., and Lee, H.G. (2008) Antioxidant and ACE inhibitory activities of soybean hydrolysates: Effect of enzyme and degree of hydrolysis. *Food Science and Biotechnology*, **17**: 873–877.
- Lee, K.J., Inoue, M., Otani, T., Iwasaki, M., Sasazuki, S., and Tsugane, S. (2007) Coffee consumption and risk of colorectal cancer in a population-based prospective cohort of Japanese men and women. *International Journal of Cancer*, **121**: 1312–1318.
- Lee, M.M., Gomez, S.L., Chang, J.S., Wey, M., Wang, R., and Hsing, A.W. (2003) Soy and isoflavone consumption in relation to Prostate cancer risk in China. *Cancer Epidemiology, Biomarkers and Prevention*, **12**: 665–668.
- Li, J.Y., Taylor, P.R., Li, B., Dawsey, S., Wang, G.Q., Ershow, A.G., Guo, W., Liu, S.F., and Yang, C.S. (1993) Nutrition Intervention Trials in Linxian, China: Multiple Vitamin/Mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *Journal of the National Cancer Institute*, **85**: 1492–1498.
- Li, Y., Ahmed, F., Ali, S., Philp, P.A., Kucuk, O., and Sarkar, F.H. (2005) Inactivation of Nuclear factor  $\kappa$ B by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. *Cancer Research*, **65**: 6934–6940.
- Liang, Y.C., Huang, Y.T., Tsai, S.H., Lin-Shiau, S.Y., Chen, C.F., and Lin, J.K. (1999) Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis*, **20**: 1945–1952.
- Libby, P. (1995) Molecular basis of the acute coronary syndromes. *Circulation*, **91**: 2844–2850.
- Lichtenstein, A.H. (1998) Soy protein isoflavones and cardiovascular disease risk. *Journal of Nutrition*, **128**: 1589–1592.
- Ling, W.H., Cheng, Q.X., Ma, J., and Wang, T. (2001) Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *Journal of Nutrition*, **131**: 1421–1426.
- Liu, S., Sesso, H.D., Manson, J.E., Willet, W.C., and Buring, J.E. (2003) Is intake of breakfast cereals related to total and cause-specific mortality in men? *American Journal of Clinical Nutrition*, **77**: 594–599.
- Liyana-Pathirana, C. and Shahidi, F. (2005) Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry*, **53**: 2433–2440.
- Liyana-Pathirana, C. and Shahidi, F. (2006) Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry*, **54**: 1256–1264.
- Lorenz, P., Roychowdhury, S., Engelmann, M., Wolf, G., and Horn, T.F.W. (2003) Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: Effect on nitrosative and oxidative stress derived from microglial cells. *Nitric Oxide*, **9**: 64–76.

- Luceri, C., Giannini, L., Lodovici, M., Antonucci, E., Abbate, R., Masini, E., and Dolara, P. (2007) p-Coumaric acid, a common dietary phenol, inhibits platelet activity in vitro and in vivo. *British Journal of Nutrition*, **97**: 458–463.
- Luceri, C., Guglielmi, F., Lodovici, M., Giannini, L., Messerini, L. and Dolara, P. (2004) Plant phenolic 4-coumaric acid protects against intestinal inflammation in rats. *Scandinavian Journal of Gastroenterology*, **39**: 1128–1133.
- Madhujith, T. and Shahidi, F. (2005) Antioxidant potential of pea beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, **70**: 80–90.
- Madhujith, T. and Shahidi, F. (2009) Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry*, **117**: 615–620.
- Madhujith, T., Naczki, M., and Shahidi, F. (2004) Antioxidant activity of common beans (*Phaseolus vulgaris* L.). *Journal of Food Lipids*, **11**: 220–233.
- Manach, C., Mazur, A., and Scalbert, A. (2005) Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology*, **16**: 77–84.
- Mann, G., Spoerri, A., Gray, M., and Jarashow, D. (1972) Atherosclerosis in the Masai. *American Journal of Epidemiology*, **95**: 26–37.
- Mann, J.R., Backlund, M.G., and DuBois, R.N. (2005) Mechanisms of disease: Inflammatory mediators and cancer prevention. *Nature Clinical Practice Oncology*, **2**: 202–210.
- Manthey, J. A., Guthrie, N., and Grhmann, K. (2001) Biological properties of Citrus flavonoids pertaining to cancer and inflammation. *Current Medicinal Chemistry*, **8**: 135–153.
- Markku, L. (1995) Epidemiology of diabetes dyslipidemia. *Diabetic Review*, **3**: 408–422.
- Márquez, N., Sancho, R., Macho, A., Calzado, M.A., Fiebich, B.L., and Munoz, E. (2004) Caffeic acid phenethyl ester inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF- $\kappa$ B transcription factors. *Journal of Pharmacology and Experimental Therapeutics*, **308**: 993–1001.
- Master, R.C., Liese, A.D., Haffner, S.M., Wagenknecht, L.E., and Hanley, A.J. (2010) Whole and refined grain intakes are related to inflammatory protein concentrations in human plasma. *Journal of Nutrition*, **140**: 587–594.
- Mathews-Roth, M.M. (1982) Antitumor activity of  $\beta$ -carotene, canthaxanthin and phytoene. *Oncology*, **39**: 33–37.
- Mellen, P.B., Liese, A.D., Toozé, J.A., Vitolins, M.Z., Wagenknecht L.E., and Herrington, D.M. (2007) Whole-grain intake and carotid artery atherosclerosis in a multiethnic cohort: The Insulin Resistance Atherosclerosis Study. *American Journal of Clinical Nutrition*, **85**: 1495–1502.
- Michels, K.B., Willett, W.C., Fuchs, C.S., and Giovannucci, E. (2005) Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. *Journal of the National Cancer Institute*, **97**: 282–292.
- Miles, E.A., Zoubouli, P., and Calder, P.C. (2005) Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. *Nutrition*, **21**: 389–394.
- Minhajuddina, M., Begb, Z., and Iqbal, J. (2005) Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food and Chemical Toxicology*, **43**: 747–753.
- Mohamed-Ali, V., Pinkney, J.H., and Coppack, S.W. (1998) Adipose tissue as an endocrine and papracrine organ. *International Journal of Obesity and Related Metabolic Disorders*, **22**: 1145–1158.
- Molan, A.L., Lila, M.A., and Mawson, J. (2008) Satiety in rats following blueberry extract consumption induced by appetite-suppressing mechanisms unrelated to in vitro or in vivo antioxidant capacity. *Food Chemistry*, **107**: 1039–1044.
- Moncada, S. (1999) Nitric oxide: Discovery and impact on clinical medicine. *Journal of the Royal Society of Medicine*, **92**: 164–169.
- Mousavi, Y., and Adlercreutz, H. (1992) Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *Journal of Steroid Biochemistry and Molecular Biology*, **41**: 615–619.
- Mukamal, K.J., Maclure, M., Muller, J.E., Sherwood, J.B., and Mittleman, M.A. (2002) Tea consumption and mortality after acute myocardial infarction. *Circulation*, **105**: 2476–2481.
- Natarajan, K., Singh, S., Burke, T.R. Jr, Grunberger, D., and Aggarwal, B.B. (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF- $\kappa$ B. *Proceedings of the National Academy of Sciences of the United States of America*, **93**: 9090–9095.
- Newairy, A.S. and Abdou, H.M. (2009) Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. *Food Chemistry and Toxicology*, **47**: 813–818.

- Niemeyer, H.B., and Metzler, M. (2003) Differences in the antioxidant activity of plant and mammalian lignans. *Journal of Food Engineering*, **56**: 255–266.
- Novelli, M., Bonamassa, B., Masini, M., Funel, N., Canistro, D., De Tata, V., Martano, M., Soleti, A., Campani, D., Paolini, M., and Masiello, P. (2010) Persistent correction of hyperglycemia in streptozotocin-nicotinamide-induced diabetic mice by a non-conventional radical scavenger. *Naunyn-Schmiedeberg's Archives of Pharmacology*, DOI 10.1007/s00210-010-0524-7.
- Omenn, G.S., Goodman, G., Thomquist, M., Grizzle, J., Rosenstock, L., Barnhart, S., Balmes, J., Cherniack, M.G., Cullen, M.R., Glass, A., Keogh, J., Meyskens, F. Jr., Valanis, B., and Williams, J. Jr. (1994) The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: Smokers and asbestos-exposed workers. *Cancer Research*, **54**: 2038s–2043s.
- Onozawa, M., Fukuda, K., Ohtani, M., Akaza, H., Sugimura, T., and Wakabayashi, K. (1998) *Japanese Journal of Clinical Oncology*, **28**: 360–363.
- Osganian, S.K., Stampfer, M. J., Rimm, E., Spiegelman, D., Hu, F.B., Manson, J.E., and Willett, W.C. (2003) Vitamin C and risk of coronary heart disease in women. *Journal of the American College of Cardiology*, **42**: 246–252.
- Pan, M.H., Lai, C.S., Dushenkov, S., and Ho, C.T. (2009a) Modulation of inflammatory genes by natural dietary Bioactive compounds. *Journal of Agricultural and Food Chemistry*, **57**: 4467–4477.
- Pan, M.H., Yang, J.R., Tsai, M.L., Sang, S., and Ho, C.T. (2009b) Anti-inflammatory effect of Momordica grosvenori Swingle extract through suppressed LPS-induced upregulation of iNOS and COX-2 in murine macrophages. *Journal of Functional Foods*, **1**: 145–152.
- Park, K.G., Min, A.K., Koh, E.H., Kim, H.S., Kim, M.O., Park, H.S., Kim, Y.D., Yoon, T.S., Jang, B.K., Hwang, J.S., Kim, J.B., Choi, H.S., Park, J.Y., Lee, I.K., and Lee, K.U. (2008) Alpha-lipoic acid decreases hepatic lipogenesis through adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK independent pathways. *Hepatology*, **48**: 1477–1486.
- Parka, H.J., Davisa, S.R., Lianga, H., Rosenbergb, D.W., and Brunoa, R.S. (2010) Chlorogenic acid differentially Alters Hepatic and Small Intestinal Thiol Redox Status Without Protecting Against Azoxymethane-Induced Colon Carcinogenesis in Mice. *Nutrition Cancer*, **62**: 362–370.
- Patrick, L. and Uzick, M. (2001) Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the Literature. *Alternative Medicine Review*, **6**: 248–271.
- Patrignani, P., Tacconelli, S., Sciulli, M.G., and Capone, M.L. (2005) New insights into COX-2 biology and inhibition. *Brain Research Reviews*, **48**: 352–359.
- Pellegrini, N., Valtuen, S., Ardigo, D., Brighenti, F., Franzini, L., Del Rio, D., Scazzina, F., Piatti, P. M., and Zavaroni, I. (2010) Intake of the plant lignans matairesinol, secoisolariciresinol, pinoresinol, and lariciresinol in relation to vascular inflammation and endothelial dysfunction in middle age-elderly men and postmenopausal women living in Northern Italy. *Nutrition, Metabolism, and Cardiovascular Diseases*, **20**: 64–71.
- Peters, U., Foster, C.B., Chatterjee, N., Schatzkin, A., Reding, D., Andriole, G.L., Crawford, D., Sturup, S., Chanock, S.J., and Hayes, R.B. (2007) Serum selenium and risk of prostate cancer – a nested case-control study. *American Journal of Clinical Nutrition*, **85**: 209–217.
- Peterson, g. and Barnes, S. (1991) Genistein inhibition of the growth of human breast cancer cells: Independence from estrogen receptors and the multi-drug resistance gene. *Biochemical and Biophysical Research Communications*, **179**: 661–667.
- Pierini, R., Gee, J.M., Belshaw, N.J., and Johnson, I.T. (2008) Flavonoids and intestinal cancers. *British Journal of Nutrition*, **99** E-Suppl. 1: ES53–ES59.
- Pietinen, P. and Kilkkinen, A. (2002) Enterolactone and risk of cancer. *Journal of Nutrition*, **132**: 3535S–3536S.
- Pietinen, P., Stumpf, K., Mannisto, S., Kataja, V., Uusitupa, M., and Adlercreutz, H. (2001) Serum enterolactone and risk of breast cancer: A case-control study in eastern Finland. *Cancer Epidemiology, Biomarkers and Prevention*, **10**: 339–344.
- Pihlanto, A., Akkanen, S., and Korhonen, H.J. (2008) ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). *Food Chemistry*, **109**: 104–112.
- Plantinga, Y., Ghiadoni, L., Magagna, A., Giannarelli, C., Franzoni, F., Taddei, S., and Salvetti, A. (2007) Supplementation with vitamins C and E improves arterial stiffness and endothelial function in essential hypertensive patients. *American Journal of Hypertension*, **20**: 392–397.
- Poirier, J., Cockell, K., Hidioglou, N., Madere, R., Trick, K., and Kubow, S. (2002) The effects of vitamin E and selenium intake on oxidative stress and plasma lipids in hamsters fed fish oil. *Lipids*, **37**: 1124–1132.

- Prochaska, H.J. and Talalay, P. (1988) Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Research*, **48**: 4776–4782.
- Psaltopoulou, T., Panagiotakos, D.B., Pitsavos, C., Chrysochoou, C., Detopoulou, P., Skoumas, J., and Stefanadis, C. (2010) Dietary antioxidant capacity is inversely associated with diabetes biomarkers: The ATTICA study. *Nutrition, Metabolism and Cardiovascular Diseases*, doi:10.1016/j.numecd.2009.11.005.
- Qi, L. and Hu, F.B. (2007) Dietary glycemic load, whole grains, and systemic inflammation in diabetes: The epidemiological evidence. *Current Opinion in Lipidology*, **18**: 3–8.
- Qu, H., Madl, R.L., Takemoto, D.J., Baybutt, R.C., and Wang, W. (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. *Journal of Nutrition*, **135**: 598–602.
- Raghavendra, M.P., Kumar, P.R., and Prakash, V. (2007) Mechanism of inhibition of rice bran lipase by polyphenols: A case study with chlorogenic acid and caffeic acid. *Journal of Food Science*, **72**: E412–419.
- Rahman, I. and MacNee, W. (2000) Oxidative stress and regulation of glutathione in lung inflammation. *European Respiratory Journal*, **16**: 534–554.
- Randhir, R., Kwon, Y.I., and Shetty, K. (2008) Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science and Emerging Technologies*, **9**: 355–364.
- Ranilla, L.G., Apostolidis, E., Genovese, M.I., Lajolo, F.M., and Shetty, K. (2009) Evaluation of indigenous grains from the Peruvian Andean region for antidiabetes and antihypertension potential using in vitro methods. *Journal of Medicinal Food*, **12**: 704–713.
- Ranka, S., Gee, J.M., Johnson, I.T., Skinner, J., Hart, A.R., and Rhodes, M. (2006) Non-Steroidal Anti-Inflammatory Drugs, Lower Oesophageal Sphincter-Relaxing Drugs and Oesophageal Cancer. A Case-Control Study. *Digestion*, **74**: 109–115.
- Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J., and Willett, W.C. (1996) Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals of Internal Medicine*, **125**: 384–389.
- Romani, A., Vignolini, P., Galardi, C., Nulinacci, N., Benedettelli, S., and Heimler, D. (2004) Germplasm characterization of Zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. *Journal of Agricultural and Food Chemistry*, **52**: 3838–3842.
- Ross, R. (1999) Atherosclerosis – an inflammatory disease. *New England Journal of Medicine*, **340**: 115–126.
- Rumpler, W., Seale, J., Clevidence, B., Judd, J., Wiley, E., Yamamoto, S., Komatsu, T., Sawaki, T., Ishikura, Y., and Hosoda, K. (2001) Oolong tea increases metabolic rate and fat oxidation in men. *Journal of Nutrition*, **131**: 2848–2852.
- Rushmore, T.H., Morton, M.R., and Pickett, C.B. (1991) The antioxidant responsive element. *Journal of Biological Chemistry*, **266**: 11632–11639.
- Rushmore, T.H. and Pickett, C.B. (1990) Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. *Journal of Biological Chemistry*, **265**: 14648–14653.
- Rushmore, T.H. and Pickett, C.B. (1993) Glutathione S-transferases, structure, regulation, and therapeutic implications. *Journal of Biological Chemistry*, **268**: 11475–11478.
- Saarinen, N.M., Warri, A., Makela, S.I., Eckerman, C., Reunanen, M., Ahotupa, M., Salmi, S.M., Franke, A.A., Kangas, L., and Santti, R. (2000) Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutrition Cancer*, **36**: 207–216.
- Salganik, R.I. (2001) The Benefits and Hazards of Antioxidants: Controlling Apoptosis and Other Protective Mechanisms in Cancer Patients and the Human Population. *Journal of the American College of Nutrition*, **20**: 464 S–472 S.
- Salinas-Moreno, Y., Rojas-Herrera, L., Sosa-Montes, E., and Perez-Herrera, P. (2005) Anthocyanin composition in black bean varieties grown in Mexico. *Agrociencia*, **39**: 385–394.
- Scalbert, A. and Williamson, G. (2000) Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, **130**: 2073 S–2085 S.
- Schubert, S.Y., Lansky, E.P., and Neeman, I. (1999) Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of Ethnopharmacology*, **66**: 11–17.
- Sefi, M., Fetoui, H., Makni, M. and Zeghal, N. (2010) Mitigating effects of antioxidant properties of *Artemisia campestris* leaf extract on hyperlipidemia, advanced glycation end products and oxidative stress in alloxan-induced diabetic rats. *Food and Chemical Toxicology*, **48**: 1986–1993.
- Serafini, M., Bellocco, R., Wolk, A., and Ekstrom, A.M. (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology*, **123**: 985–991.

- Sesso, H.D., Gaziano, J.M., Buring, J.E., and Hennekens, C.H. (1999) Coffee and tea intake and the risk of myocardial infarction. *American Journal of Epidemiology*, **149**: 162–167.
- Sesso, H.D., Gaziano, J.M., Liu, S., and Buring, J.E. (2003a) Flavonoid intake and the risk of cardiovascular disease in women. *American Journal of Clinical Nutrition*, **77**: 1400–1408.
- Sesso, H.D., Paffenbarger, R.S. Jr, Oguma, Y., and Lee, I.M. (2003b) Lack of association between tea and cardiovascular disease in college alumni. *International Journal of Epidemiology*, **32**: 527–533.
- Shahidi, F. and Zhong, Y. (2007) Measurement of antioxidant activity in food and biological systems. In *Antioxidant measurement and applications* (eds. Shahidi, F. and Ho, C.T.), ACS Symposium Series 956, American Chemical Society, Washington, DC, pp. 36–66.
- Shahidi, F. and Zhong, Y. (2009) Antioxidants, polyphenols, and adipose inflammation. in *Adipose Tissue and Inflammation* (eds A.B. Awad and P.G. Bradford ), CRC Press, Taylor & Francis, Inc., Boca Raton, FL, pp. 233–254.
- Shahidi, F., Chavan, U.D., Naczki, M., and Amarowicz, R. (2001) Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). *Journal of Agricultural Food Chemistry*, **49**: 926–933.
- Sharma, B., Viswanath, G., Salunke, R., and Roy, P. (2008) Effects of flavonoid-rich extract from seeds of *Eugenia jambolana* (L.) on carbohydrate and lipid metabolism in diabetic mice. *Food Chemistry*, **110**: 697–705.
- Shaughnessy, K.S., Boswall, I.A., Scanlan, A.P., Gottschall-Pass, K.T., and Sweeney, M.I. (2009) Diets containing blueberry extract lower blood pressure in spontaneously hypertensive stroke-prone rats. *Nutrition Research*, **29**: 130–138.
- Shen, X.H., Tang, Q.Y., Huang, J., and Cai, W. (2010) Vitamin E regulates adipocytokine expression in a rat model of dietary-induced obesity. *Experimental Biology and Medicine*, **235**: 47–51.
- Sheu, F., Lai, H.H., and Yen, G.C. (2001) Suppression Effect of Soy Isoflavones on Nitric Oxide Production in RAW 264.7 Macrophages. *Journal of Agricultural and Food Chemistry*, **49**: 1767–1772.
- Sies, H. (1993) Strategies of antioxidant defense. *European Journal of Biochemistry*, **215**: 213–219.
- Sirtori, C.R., Galli, C., Anderson, J.W., Sirtori, E., and Arnoldi, A. (2009) Functional foods for dyslipidaemia and cardiovascular risk prevention. *Nutrition Research Reviews*, **22**: 244–261.
- Slavin, J. (2003) Why whole grains are protective: Biological mechanisms. *Proceedings of the Nutrition Society*, **62**: 129–134.
- Sohn, O.S., Fiala, E.S., Requeijo, S.P., Weisburger, J.H., and Gonzalez, F.J. (2001) Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer Research*, **61**: 8435–8440.
- Song, Y., Cook, N.R., Albert, C.M., Van Denburgh, M. and Manson, J.E. (2009). Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. *American Journal of Clinical Nutrition*, **90**: 429–437.
- Sosulski, F.W. and Dabrowski, K.J. (1984) Composition of free and hydrolyzable phenolic acids in the flours and hulls of ten legume species. *Journal of Agricultural and Food Chemistry*, **32**: 131–133.
- Squadrito, F., Altavilla, D., Morabito, N., Crisafulli, A., D'Anna, R., Corrado, F., Ruggeri, P., Campo, G.M., Calapai, G., Caputi, A.P., and Squadrito, G. (2002) The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. *Atherosclerosis*, **163**: 339–347.
- Stahl, W. and Sies, H. (1993) Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. *Annals of the New York Academy of Sciences*, **691**: 10–19.
- Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989) Beyond cholesterol. *Modifications of low-density lipoprotein that increase its atherogenicity. New England Journal of Medicine*, **320**: 915–924.
- Stensvold, I. and Jacobsen, B.K. (1994) Coffee and cancer: A prospective study of 43,000 Norwegian men and women. *Cancer Causes Control*, **5**: 401–408.
- Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T., and Ohtake, Y. (2007) Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *Journal of Agricultural Food Chemistry*, **55**: 4604–4609.
- Surh, Y. (2003) Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer*, **3**: 768–780.
- Surh, Y.J. (2002) Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. *Food and Chemical Toxicology*, **40**: 1091–1097.
- Suzuki, K., Koike, H., Matsui, H., Ono, Y., Hasumi, M., Nakazato, H., Okugi, H., Sekine, Y., Oki, K., Ito, K., Yamamoto, T., Fukabori, Y., Kurokawa, K., and Yamanaka, H. (2002) Genistein, a soy isoflavone, induces

- glutathione peroxidase in the human prostate cancer cell lines LNCAP and PC-3. *International Journal of Cancer*, **99**: 846–852.
- Szkudelska, K., Nogowski, L., and Szkudelski, T. (2009) Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. *Journal of Steroid Biochemistry and Molecular Biology*, **113**: 17–24.
- Szocs, K. (2004) Endothelial dysfunction and reactive oxygen species production in ischemia/reperfusion and nitrate tolerance. *General Physiology and Biophysics*, **23**: 265–95.
- Takahata Y., Ohnishi-Kameyama M., Furuta S., Takahashi M., and Suda I. (2001) Highly polymerized pro-cyanidins in brown soybean seed coat with a high radical-scavenging activity. *Journal of Agricultural Food Chemistry*, **49**: 5843–5847.
- Takeoka, G. R., Dao, L. T., Full, G. H., Wong, R. Y., Harden, L. A., Edwards, R. H., Berrios, J., and De, J. (1997) Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. *Journal of Agricultural Food Chemistry*, **45**: 3395–3400.
- Takikawa, M., Inoue, S., Horio, F., and Tsuda, T. (2010) Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *Journal of Nutrition*, **140**: 527–533.
- Tavani, A. and La Vecchia, C. (2004) Coffee, decaffeinated coffee, tea, and cancer of the colon and rectum: A review of epidemiological studies, 1990–2003. *Cancer Causes and Control*, **15**: 743–757.
- Terao, J. (1989) Antioxidant activity of beta-carotene-related carotenoids in solution. *Lipids*, **24**: 659–661.
- The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. (1994) The effects of vitamin E and  $\beta$ -carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine*, **330**: 1029–1035.
- The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. (1994) The alpha-tocopherol, beta-carotene lung cancer prevention study: Design, methods, participant characteristics, and compliance. *Annals of Epidemiology*, **4**: 1–10.
- Tiwari, A.K. and Rao, J.M. (2002) Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, **83**: 30–38.
- Troszynska, A. and Ciska, E. (2002) Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. *Czech Journal of Food Sciences*, **20**: 15–22.
- Turini, M.E. and DuBois, R.N. (2002) Cyclooxygenase-2: A therapeutic target. *Annual Review of Medicine*, **53**: 35–57.
- Tylicki, L., Rutkowski, B., and Horl, W.H. (2003) Antioxidants: A possible role in kidney protection. *Kidney and Blood Pressure Research*, **26**: 303–314.
- Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., and Telser, J. (2004) Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and Cellular Biochemistry*, **266**: 37–56.
- Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., and Mazur, M. (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, **160**: 1–40.
- Vanharanta, M., Voutilainen, S., Lakka, T., van der Lee, M., Adlercreutz, H., and Salonen, J. (1999) Risk of acute coronary events according to serum concentrations of enterolactone: A prospective population-based case-control study. *Lancet*, **354**: 2112–2115.
- Vaughan, T.L., Dong, L.M., Blount, P.L., Ayub, K., Odze, R.D., Sanchez, C.A., Rabinovitch, P.S., and Reid, B.J. (2005) Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: A prospective study. *Lancet Oncology*, **6**: 945–952.
- Visioli, F. and Galli, C. (2002) Biological properties of olive oil phytochemicals. *Critical Reviews in Food Science and Nutrition*, **42**: 209–221.
- Visioli, F., Poli, A., and Galli, C. (2001) Antioxidant and other biological activities of phenols from olives and olives oil. *Medical Research Review*, **22**: 65–75.
- Vita, J.A. (2005) Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. *American Journal of Clinical Nutrition*, **81** (suppl): 292S–297S.
- Vita, J.A. and Keaney, J.F. Jr. (2002) Endothelial function: A barometer for cardiovascular risk? *Circulation*, **106**: 640–652.
- Wadsworth, T.L., McDonald, T.L., and Koop, D.R. (2001) Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor- $\alpha$ . *Biochemical Pharmacology*, **62**: 963–974.
- Wenzel, U., Kuntz, S., Brendel, M.D., and Daniel, H. (2000) Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. *Cancer Research*, **60**: 3823–3831.

- Widlansky, M.E., Gokce, N., Keaney, J.F. Jr, and Vita, J.A. (2003) The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology*, **42**: 1149–1160.
- Witztum, J.L. and Steinberg, D. (1991) Role of oxidized low density lipoprotein in atherogenesis. *Journal of Clinical Investigation*, **88**: 1785–1792.
- Wong, A., Luth, H.J., Deuther-Conrad, W., Dukic-Stefanovic, S., Gasic-Milenkovic, J., Arendt, T., and Münch, G. (2001) Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease. *Brain Research*, **920**: 32–40.
- Wu, K., Willett, W.C., Chan, J.M., Fuchs, C.S., Colditz, G.A., and Giovannucci, E.L. (2002) A prospective study on supplemental vitamin E intake and risk of colon cancer in women and men. *Cancer Epidemiology, Biomarkers and Prevention*, **11**: 1298–1304.
- Yamamoto, S., Sobue, T., Kobayashi, M., Sasaki, S., and Tsugane, S. (2003) Soy, Isoflavones, and breast cancer risk in Japan. *Journal of National Cancer Institute*, **95**: 906–912.
- Yanagihara, K., Ito, A., Toge, T., and Numoto, M. (1993) Antiproliferative effects of isoflavones on Human Cancer cell lines established from the gastrointestinal tract. *Cancer Research*, **53**: 5815–5821.
- Yang, J.L., Jang, J.H., Radhakrishnan, V., Kim, Y.H., and Song, Y.S. (2008)  $\beta$ -glucan suppresses LPS-stimulated NO production through the down-regulation of iNOS expression and NF $\kappa$ B Transactivation in RAW 264.7 macrophages. *Food Science and Biotechnology*, **17**: 106–113.
- Yeh, Y.H., Lee, Y.T., Hsieh, H.S., and Hwang, D.F. (2009) Dietary caffeic acid, ferulic acid and coumaric acid supplements on cholesterol metabolism and antioxidant activity in rats. *Journal of Food and Drug Analysis*, **17**: 123–132.
- Yochum, L., Kushi, L.H., Meyer, K., and Folsom, A.R. (1999) Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *American Journal of Epidemiology*, **149**: 943–949.
- Yousef, M.I., Kamel, K.I., Esmail, A.M., and Baghdadi, H.H. (2004) Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. *Food and Chemical Toxicology*, **42**: 1497–1503.
- Youssef, F., Gupta, P., Mikhailidis, D.P., and Hamilton, G. (2005) Risk modification in patients with peripheral arterial disease: A retrospective survey. *Angiology*, **56**: 279–287.
- Zern, T.L. and Fernandez, M.L. (2005) Cardioprotective effects of dietary polyphenols. *Journal of Nutrition*, **135**: 2291–2294.
- Zhang, J., Stanley, R.A., Adaim, A., Melton, L.D., and Skinner, M.A. (2006) Free radical scavenging and cytoprotective activities of phenolic antioxidants. *Molecular Nutrition and Food Research*, **50**: 996–1005.
- Zhang, Y., Vareed, S.K., and Nair, M.G. (2005) Human tumor cell growth inhibition by non-toxic anthocyanidins, the pigments in fruits and vegetables. *Life Sciences*, **76**: 1465–1472.
- Zielinski, H. (2002) Low molecular weight antioxidants in the cereal grains – a review. *Polish Journal of Food and Nutrition Sciences*, **11/52**: 3–9.

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