Flavor Chemistry of Ethnic Foods

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Edited by

Fereidoon Shahidi

Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland
A1B 3X9 Canada

Chi-Tang Ho

Department of Food Science
Cook College
Rutgers, The State University of New Jersey
P.O. Box 231
New Brunswick, NJ 08903-0231

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PREFACE

Flavor is an important quality characteristic of all foods. Both odor- and taste-active components are involved. Although perception of flavor is a chemically-derived phenomenon, a flavor's acceptability and desirability is highly affected by the cultural background and ethnic diversity of the consumers. On this basis, there are distinct differences and particularities in the ingredients as well as the methods of preparation of foods in different regions of the world, and these are also affected by the specific environment, culture, and ethnicity of the people. Changes in the flavors of foods, induced by processing, are also important factors affecting their diversity and characteristics. The use of different types of sauce, such as soy sauce, tomato sauce, and fish sauce, as well as condiments and spices, is of particular importance in defining the cultural and international nature of prepared foods. The source of raw material, particularly when they are available only in certain parts of the world, can also have a profound effect on the availability and popularity of such products. The availability of better and faster modes of transportation and the migration of individuals, each resulting in the intermingling of different cultures, have allowed production of a new generation of prepared foods such as American-Chinese, among others.

The present monograph assembles a collection of scientific contributions on flavor and chemistry of international foods presented during the 5th Chemical Congress of North America, held in November 1997, in Cancun, Mexico, or subsequently solicited by the editors. We are grateful to the authors for their outstanding cooperation and contributions that made the production of this state-of-the-art monograph possible.

Fereidoon Shahidi
Chi-Tang Ho

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FLAVOR CHEMISTRY OF ETHNIC FOODS

An Overview

F. Shahidi[1] and C.-T. Ho[2]

[1]Department of Biochemistry, Memorial University of Newfoundland
St. John's, Newfoundland, A1B 3X9, Canada
[2]Department of Food Science, Cook College, Rutgers
The State University of New Jersey, New Brunswick, New Jersey 08903

Ethnic and international foods have gradually been integrated into our daily diets in North America. However, the existing literature on flavor characteristics and chemistry of such foods remains fragmentary and diverse. An attempt has been made to present a summary of the current status of knowledge in this area.

INTRODUCTION

Flavor is an important sensory aspect of the overall acceptability of foods. The flavor of food is influenced by both its odor-active volatiles and taste-active non-volatiles. However, methods of preparation and heat processing as well as presence of other ingredients and seasonings might exert a profound effect on the characteristics and "ethnic" flavor of certain foods. Proximity of cultures and ethnic groups has also caused modification in certain food formulations in order to offer an "international" flavor to them. As an example, while Chinese food has preserved its overall authenticity, it has undergone certain modification and transformation which has led to the availability of the so-called American-Chinese or North American-Chinese cuisines.

FLAVOR GENERATION IN FOODS — ROLE OF INGREDIENTS AND PROCESSING METHODS

Flavor of foods is generated by the action of enzymes, fermentation or heat-processing operations. Ingredients or combination of specific ingredients present in different food formulations of ethnic origin may influence the formation of Maillard reaction products as

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well as effects on lipids via oxidation or participation in Maillard reactions as well as reaction of amino acids and proteins with carbohydrates. Other food components such as spices and condiments as well as certain vitamins and specific amino acids may modify the flavor of foods and thus lead to generation of specific "ethnic", such as Chinese, Japanese, Indian, Thai, etc., aromas in selected foods.

The Maillard reaction in relation to flavor has been the subject of a number of reviews (Bailey, 1998; Hurrell, 1982; Mottram, 1994; Nursten, 1986; Tressl *et al.*, 1993). An important reaction associated with the Maillard reaction is Strecker degradation which involves oxidative deamination and decarboxylation of α-amino acids in the presence of a dicarbonyl compound. This leads to the formation of an aldehyde containing one less carbon than the original amino acid and an α-aminoketone. Strecker aldehydes, mercaptoaldehydes and α-aminoketones as well as hydrogen sulfide, ammonia and acetaldehyde are formed from degradation of cysteine. Lipid degradation may also afford aldehydes that could participate in the Maillard reaction.

These compounds serve as important intermediates for the formation of many odoriferous compounds. Breakdown of methionine as well as thiamine may also provide a number of intermediates as well as heterocyclic and sulfurous compounds (Shahidi, 1989). The Maillard products formed may further react with one another. Thus, interaction of furfural, furanones and dicarbonyl compounds with other reactive compounds such as amino compounds, hydrogen sulfide, thiols, ammonia and aldehydes may produce an array of flavor-active compounds, including heterocyclics, among others.

Lipid components of food may also undergo different reactions via autoxidation, thermal oxidation, photooxidation and lipoxygenase-assisted oxidation to produce a wide range of flavor-active compounds. While the primary products of lipid oxidation, hydroperoxides, are odorless, their breakdown leads to the production of aldehydes, ketones, alcohols and hydrocarbons, among others. Free fatty acids may also be formed via thermal hydrolysis of lipids. Substituted fatty acids may further cyclize to produce lactones and other flavor-active compounds. The role of lipids in aroma generation has been reviewed in the literature (Chen and Ho, 1998; Mottram, 1998; Shahidi and Cadwallader, 1997; Skibsted *et al.*, 1998).

Different sauces produced via brine fermentation processes are used in many ethnic foods. Shoyu is a popular Japanese sauce from soybean that is used widely in Japan and other South East Asian countries, but Miso is not internationally as popular because of its paste consistency (Chapter 2). Meanwhile, black soybeans are brine fermented to produce *moromi* which is subsequently cooked with coconut sugar and spices to afford Indonian soy sauce known as *Kecap manis* (Chapter 3). Meanwhile, a complex ingredient which includes dried shrimp, fish, sugar and roasted sesame, among others affords Sa Cha sauce (Chapter 4) which is important in Chinese cuisine. Meanwhile, brine fermentation of fish affords fish sauce which is again very popular in Vietnam and other South East Asian countries (Chapter 5). Furthermore, soy sauce, garlic, scallion and shallot as well as ginger, star anise and black or Shiitake mushroom are among the condiments and ingredients used in Chinese foods and are responsible for many volatile and non-volatile components responsible for the rich and delicate flavor of Chinese dishes that are popular worldwide (Chapter 6). In addition, coriander fruit is used as a spice while its fresh leaves serve as important culinary herbs, more commonly known as Chinese parsley (Potter and Fagerson, 1990; Chapter 7) . Meanwhile, the importance of Wasaki and Japanese horseradish in food flavors has been documented (Chapter 8). The rhizomes of greater galangal are also widely used throughout the South East Asian countries (Chapter 9) and pandan leaves have a strong characteristic aroma which is desirable in various bakery products, sweets and home cooking (Chapter 10).

Cooking of broccoli, an important vegetable, affords a range of volatiles, including glucosinolate degradation products with sulfur or nitrogen heteroatoms (Chapter 11). Meanwhile, flavor extracts from fruits such as kweni, an exotic tropical fruit may find application in the food and flavor industries (Chapter 12). Flavor quality of foods, including fruits may be assessed and compared using an electronic nose apparatus (Chapter 13).

Meat products from different sources such as those from harp seal (Chapter 14) and as affected by nitrite curing (Chapter 14) or use of organic acids (Chapter 15) or pan-frying (Chapter 16) and fermentation (Chapter 17) is of interest. Role of lipids in these meat-based foods is also quite important.

Tea is one of the most popular drinks in the world. The flavor and astringency of tea is affected by the method of preparation as well as the type and content of phenolics present (Chapters 18 and 19). An alcoholic beverage, tequila (Chapter 20) is found to have a typical flavor, but compounds involved are similar to those found in wines and sake.

Many of the foods are prepared using oil as a frying medium. The release of odor-active compounds during oxidation of vegetable oils (Chapter 21) as well as those arising from roasting of seeds, such as sesame, prior to oil removal may play an important role in the aroma of final products (Chapter 22). In addition various sugars in foods may interact with specific amino acids such as β-alanine to produce maltol which is responsible, at least in part, for the flavor of cookies (Chapter 23).

Finally, flavor of certain exotic food products such as dried roe mullet, which is considered a delicacy in Japan and Taiwan, similar to sturgeon caviar in Europe, is especially affected by its high content of unsaturated fatty acids and free amino acids (Chapter 24). Preservation of duck egg or pidan by covering them in a paste of lime, charcoal, caustic soda and salt affords a product which has a transparent and brown egg while and a semi-solid and hard egg yolk which possesses a fresh sulfur odor with some alkaline sense (Chapter 25).

In all international food preparations, specific aromas and flavor volatiles are generated via interaction of ingredients or are formed as a result of chemical changes induced during processing and preparation as well as use of spices and condiments. Therefore, study of flavor attributes of international foods opens a new area of research which could shed light, not only on the basic reactions and interactions involved in their generation, but would also improve our appreciation of existing complexities of cumulative role of chemicals involved in flavor perception.

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FLAVOR COMPONENTS OF SHOYU AND MISO JAPANESE FERMENTED SOYBEAN SEASONINGS

Akio Kobayashi[1] and Etsuko Sugawara[2]

[1]Ochanomizu University, Department of Nutrition and Food Science
Laboratory of Food Chemistry
2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan
[2]Iwate University, Faculty of Education
3-18-33 Ueda, Morioka, Iwate 020, Japan

The complexity of shoyu and miso flavors originates from the many fungi, yeast and other bacteria participating in fermentation controlled under a high concentration of sodium chloride. Volatile compounds with various concentrations have already been identified from both shoyu and miso, however, not all of them contribute to their flavors.

A simple separation method of adsorption of the volatiles to a porous polymer adsorbent is useful to analyze the heating labile compounds in shoyu and miso volatiles. The results obtained by this method could well explain the common and different aroma characters of shoyu and miso, among which HEMF is one of the physiologically active components as well as a flavor impact compound.

INTRODUCTION

The name soybean comes from "bean of soy" which is derived from Portuguese *Soya*. In the 16th century, Portuguese imported Japanese soy sauce (shoyu) and distributed it in the European countries. The name *Soya* is an alteration of the Japanese word "shoyu." In this respect, shoyu has been an international food item since that time.

Japanese live on rice as the principal food, whose protein has low concentrations of some essential amino acids. It is thus necessary to make up for the nutritional deficiencies by taking other plant protein foods, for example, shoyu, miso, natto and tofu, all of which are soybean products.

Shoyu and miso are manufactured by the same fermentation process under a high concentration of salt and with many different microbial activities. Shoyu is indispensable for everyday Japanese cooking, and is also becoming popular in Western cuisine. While the total production of shoyu in Japan was 1,300,000 kl in 1996, the production in the

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United States has been estimated to be 150,000 kl in the same year. Miso is not as internationally popular as shoyu because its paste consistency is sufficiently difficult to sterilize as to limit its range of distribution and because miso soup, for which most miso is used, is unfamiliar to the western taste and is regarded as being not suitable for western dishes. However, miso has a common aroma and taste to that of shoyu, and a variety of miso products can be used as a new seasoning for ethnic dishes. There are similar food materials to shoyu and miso in China and Southeast Asian countries, and these have recently been introduced to western countries as ethnic foods or food materials.

The historical aspects, manufacturing processes and microbiological problems of these fermented soybean products have been reviewed in detail by Fukushima (1985). We describe here the flavor and chemistry of these products.

SHOYU (SOYBEAN SAUCE)

There are various types of shoyu sold in Japan, although the main product is Koikuchi-shoyu, a deep brown-colored shoyu, whose typical composition at pH 4.7 is as follows: NaCl content, 16.9 g/100 ml; total nitrogen, 1.57 g/100 ml; reducing sugar, 3.0 g/100 ml; alcohol, 2.3 ml/100 ml.

The manufacturing process basically consists of three stages: koji-making, brine fermentation and refining. In the first process, a mixture of cooked soybean and roasted wheat is inoculated with a starter culture of *Aspergillus oryzae* or *A. sojae*. After 3 or 4 days, a fungus grows over the culture mixture which is called "koji." Brine is then added (16–19 g of salt/100 ml) to facilitate the fermentation by osmophilic lactic acid bacteria and yeast and to preclude any undesirable fermentation. This fermentation is continued for 6–8 months, during which starch is changed to lactic acid, alcohol and carbon dioxide by conversion of simple sugars from starch as well as by enzymatic hydrolysis of protein to peptides and amino acids. At the same time, various kinds of volatile compounds are formed as fermented products. The refining process includes filtration and pasteurization, the latter involving heating to 70–80 to develop the flavor of shoyu. Many flavor compounds appear as the result of non-enzymatic or amino-carbonyl reaction at this stage, some of them being typical thermally generated aroma compounds which change with stronger heating.

Table 1 shows the comparative amino acid composition of the two starting materials, soybean and wheat, which are generally used in equal amounts in the mixture. Therefore, the average amino acid composition of these two should correspond to that of shoyu if there are no chemical changes to amino acids during the fermentation process to hydrolyse the protein to the water-soluble peptides and amino acids. The great decrease in the arginine content is thought to be related to the increase in ornithin as this type of conversion is observed in lactic acid fermentation. The instability of tryptophan and cystine under acidic conditions with a long-period fermentation dropping to pH 4.7–4.8 might be the reason for the decrease in the concentration of these two amino acids. The decrease in methionine content is marginal. As discussed later, the Strecker degradation product of methionine, methional, and its reduced product methionol are key aroma components of shoyu flavor. However, the degradation of methionine is perhaps statistically in error considering of the very low threshold values of methional and methionol (2.0×10^{-1} and 3.0×10^{-1} ppb in water, respectively; Guadagni 1972).

The palatability of shoyu is greatly enhanced by the high concentration (ca. 7.5%) of amino acids and particularly by the presence of glutamic acid (Yamaguchi, 1987), in spite of the 17 % sodium chloride content in shoyu.

Table 1. Amino acid composition of Koikuchi-shoyu and its starting materials

Amino acid	Soybean (%)	Wheat (%)	Koikuchi-shoyu (%)
Arginine	8.42	4.71	2.6
Histidine	2.55	2.12	1.5
Lysine	6.86	2.67	6.5
Tyrosine	3.90	3.19	1.0
Tryptophan	1.28	1.13	–
Phenylalanine	5.01	4.43	4.2
Cystine	1.58	1.80	0.9
Methionine	1.56	1.74	1.4
Serine	5.57	5.22	5.3
Threonine	4.31	2.76	4.2
Leucine	7.72	6.52	7.3
Isoleucine	5.10	3.78	4.8
Valine	5.38	4.69	5.5
Glutamic acid	21.00	29.30	22.5
Aspartic acid	12.01	4.85	10.5
Glycine	4.52	3.94	3.9
Alanine	4.51	3.37	4.4
Proline	6.28	9.94	6.5
Ornithine	–	–	5.7
Total	106.56	96.16	100.1

The composition of flavor volatiles is complicated and some of them are unstable to heating. Yokotsuka *et al.* (1981) first applied steam distillation under reduced pressure (15 mmHg at 40) and extraction of the distillate with dichloromethane to isolate the shoyu volatiles. They listed up to 267 compounds as shoyu volatiles, among which 37 hydrocarbons, 29 alcohols, 40 esters, 15 aldehydes, 3 acetals, 17 ketones, 24 acids, 16 phenols, 16 furans, 4 lactones, 4 furanones, 5 pyrones, 25 pyrazines, 7 pyridines, 6 miscellaneous N-compounds, 11 S-containing compounds, 3 thiazoles, 3 terpenes and 2 other compounds were described. Not all of these volatiles would contribute to the shoyu aroma, particularly 37 hydrocarbons; however, almost all aroma compounds are popular in fermented food products or in thermally generated aroma compounds. The characteristic compound of shoyu flavor will be discussed later in comparison to the aroma impact compounds of miso.

MISO (FERMENTED SOYBEAN PASTE)

The original meaning of miso in Japanese is "immature shoyu." It is also a fermentation product of soybean and other cereals in the presence of salt; however, the current manufacturing process for miso differs from that of shoyu in terms of koji making and the fermentation process. Miso koji is made from a single cereal or bean, that is, cooked rice, barley or soybean itself, by inoculating with a koji starter of *A. oryzae*, which is added later to cooked soybean and salt and then fermented for a longer period of 3–12 months. During this time, yeast and lactic acid bacteria grow and hydrolyze soybean carbohydrate and protein in a similar way to that of shoyu manufacturing. The species of fungus, bacterium and yeast are almost the same as those for shoyu; however, the degree of hydrolysis is much lower in miso than in shoyu, and the product is a solid paste. Many types of miso

other than shoyu are available. This report addresses the flavor components of salty miso made from rice koji, which corresponds to koikuchi-shoyu in its manufacturing processes, and is the predominant product in the Japanese market.

As miso is a paste, it cannot be pasteurized. Therefore, the flavor of miso is much more labile to heating than that of shoyu whose instability has already been mentioned.

COMPARISON OF THE AROMA COMPONENTS IN SHOYU AND MISO

To avoid decomposition of the amino-carbonyl type products while extracting the aroma components from shoyu and miso, we compared several extraction methods.The adsorption of aroma compounds from the aqueous phase by a porous polymer resin was thought to be most reasonable with respect to the recovery of fresh aroma and ease of sampling (Sugawara *et al.*, 1990).

SAMPLE PREPARATION

Two types of salty rice-miso were used: one was reddish-brown in color resulting from a long fermentation period of 5–12 months (Miso 1), while the other was bright light yellow in color from fermentation for 2–6 months (Miso 2) under nearly the same conditions. Miso (40 g) was finely ground and suspended in 160 ml of distilled water, the suspension was centrifuged at 3000 rpm for 15 min. The supernatant was passed through a Tenax GC (2.0 g) packed column. The adsorbed material was eluted with 50 ml of diethyl ether to which n-decanol had been added as an internal standard. After drying and evaporating the ether solution, the concentrate was analyzed by capillary gas chromatography (GC) and GC-mass spectrometry. The amount of each compound was calculated from the ratio of the compound peak to that of the internal standard.

The shoyu volatiles were prepared by mixing 20 g of shoyu with 180 ml water to give the same content of salt as that in miso and were passed through the Tenax column.

Gas chromatograms of the volatiles obtained from shoyu (Koikuchi-shoyu) and two of the salty rice-miso samples (Miso 1 and 2) are shown in Figure 1. Sixty eight compounds were identified on these gas chromatograms among which 44 compounds that are thought to have contributed or to be related to each aroma character are summarized in Table 2 according to their functional groups. The concentration of each compound was calculated from the ratio of the peak area to that of the internal standard and was converted to ppm of the original amount of shoyu or miso used.

Among the aliphatic alcohols, those common to both shoyu and miso were typical aroma products of fermentation. The lack of hexanol and 1-octen-3-ol, which are the major aroma constituents of soybean, suggests the degradation of those compounds at some stage of the manufacturing process. While whole soybean is generally used in miso manufacturing, defatted soybean is occasionally used as the fermentation material for shoyu making, or in the case of using whole soybean, the transesterified product of triacylglycerols with alcohol (ethyl esters of the higher-number fatty acids) is separated during the early stages of the refining process. This is the reason why many high fatty acid esters appear only in miso 1 and 2, while the short fermentation process for Miso 2 results in a lower amount of transesterified products.

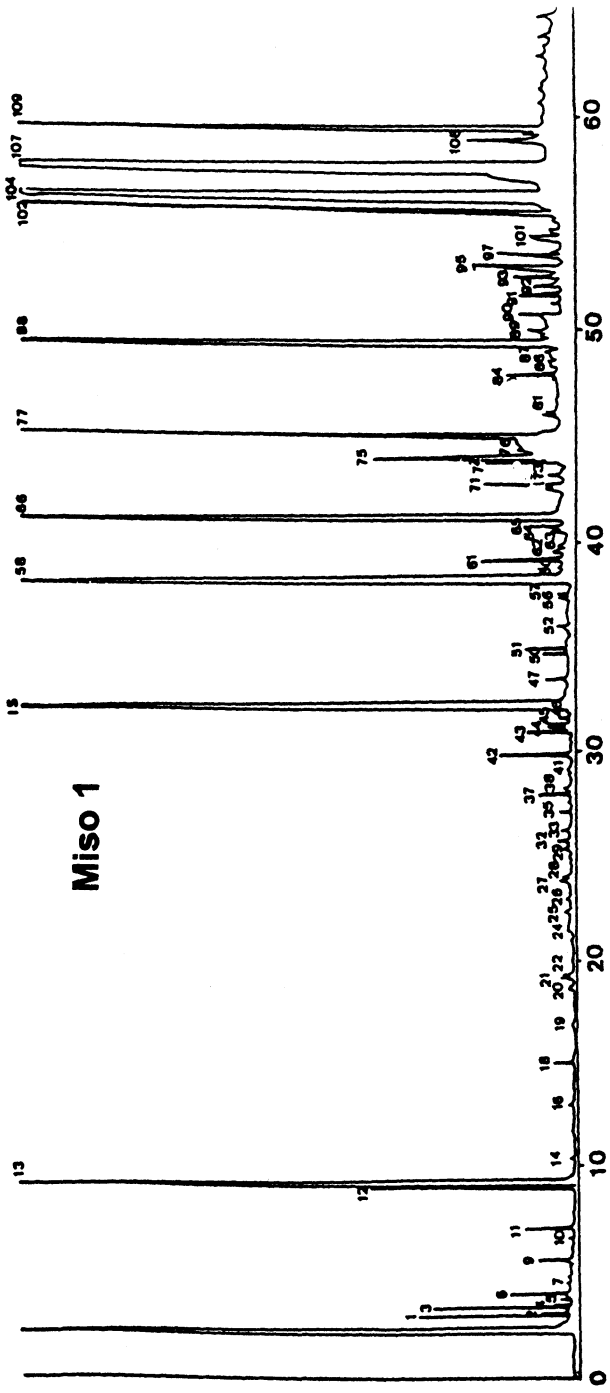

Figure 1A-C. (B and C on subsequent pages.) Gas chromatograms of the volatile compounds in shoyu and miso is = internal standard.

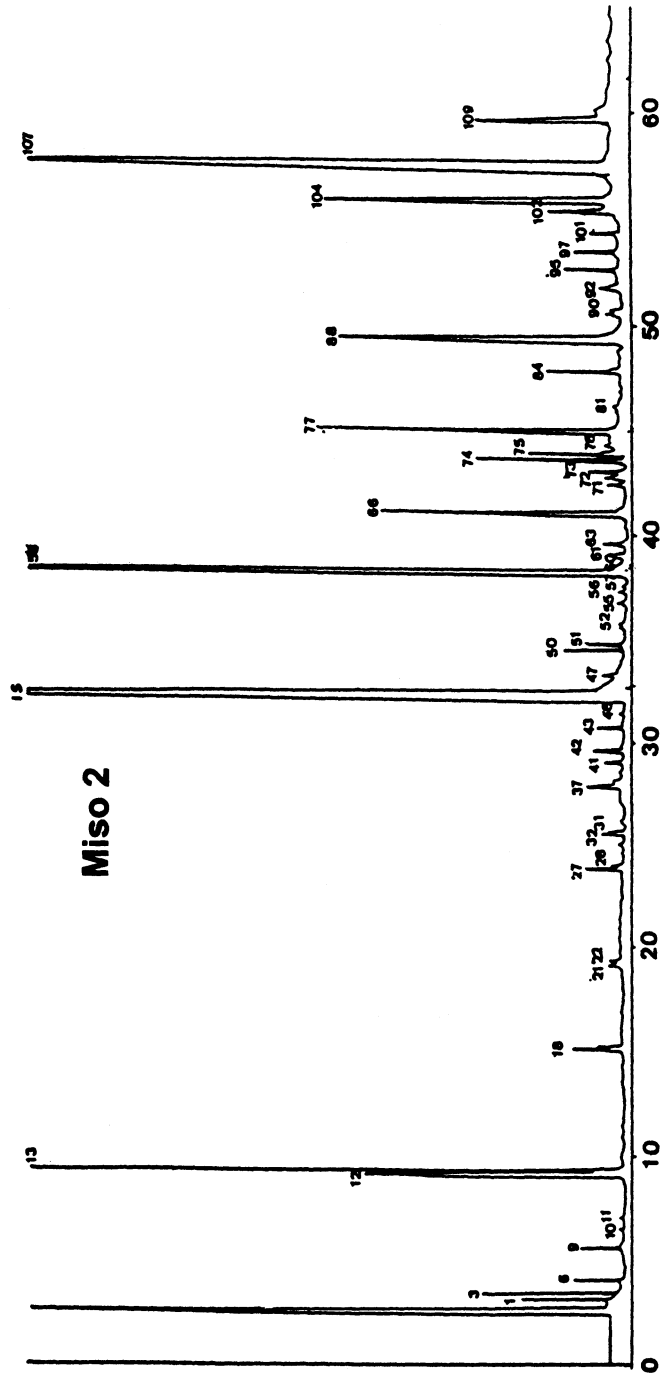

Figure 1B.

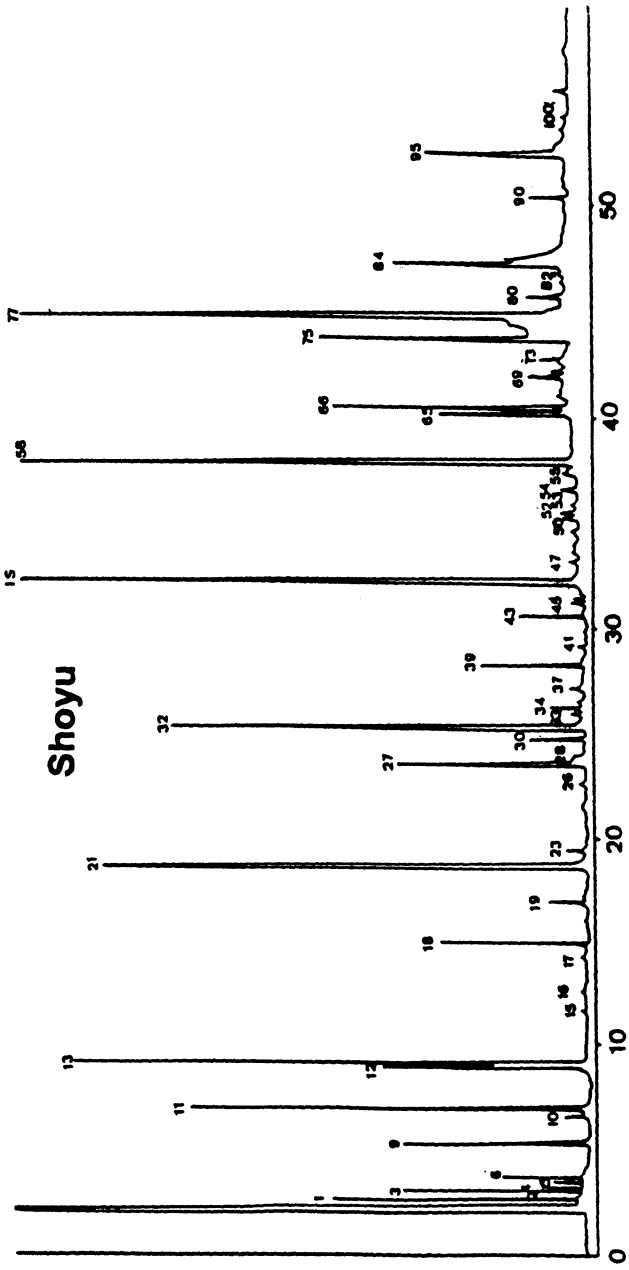

Figure 1C.

Table 2. Main volatile compounds in shoyu and miso

Peak no.	Compound	Miso 1	Miso 2	Shoyu
A. Aliphatic alcohols (8)				
9	methyl-1-propanol	0.20	0.22	0.62
11	butanol	0.24	0.03	0.59
12	2-methylbutanol	1.24	0.82	0.42
13	3-methylbutanol	3.99	3.62	1.75
18	hexanol	0.13	0.13	0.77
22	1-octen-3-ol	0.27	0.10	
27	2,3-butanediol	0.08	+	1.50
32	meso-2,3-butandiol	0.18	0.08	2.86
B. Esters (11)				
10	3-methylbutyl acetate	0.03	0.02	0.01
7	ethyl 3-methylpentanoate	+	+	0.01
45	ethyl octanoate	0.08	+	0.01
42	diethyl succinate	0.42	0.11	
71	ethyl tetradecanoate	0.46	0.32	
88	ethyl hexadecanoate	28.26	2.46	0.68
97	ethyl nonadecanoate	0.38	0.21	
102	ethyl octadecanoate	4.50	0.40	
104	ethyl (Z)-9-octadecanoate	24.72	2.33	
107	ethyl (Z,Z)-9,12-octadecadienoate	88.80	6.13	
109	ethyl (Z,Z,Z)-9,12,15-octadecatrienoate	12.64	0.73	
C. Carbonyl and acid compounds (3)				
16	3-hydroxy-2-butanone	+	0.13	0.01
21	acetic acid	+	+	0.37
28	2-methylpropanoic acid	0.02	0.03	0.01
D. Aromatic compounds (13)				
53	2-methoxyphenol			0.04
69	4-ethyl-2-methoxyphenol			0.17
82	4-ethyl-2-phenol			0.01
84	2-methoxy-4-vinylphenol	0.35	0.19	2.48
90	2,6-dimethoxyphenol	0.21	+	2.59
26	benzaldehyde	0.23	0.09	0.03
37	phenylacetaldehyde	+	+	0.10
61	2-phenyl-2-butenal	0.45	0.05	
54	benzylalcohol		+	0.01
58	2-phenylethanol	11.68	10.17	2.95
38	ethyl benzoate	0.01	0.08	
47	2-phenylethyl acetate	0.14	0.28	0.18
100	benzoic acid			0.01
E. Furans (2)				
29	5-methyl-2-furfural	0.03		
39	furfuryl alcohol	+	+	0.13
F. Nitrogen containing compound (1)				
65	acetyl pyrrole	0.22	0.03	0.30
G. Sulfur containing compounds (2)				
23	3-methylthiopropanal	0.11	+	
43	3-methylthiopropanol	0.22	0.10	0.30
H. Pyrones and furanones (4)				
66	maltol	4.16	0.46	6.96
73	HDMF	0.03	+	0.05
77	HEMF	6.42	0.75	5.47
80	HMF			1.51

HDMF: 4-hydroxy-2,5-dimethyl-3(2H)-furanone
HEMF: 4-hydroxy-2(or5)-ehtyl-5(or2)-methyl-3-(2H)-furanone
HMF: 4-hydroxy-5-methyl-3(2H)-furanone

Among the aromatic compounds, phenols were predominantly present in shoyu. These have already been reported to be formed from the degradation of lignin glycosides during fermentation (Yokotsuka *et al.*, 1981). Lignins are components of cereal bran, and whole wheat is used as koji from the early stage of shoyu fermentation; on the other hand, rice miso koji is made from polished white rice. The difference in the content of phenols in shoyu and miso is thought to be one of the factors differentiating the flavor character of each of these products.

The sulfur-containing compounds, 3-methylthiopropanal (methional) and 3-methylthiopropanol (methionol), were other flavor impact compounds. The low threshold value for methionol (0.3 ppb in water) and its odor character is a conclusive factor determining the common characteristic aromas of shoyu and miso. Methionol is thought to be derived from methional under the reducing conditions of fermentation. The latter is formed from methionine by Strecker degradation and has a more off-flavor character than that of methionol. The absence or low concentration of this aldehyde in shoyu results from the exhaustive fermentation and gives a mild and acceptable shoyu aroma.

The group of 4H-pyrone and 3(2H)-furanone are most intresting with respect to the common sweet and burnt aroma character of shoyu and miso. 3-Hydroxy-2-methyl-4H-pyran-4-one (maltol) and 4-hydroxy-2,5-dimethyl-3(2H)furanone (HDMF) are thought to be formed from C_6-monosaccharides through an amino-carbonyl reaction.

Sugawara (1991) has shown that maltol appears under the usual cooking conditions of soybean and we think that, in addition to an amino-carbonyl reaction, the hydrolysis of a soybean saponin, which has a maltol structure as its aglycon (Kudou *et.al.*, 1992) is responsible for maltol formation.

Table 3 shows the concentrations of maltol and 3(2H)-furanones in cooked soybean and following fermentation processes of miso and shoyu. The content of 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF) only increased during the later stages of the fermentation and its yield was highest after 60 days. This means that the formation of HEMF is different from that of other components which are present in cooked soybean and their content decreases during the fermentation. The presence of HEMF in miso was reported by Sugawara (1991) and biosynthetic route of HEMF was claimed to be through pentose-phosphate cycle by shoyu yeast (Sasaki, 1996). Therefore, its formation by yeast via a pathway may occur during the fermentation of miso (Sugawara, *et al.*, 1994).

In Japan, large amounts of miso are consumed in the form of miso soup. Hirayama (1982) already pointed out that daily intake of miso soup significantly reduces the standardized mortality rate from gastric cancer. More recently, HEMF was found to possess antitumor activity (Nagahara *et al.*, 1992). Therefore, HEMF is not only a significant contributor to shoyu and miso flavor, but is also thought to be participating in their antitumor activities.

Table 3. Changes in the flavor impact compounds during miso fermentation*

Compounds	Rice koji	Cooked soybean	Start	30 days	60 days	90 days	120 days
maltol	0	2.98	1.43	0.78	0.58	0.65	0.24
HMF	0	0.01	0	0	0	0	0
HDMF	0	0.03	tr	tr	tr	tr	tr
HEMF	0	0	0	2.31	2.33	1.84	0.81

* ppm in rice koji, cooked soybean and miso, respectively.
tr: trace

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3

FLAVOR CHARACTERISTICS OF INDONESIAN SOY SAUCE (*KECAP MANIS*)

A. Apriyantono, H. Husain, L. Lie, M. Jodoamidjojo, and
N. L. Puspitasari-Nienaber

Department of Food Technology and Human Nutrition
Bogor Agricultural University
Kampus IPB Darmaga, P.O. Box 220
Bogor, 16002 Indonesia

Kecap manis is a typical Indonesian soy sauce prepared from black soybeans. The beans are mold and brine fermented to produced *moromi* which is then filtered and cooked with coconut sugar and spices for 1 h to afford *kecap manis*. The brine fermentation took up to 4 months, during the first month of which the content of glutamic acid reached a maximum of 14.33%, based on the dried matter. The content of glutamic acid was then decreased and a similar trend was observed for some of the amino acids. The flavor volatiles of *kecap manis* were then studied after different fermentation periods for *moromi* preparation (0, 1, 2, 3, 4 months). The volatiles contained 16 acids, 4 aliphatic aldehydes, 1 phenol, 6 aliphatic ketones, 21 furans, 10 pyrazines, 1 pyran, 3 pyrroles, 1 thiazole, 1 pyridine, 4 alicyclic hydrocarbons, and 11 unknowns. While these volatiles were qualitatively similar, there were quantitative differences. Thus, the content of pyrazines and acids increased as the fermentation progressed. However, the highest *gurih* (tasty, *umami*) taste was attained for *kecap manis* prepared from 1-month-fermented *moromi*. Analysis of sensory description, consumer preference and volatiles composition of seven commercial *kecap manis* showed that the preference of consumer to *kecap manis* was more affected by its taste where sweet and *gurih* (tasty, *umami*) were the taste that the consumer like. Volatile composition apparently do not correlated well with the aroma of *kecap manis*.

INTRODUCTION

Soy sauce is one of the most popular seasoning uses, especially in the Asian countries. The most known type of soy sauce is the salty soy sauce which possesses mainly salty and *umami* taste with appetizing aroma. This soy sauce can be grouped further into two types, i.e. a Japanese type (only found in Japan) and a Chinese type (produced in China and South East Asia). The Japanese soy sauce uses soybeans and wheat (ratio 1:1) as the main raw material, whereas the Chinese type uses no or very little wheat beside soybeans (Röling, 1995). The main preparation of this soy sauce involves mold and brine fermentation and the preparation is then pasteurized during the final stage.

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Unlike the salty soy sauce, the taste of *kecap manis* (a typical Indonesian soy sauce) is mainly sweet and *gurih* (tasty, *umami*) with a typical aroma. Its preparation involves also mold and brine fermentation to produce moromi, as applied for the salty soy sauce, however, at the final stage the filtered moromi are cooked with coconut sugar (45–50% of the total raw materials) and spices for 1 h. A similar type of soy sauce to *kecap manis* is also produced in Malaysia and Singapore named *kicap* (Röling, 1995), however, its preparation does not involve cooking for a long time and no spices, but some caramel, are added to it.

Previous studies on *kecap manis* showed that the Maillard reaction plays a major role in the formation of volatiles and color of *kecap manis* involving coconut sugar (as a source of sugar) and moromi (as a source of amino acids and peptides) (Apriyantono *et al.*, 1996, 1997). Therefore, focused on analyzing the changes of free amino acids during brine fermentation (*moromi* preparation) for 0, 0.5, 1, 1.5, 2, 3 and 4 months. *Kecap manis* produced from *moromi* obtained from those fermentation times were also analyzed for their volatiles composition and consumer acceptance as well as quantitative sensory description. In addition, volatiles composition of seven brands of commercial *kecap manis* and correlations with their sensory description and consumer preference were also examined.

EXPERIMENTAL

Materials

Materials for *kecap manis* preparation, i.e. black soybeans, coconut sugar, salt, Chinese star anise, fennel and *Aspergillus sojae* starter, were obtained either from a local market in Bogor (Indonesia), or traditional *kecap manis* industries located in Bogor. Seven samples of commercial *kecap manis* were also obtained from local markets in Bogor.

Starter Preparation

Ten grams rice and 10 ml water were put in a petri dish, sterilized at 121 °C for 20 min, cooked and then inoculated with *Aspergillus sojae*. The mole were incubated at room temperature for 4 days and then dried in an oven at 45 °C for 3 days. Finally, the starters were powdered with a dry blender.

Moromi Preparation

One kilogram black soybeans were cooked with 3 L water for 1 h, cooled and drained. The soybeans were then inoculated with 0.5% *Aspergillus sojae* starter, spread in bamboo trays and finally incubated at room temperature for 72 h to give *koji*. One kilogram *koji* was soaked in 3L water and 600 g salt in a bucket and then incubated at room temperature for given times for each bucket, i.e. 0, 0.5, 1, 1.5, 2, 3 and 4 months. The *moromi* were stirred twice a day and exposed to sunlight every day for about 7 h. Water was added to each bucket once a week to replace the evaporated volume. Preparation was done in triplicate for each fermentation time.

Moromi Filtrate Sample Preparation

The *moromi* from each fermentation time batch was filtered using cheese cloth. Seven hundred fifty milliliters were added to the residue and then the residue was pressed

using an hydraulic press at an increasing pressure from 0 to 100 kg/cm^2 · 10 min. Seven hundred fifty milliliters were added again to the residue and the residue was pressed again with the hydraulic press at the same increasing pressure. All the filtrate obtained were combined and ready for preparation of *kecap manis* and analysis of pH, total nitrogen and formol nitrogen. Part of the filtrates were also freeze dried, kept in plastic bags and stored in a freezer until time for free amino acids analysis.

Kecap Manis Preparation

Coconut sugar (1 kg) was dissolved in water (300 ml) and boiled for 10 min. The solution was mixed *moromi* filtrate (750 ml), boiled for 50 min, and then mixed with 4 ml spice extract (prepared by heating until boiling for 15 min and filtering a mixture of 25 g fennel and 6 g Chinese star anise in 100 ml water). Finally, the mixture was boiled for another 10 min, filtered, cooked, and bottled.

Free Amino Acids Analysis

The method used was a modification method suggested by Bidlingmeyer *et al.* (1987). To separate the free amino acids, the freeze dried filtrate was dissolved in 5 ml solution containing internal standard, i.e. α-aminobutyric acid (0.24 mM internal standard in 0.01 N HCl). Two milliliters of acetonitrile were added to 2 ml of the solution, centrifuged at 10,000 rpm for 45 min. The filtrate was dried in air, dissolved in sodium citrate buffer pH 2.2 and injected to HPLC with a post derivatization technique and OPA (ortho pthalaldehyde) was used as the derivatizing agent. Ion exchange column (Shimpack, Shimadzu), mobile phase of 1M sodium citrate buffer pH 3.2, 4.2, 9.0, and 11.0 with a multistep gradient, and a fluorescence detector was used for the analysis.

Volatiles Analysis

Volatiles of *kecap manis* were extracted using a Likens-Nickerson apparatus with diethyl ether as the extraction solvent. The extracts were then dried with anhydrous sodium sulfate, concentrated using a rotary evaporator followed by flushing with nitrogen gas until the volume was reduced to approximately 0.5 ml. The extracts were analyzed using a GC-MS and identification was done by matching the mass spectra obtained with those present in the NIST Library or published literature. Identification was confirmed by matching their LRI values with those reported in the literature. Quantification of volatiles was carried out using GC-MS with 1,4-dichlorobenzene as the internal standard.

Other Chemical Analysis

The pH was measured using a pH meter, total nitrogen, and formol nitrogen were determined using Kjeldahl method, and formol titration method (Judoamidjojo, 1986), respectively.

Sensory Analysis

Consumer preference for *kecap manis* was assessed by 20 or 25 semi-trained panelists, whereas for quantitative flavor profiling 10 trained panelists and a series of standard for sensory description were used (Table 1) as given by Hashim *et al.* (1995).

Table 1. Standards used for sensory description

Description	Score[a]	Standard
Taste		
Sweet	80	7.5% sugarcane solution
	50	2.5% sugarcane solution
Salty	80	0.5% sodium salt solution
	50	0.25% sodium salt solution
Gurih (tasty, *umami*)	150	50% Tetrapack liquid coconut milk (Kara brand)
	50	6.25% Tetrapack liquid coconut milk (Kara brand)
Bitter or bitter aftertaste	50	0.05% caffeine solution
	20	0.025% caffeine solution
Sour	50	0.1% citric acid solution
	20	0.05% citric acid solution
Aroma		
Coconut sugar, palm sugar, or sugarcane	80	coconut sugar, palm sugar, or sugarcane (undissolved)
Caramel	75	sugarcane caramel (prepared by heating sugarcane until the caramel was formed where all sugar was melted)
Acid	75	3.75% acetic acid solution
Koji	50	*koji* obtained from a local *kecap manis* industry in Bogor
Moromi	75	*moromi* obtained from 1.5 month brine fermentation time
Spice	80	a mixture of roasted fennel and Chinese star anise (1:1)
Smoky	50	2.5% charsol C-10[b] solution

[a]The score was selected arbitrarily based on preliminary examination of the relative intensity of each description and the logarithmic relationship between concentration and intensity.
[b]A flavoring obtained from Quest International Indonesia.

Statistical Analysis

Multivariate analysis was performed using a computer software, i.e., The Unscrambler version 6.0 (Camo AS, Norway, 1996).

RESULTS AND DISCUSSION

The pH, Total Nitrogen, and Formol Nitrogen

The pH of *moromi* filtrate of 0 month brine fermentation time was 5.37. The pH dropped drastically to 4.30 for *moromi* filtrate of 0.5 month brine fermentation time and then remained relatively unchanged until 2 months brine fermentation time (Figure 1). A similar trend was observed by Röling *et al.* (1994) who observed the pH change during brine fermentation of *Kecap* (Indonesian soy sauce) either prepared in the laboratory, or taken from a traditional manufacture. The decrease of pH is expected since lactic acid bacteria grow during brine fermentation of soy sauce and produce high amounts of lactic acid. However, the pH was then increased from 2 to 4 months fermentation time (Figure 1). This increase indicates two things, i.e., a great reduction of lactic acid bacteria activity (Röling *et al.*, 1994) and a possible formation of basic compounds as a result of degradation of soybean components which are more likely to be components derived from proteins.

Total nitrogen of *moromi* filtrate increased up to 2 months and then decreased, whereas formol nitrogen increased up to 1 month and then decreased slightly up to 4

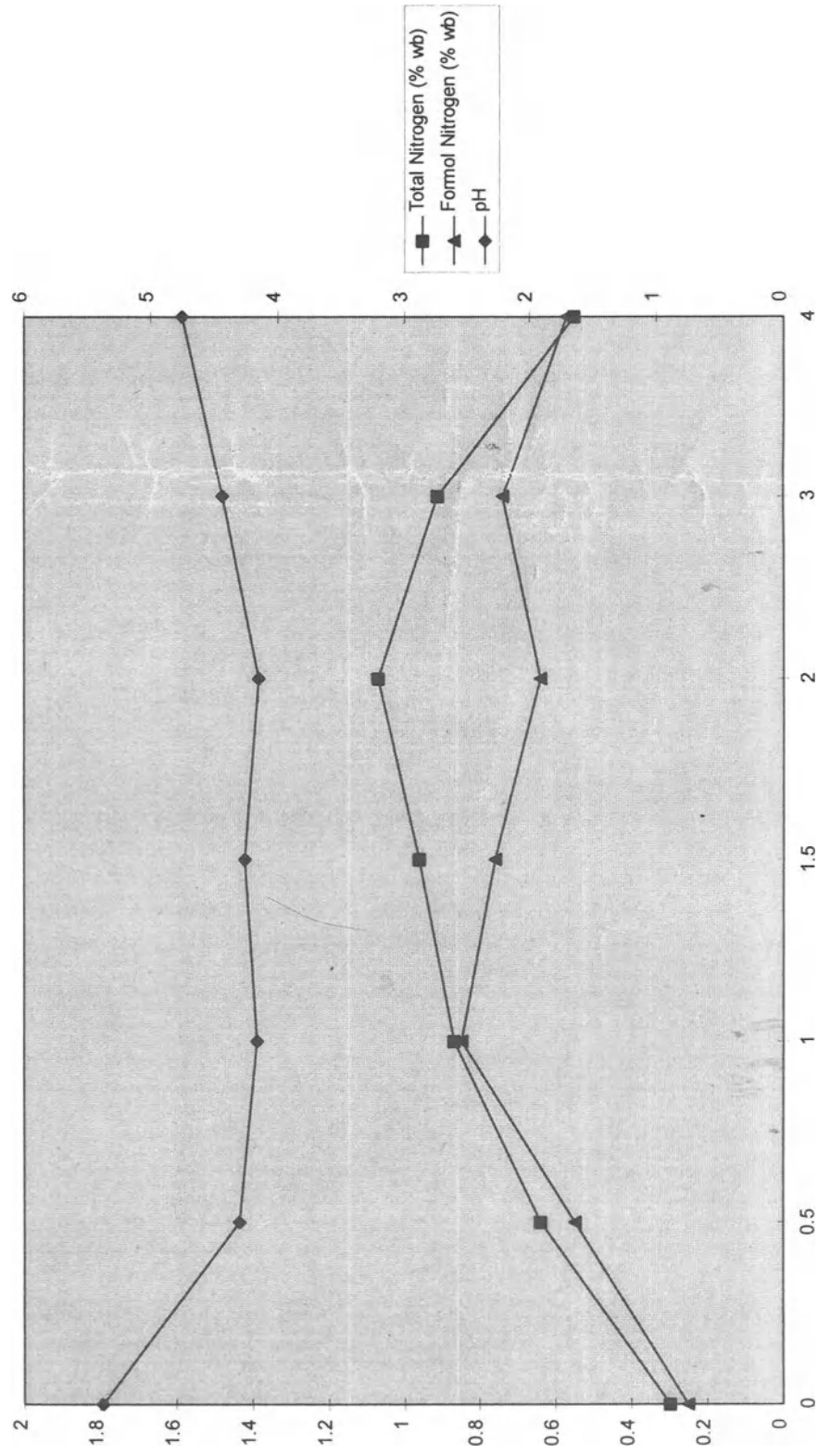

Figure 1. Change of pH, total nitrogen and formol nitrogen with increasing brine fermentation time (left Y-axis is for total and formol nitrogen and right-Y axis is for pH).

months fermentation time (Figure 1). The increase of total nitrogen is expected, but the decrease of total nitrogen after 2 months fermentation time is quite surprising. Previous observations by Poesponegoro *et al.* (1978) revealed that total nitrogen of *moromi* increased up to 1 month and then remained relatively unchanged up to 4 month brine fermentation time. A similar trend for change of soluble proteins during brine fermentation time was also investigated by Röling *et al.* (1994). The decrease of total nitrogen after 2 months brine fermentation time observed in this study was concomitant with the increase of pH as has been discussed above. Therefore, the formation of basic compounds derived from proteins after 2 months brine fermentation is very likely. Ammonia formed during brine fermentation is the most possible compound responsible for the increase of pH, since formation of ammonia during brine fermentation of Indonesian traditional soy sauce increased constantly, whereas lactate and acetate increased markedly up to 20 days and then increased slightly up to 70 days (Röling *et al.*, 1994). However, ammonia is volatile, therefore, some ammonia may be released from the solution causing the decrease of the total nitrogen.

It is quite interesting to note that the highest value of formol nitrogen was attained by *moromi* filtrate of 1 month brine fermentation time. The value of formol nitrogen could express the amount of free amino acids and ammonia, therefore, the formation of free amino acids had already reached a maximum at 1 month brine fermentation time. Since free amino acids, especially glutamic acid, are the components responsible for the taste of soy sauce, therefore, 1 month is apparently the optimum brine fermentation time. This is supported by the conclusion given by Röling *et al.* (1996) who stated that brine fermentation of 1 month is sufficient for industrial Indonesian soy sauce production. Their conclusion was based on the fact that during industrial brine fermentation the contents of formol nitrogen and glutamic acid increased up to 20 days and then leveled off up to 120 days brine fermentation time whereas other parameters such as activity of glutaminase, protease and leucine amino-peptidase enzymes also followed a similar trend.

Free Amino Acids

In total, 15 free amino acids were identified in the *moromi* filtrate and another 4 peaks were present in the HPLC chromatogram of the *moromi* filtrate were not identified. The identified amino acids were aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine (Table 2). The free amino acids present in the *moromi* filtrate with the highest amount were glutamic acid, aspartic acid and alanine.

Total free amino acids present in the *moromi* filtrate increased up to 1 month brine fermentation time and then decreased (Table 2). This was in line with the increase of formol nitrogen up to 1 month brine fermentation and then decreased (Figure 1). Many individual amino acids followed this trend including glutamic acid, alanine, phenylalanine and glycine. Some others reached maximum at 0.5 month brine fermentation time, i.e. arginine, lysine, serine and leucine, whereas the rest amino acids either relatively unchanged (aspartic acid, methionine, and histidine), or increased slightly (tyrosine). The decrease of the amount of amino acids after 1 month brine fermentation time indicates two things, i.e. the use of amino acids as a carbon source of microorganisms grown during brine fermentation, and/or amino acids react with sugars to form brown color compounds as indicated by the increase of brown color of *moromi* filtrate during brine fermentation of industrial Indonesian soy sauce (Röling *et al.*, 1996) or Indonesian soy sauce prepared in the laboratory (Apriyantono and Marianti, 1999).

Table 2. Amount of free amino acid[a,b] present in *moromi* filtrate brine fermented for 0–4 months

Amino acid	Brine fermentation time (month)						
	0	0.5	1	1.5	2	3	4
Aspartic acid	6.26	4.61	6.89	5.45	4.69	3.64	5.63
Threonine	2.58	3.52	3.55	2.87	2.04	0.37	1.11
Serine	4.04	4.58	4.13	4.03	3.72	0.20	0.30
Glutamic acid	7.05	10.6	14.33	9.43	9.49	4.97	6.87
Glycine	1.86	2.40	3.12	2.83	3.16	1.93	2.04
Alanine	5.49	10.42	13.13	11.63	11.16	7.27	9.04
Valine	3.12	3.56	5.02	3.88	3.77	2.62	3.49
Methionine	0.56	0.64	0.83	0.62	0.58	0.53	0.40
Isoleucine	1.31	2.15	2.48	2.08	3.27	2.62	1.79
Leucine	2.10	5.95	4.82	5.20	3.38	3.27	3.72
Tyrosine	0.86	1.20	1.27	1.52	0.93	1.05	1.73
Phenylalanine	0.76	2.59	2.93	2.71	2.24	1.55	2.11
Histidine	1.59	1.61	2.04	1.69	1.63	1.19	1.52
Lysine	0.76	2.60	1.41	1.40	2.45	1.50	1.22
Arginine	0.35	3.12	1.64	2.12	2.38	2.08	1.48
Total	38.69	59.55	67.59	57.46	54.89	34.79	42.45

[a]% weight of dried material.
[b]Average of 2 replicates.

It has been well known that amino acids play a role in taste of foods where they can have sweet, salty, bitter and *umami* effects. However, special attention should be paid to glutamic acid since this amino acid and its sodium salt possess *umami* tastes (Kirimura *et al.*, 1969; Solm, 1969). *Umami* taste is the taste expected to be the dominant and important taste of soy sauce. Therefore, the maximum time of the brine fermentation for glutamic acid formation, in particular, and free amino acids in general, should be considered to be the optimum time of brine fermentation as far as the taste of soy sauce is concerned. Since the formation of free glutamic acid reached maximum at 1 month brine fermentation time, accordingly, 1 month is the optimum brine fermentation time for production of Indonesian soy sauce.

Volatiles Composition

In total, 98 volatiles were detected in *kecap manis* prepared from *moromi* brine fermented for 0, 1, 2, 3 and 4 months. They included 4 aliphatic aldehydes, 6 aliphatic ketones and lactone, 4 aliphatic alcohols, 16 acids, 5 esters, 21 furans, 10 pyrazines, 3 pyrroles, 1 thiazole, 1 pyran, 1 pyridine, 11 benzene derivatives, 4 alicyclic hydrocarbons and 11 unknowns (Table 3). A similar composition was also found in 7 commercial *kecap manis* where 77 volatiles were detected including 5 aliphatic alcohols, 6 aliphatic ketones, 1 lactone, 2 aliphatic aldehydes, 18 acids, 4 aliphatic esters, 1 monoterpenoid, 8 alicyclics, 8 benzene derivatives, 9 phenols, 8 furans, 1 pyrrole, 5 pyrazines, and 1 unknown (Table 4). From this composition, it is clear that many volatiles are derived from the Maillard reaction such as furans, pyrazines, pyrroles, etc. These compounds are mainly derived from coconut sugar as one of raw material of *kecap manis*, and also formed during the cooking step. Other components may be derived from spices, e.g. anethole, and *moromi*, e.g. esters, aldehydes, carboxylic acids, alcohols and benzene derivatives (Apriyantono *et al.*, 1996). A number of acids, e.g. malic and citric acids were also derived from coconut

Table 3. Volatiles composition of *kecap manis* prepared from *moromi* filtrate brine fermented for 0–4 months

No.	LRI[a]	Component	Relative percentage area[b] (%)				
			0 month	1 month	2 months	3 months	4 months
Aliphatic aldehydes							
1	925	2- and 3-Methylbutanal (E,E)-2,4-Decadienal	23.39	18.00	22.70	17.70	17.28
2	1822	2-Hexenal	nd	nd	0.05	0.09	0.03
3	2088	Tetradecanal	0.12	1.8	0.38	0.22	0.06
4	2121		0.09	0.16	0.02	0.48	nd
Aliphatic ketones and lactones							
5	992	2,3-Butanedione	1.63	0.91	1.17	0.92	1.43
6	1071	2,3-Pentanedione	0.57	0.47	0.60	0.51	0.45
7	1301	3-Hydroxy-2-butanone	0.04	0.10	0.04	0.09	0.16
8	1317	1-Hydroxy-2-propanone	0.09	0.18	0.12	0.20	0.24
9	1642	5-Methyl-3-hexen-2-one	0.10	0.07	0.11	0.03	0.04
10	1647	Butyrolactone	0.04	0.08	0.05	0.08	0.09
Aliphatic alcohols							
11	938	Ethanol	7.21	4.47	5.81	6.10	5.33
12	1050	2-Methyl-3-buten-3-ol	0.14	0.27	0.24	0.14	0.05
13	1219	3-Methyl-1-butanol	0.39	0.22	0.47	0.19	0.10
14	1335	3-Penten-2-ol	0.04	0.02	0.02	nd	nd
Carboxylic acids							
15	1467	Acetic acid	16.87	16.58	12.36	21.65	21.80
16	1564	Propanoic acid	0.24	0.19	0.27	0.64	0.49
17	1593	2-Methylpropanoic acid	0.24	0.14	0.28	0.24	0.32
18	1654	Butanoic acid	0.13	0.09	0.1	0.11	0.24
19	1693	2-Methylbutanoioc acid	0.96	0.78	1.56	3.70	2.70
20	1763	Pentanoic acid	0.02	0.03	0.02	0.06	1.38
21	1815	3-Methylbutanoic acid	nd	nd	0.07	0.07	0.07
22	1827	4-Methylpentanoic acid	0.04	0.03	0.20	0.21	0.15
23	1869	Hexanoic acid	0.24	0.39	0.15	0.13	0.30
24	1970	2-Ethylhexanoic acid	0.12	0.08	0.06	0.02	nd
25	1974	Heptanoic acid	nd	nd	0.06	0.10	nd
26	2081	Octanoic acid	0.23	0.18	0.39	0.49	0.60
27	2187	Nonanoic acid	0.04	0.10	0.09	0.03	0.12
28	>2200	A carboxylic acid	0.18	0.07	0.11	0.17	0.07
29	>2200	Decanoic acid	0.72	0.27	0.57	0.34	0.36
30	>2200	Dodecanoic acid	0.49	1.22	0.96	0.27	1.21
Esters							
31	2199	Hexadecanoic acid methyl ester	0.03	0.15	0.08	0.17	0.02
32	>2200	Hexadecanoic acid ethyl ester	0.17	0.17	0.28	0.36	0.11
33	>2200	9,15-Octadecadienoic acid methyl ester	nd	nd	0.06	0.13	nd
34	>2200	Oleic acid ethyl ester	0.66	0.25	0.32	0.03	0.16
35	>2200	Linoleic acid ethyl ester	1	0.28	0.55	0.5	0.29
Furans							
36	1241	2-Pentylfuran	nd	nd	nd	nd	0.1
37	1278	2-Methyldihydro-3(2H)-furanone	3.91	3.30	3.46	3.37	2.40
38	1448	5-Methyl-2-(3H)-furanone	0.07	0.04	0.09	0.04	0.04
39	1454	cis-5-Trimethyl-3-ethenyltetrahydro-2-furanmethanol	nd	15.49	0.13	nd	nd
40	1484	2-Furancaboxaldehyde	13.67	10.80	13.45	7.94	8.93
41	1522	2-Acetylfuran	1.95	nd	2.03	1.33	1.61
42	1546	A furan MW 124	nd	0.18	nd	nd	nd

(*continued*)

Table 3. (*Continued*)

No.	LRI[a]	Component	Relative percentage area[b] (%)				
			0 month	1 month	2 months	3 months	4 months
43	1554	2-Furanmethanol acetate	0.01	nd	0.02	0.06	0.05
44	1590	5-Methyl-2-furancarboxaldehyde	1.46	1.20	1.65	1.71	1.60
45	1611	2-2′-Bifuran	0.04	0.03	0.08	nd	0.02
46	1614	(1-Methylethyl)-2,3-dihydro-4-furan	0.05	nd	nd	0.02	nd
47	1623	A dimethylfurfural	0.08	nd	0.08	0.12	0.08
48	1681	2-Furanmethanol	5.53	4.31	4.75	5.10	5.47
49	1794	A furan MW 138	nd	0.07	0.06	0.10	0.08
50	1828	A furan	nd	0.07	1.20	nd	0.02
51	1869	3-Phenylfuran	0.12	0.04	nd	nd	0.44
52	1876	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.50	0.93	0.07	nd	nd
53	1883	A furan MW 152	0.04	0.03	0.07	0.03	0.48
54	>2200	5-(2-Octenyl)-dihydro-2(3H)-furanone	0.10	0.10	0.50	0.62	0.47
55	>2200	2,3-Dihydrobenzofuran	0.04	0.05	0.14	nd	0.02
56	>2200	5-Hydroxymethyl-2-furancarboxaldehyde	0.05	0.09	0.06	0.04	0.17
Pyrazines							
57	1322	2,5-Dimethylpyrazine	0.09	0.42	0.17	0.79	0.46
58	1338	2,6-Dimethylpyrazine	0.90	1.04	1.56	2.72	1.39
59	1344	Ethylpyrazine	0.02	0.06	0.05	0.17	0.09
60	1356	2,3-Dimethylpyrazine	0.12	0.39	0.15	0.32	0.35
61	1394	2-Ethyl-6-methylpyrazine	0.05	0.09	0.18	0.30	0.12
62	1400	2-Ethyl-5-methylpyrazine	nd	nd	0.05	0.13	0.07
63	1413	Trimethylpyrazine	0.10	0.16	0.18	0.48	0.41
64	1456	Ethenylpyrazine	nd	nd	0.04	0.13	2.51
65	1500	2-Ethenyl-6-methylpyrazine	0.08	0.12	0.20	0.34	0.17
66	1705	2-Acetyl-3-methylpyrazine	nd	nd	0.06	0.12	0.04
Pyrroles							
67	1991	2-acetylpyrrole	0.17	1.47	1.00	1.07	1.78
68	2092	1-(H-Pyrrole)-methyl-2-carboxaldehyde	0.03	0.03	0.06	0.03	0.26
69	2047	1H-pyrrole-2-carboxaldehyde	0.14	0.12	0.154	0.22	0.18
Thiazole							
70	1292	4-Methylthiazole	0.3596	0.31	0.46	0.34	0.44
Pyran							
71	1292	Maltol	0.03	0.05	nd	0.05	0.14
Pyridine							
72	1982	2-acetylpyridine	0.04	0.02	0.05	0.04	0.05
Phenol							
73	2030	Phenol	0.02	nd	nd	nd	0.13
Benzene derivatives							
74	1538	Benzaldehyde	0.25	nd	0.31	0.32	0.12
75	1841	1-Methoxy-4-(1-propenyl)benzene	1.18	1.01	1.24	1.65	1.47
75	1661	Benzeneacetaldehyde	7.01	7.39	7.78	7.35	7.43
77	1895	Benzyl alcohol	0.03	0.04	0.07	0.14	0.05
78	1947	alpha-Ethylene-benzeneacetaldehyde	nd	0.05	0.07	0.12	0.1
79	2041	4-Methoxybenzaldehyde	0.06	0.27	0.20	0.31	0.11
80	>2200	4-Methoxy-benzeneacetic acid	0.17	0.17	0.14	0.47	0.12
81	1929	Phenylethyl alcohol	nd	nd	0.16	0.14	0.16
82	1895	Benzyl alcohol	0.03	0.04	0.07	0.14	0.05
83	2172	1-(4-Methoxyphenyl)-2-propanone	0.30	0.15	0.10	0.19	0.05
Alicyclic hydrocarbons							
84	1400	1,3,3-Trimethylbicyclo(2,2,1)-2-heptanone	0.02	nd	0.07	nd	0.02
85	1203	1-Methyl-4-(1-methyl)cyclohexene	nd	nd	0.08	0.07	0.02

(*continued*)

Table 3. (*Continued*)

No.	LRI[a]	Component	Relative percentage area[b] (%)				
			0 month	1 month	2 months	3 months	4 months
86	1847	3-Methyl-2-hydroxy-1,2-cyclopenten-1-one	nd	0.24	0.08	0.09	0.14
87	1604	4-Cyclopenten-1,3-dione	1.00	0.67	0.78	0.53	0.81
Unknowns							
88	1390	Unknown MW 126	nd	nd	0.03	0.03	0.02
89	1515	Unknown MW 112	0.04	0.04	0.05	0.03	0.03
90	1583	Unknown MW 248	0.03	nd	0.06	0.12	nd
91	1794	Unknown MW 138	nd	0.02	0.58	0.12	0.09
92	1741	Unknown MW 112	2.08	1.64	1.80	2.20	1.60
93	2068	Unknown MW 160	nd	0.02	0.10	0.14	0.11
94	2078	Unknown MW 179 or 192	0.22	nd	0.27	0.38	0.24
95	2081	Unknown MW 160	nd	0.08	0.06	nd	0.06
96	2199	Unknown MW 150	0.27	0.21	0.16	0.09	0.03
97	2006	Unknown MW 146	0.07	0.10	0.07	0.14	0.09
98	1623	Unknown MW 134	nd	0.02	0.58	0.11	0.09

[a]LRI (Linear Retention Index) was determined in Carbowax BP-20 (SGE) column based on a series of standard alkanes (C_8-C_{22}).
[b]Relative percentage area of total ion chromatogram. The figures given are the average of 2 replicates.

sugar where these acids may play a role in forming the taste of *kecap manis* (Apriyantono and Wiratma, 1997).

The volatiles present in *kecap manis* prepared in the laboratory with a relative percentage area of more than 1% were 2 and 3-methylbutanal, 2,3-butanedione, ethanol, acetic acid, 3-methylbutanoic acid, 2-methyldihydro-3-(*2H*)-furanone, 2-furancarboxaldehyde, 5-methyl-2-furancarboxaldehyde, 2-furanmethanol, 2,6-dimethylpyrazine, benzaldehyde, 1-(*1H*-pyrrole)ethanone, anethole and an unknown MW 134 (Table 3). Based on Gas Chromatography-Olfactometry (GC-0) analysis, 2 methyldihydro-3(*2H*)-furanone was identified as a character impact compound of *kecap manis* (Wiratma and Apriyantono, 1995). This character impact compound of *kecap manis* is different from that of the Japanese soy sauce where 4-ethylguaiacol and 2(5)-ethyl-5(2)-methyl-4-hydroxy-3(*2H*)-furanone (HEMF) were character impact compounds (Nunomura and Sasaki, 1992)

Compared to the Japanese soy sauce, the volatiles composition of *kecap manis* are different. Therefore, alcohols, acids, esters, aldehydes and ketones were dominant in Japanese soy sauce (Nunomura and Sasaki, 1986; 1992), whereas those present in *kecap manis* were dominated by the Maillard reaction products. This difference is mainly due to the different method of their preparation. To prepare *kecap manis*, 45–50% of coconut sugar is added to the *moromi* and cooked for 1 h, whereas to prepare Japanese soy sauce either no sugar or very little at all is added to *moromi* and pasteurization, not boiling for a long time, is done in the final stage of preparation.

Volatiles composition of *kecap manis* prepared from *moromi* filtrate brine fermented at different time (0–4 months) was qualitatively similar (Table 3). However, some quantitative differences were observed. In general, the volatiles present in *kecap manis* which changed with time of brine fermentation were divided into 3 groups. The first group was those which their presence did not differ quantitatively (their relative percentage area) with time of brine fermentation up to 4 months, these included, 2,3-butanedione, 2,3-pentanedione, ethanol, 2-methyldihydro-3-(*2H*)-furanone, 2-furanmenthanol, anethole and 5-methyl-2-furancarboxaldehyde. The second group was those which their presence *kecap*

Table 4. Volatiles composition of commercial *kecap manis*

No.	Component	*Kecap manis*						
		A	B	C	D	E	F	G
1	Ethyl acetate	nd	nd	nd	0.1	nd	0.1	nd
2	2-Methylpropanal	0.13	nd	nd	nd	nd	nd	nd
3	3-Methylbuanal	17.5	8.17	18.6	17.3	6.1	17.9	2.35
4	2,3-Butanedione	0.12	0.89	0.11	0.36	0.34	0	0.16
5	1-Propanol	nd	0.1	nd	nd	nd	nd	nd
6	2,3-Pentanedione	nd	0.21	0.1	0.14	0.14	0.11	0.12
7	2-Methyl-1-propanol	nd	0.1	0.1	0.1	nd	0.1	nd
8	4-Methyl-1-pentanol	nd	0.2	nd	nd	nd	nd	nd
9	3-Methyl-1-butanol	nd	0.15	0.1	0.15	0.05	0.16	0
10	Pyrazine	nd	0	nd	nd	nd	nd	nd
11	Dihydro-2-methyl-3(*2H*)-furanone	0.56	3.1	2.37	4.69	2.27	3.43	2.47
12	3-Hydroxy-2-butanone	nd	0.16	0.1	0.1	0.06	nd	nd
13	1-Hydroxy-2-propanone	nd	0.31	0.1	0.12	0.19	0.1	0
14	2,6-Dimethylpyrazine	0.63	6.04	1.24	3.18	1.06	0.94	0.1
15	2-Hydroxypropanoic acid ethyl ester	nd	nd	0	nd	nd	0.1	nd
16	2,3-Dimethylpyrazine	nd	0.1	nd	0.1	0.04	nd	nd
17	2-Methylcyclopenten-1-one	nd	0.35	nd	nd	nd	nd	nd
18	1-Hydroxy-2-butanone	nd	nd	nd	nd	0.06	nd	nd
19	2-Ethyl-6-methyl pyrazine	nd	nd	0	0.1	nd	nd	nd
20	2-Ethyl-5-methyl pyrazine	nd	0.1	nd	nd	nd	nd	nd
21	Acetic acid	4.98	4.09	11.8	32.1	17.8	26.5	4.17
22	*cis*-Linalool oxide	nd	nd	0	nd	nd	nd	nd
23	2-Furancarboxaldehyde	8.43	4.3	6.06	9.13	3.18	5.34	6.68
24	4,4-Dimethyl-2-cyclopenten-1-one	nd	0.1	nd	nd	nd	nd	nd
25	Oxalic acid	nd	nd	0	nd	nd	nd	nd
26	1-(2-Furanyl)ethanone	2.5	1.69	1.28	2.34	1.18	1.5	1.92
27	Furan MW 124	nd	0.25	nd	nd	nd	1.61	0.1
28	Propanoic acid	0.1	0.26	0.14	0.29	0.03	0.21	0.1
29	2/3-Methyl-2-cyclopenten-1-one	nd	0.17	nd	nd	nd	nd	nd
30	2,3-Dimethyl cyclopent-2-en-1-one	nd	0.31	nd	nd	nd	nd	nd
31	2-Methylpropanoic acid	nd	0.2	0.38	1.6	0.09	0.19	nd
32	1-(2-Furanyl)-1-propanone	nd	nd	0	0.1	0.05	0.1	0.1
33	5-Methyl-2-furancarboxaldehyde	1.78	1.67	0.39	1.27	0.19	0.71	1.44
34	4-Cyclopenten-1,3-dione	0.1	0.1	0.12	0.23	0.09	0.17	0.25
35	5/6-Methyl-2-heptanol	nd	0.1	nd	nd	nd	nd	nd
36	Butanoic acid	nd	2.87	0.3	1.04	2.13	0.31	0
37	Anethole	nd	0	nd	nd	nd	nd	0.1
38	3-Ethylcyclopent-2-en-1-one	nd	0.1	nd	nd	nd	nd	nd
39	Benzeneacetaldehyde	1.3	nd	nd	nd	0.15	0.6	nd
40	2- and 3-Methylbutanoic acid	0.22	0.59	0.94	3.5	0.23	0.95	nd
41	Butyrolactone	nd	0	nd	nd	0.04	nd	nd
42	2-Furanmethanol	0.55	6.72	0.99	2.83	4.16	3.1	4.44
43	#-Ethyl-2-hydroxy-2-cyclopenten-1-one	nd	0.21	nd	nd	0.06	nd	0.1
44	Unknown MW 112	0.1	0.96	0.12	0.42	0.42	0.19	0.11
45	4-Methylpentanoic acid	nd	nd	0.1	0.1	nd	0.1	nd
46	2-Ethylbutanoic acid	nd	nd	0.1	nd	nd	nd	0
47	Furan MW 138	0.1	0.1	nd	nd	nd	nd	nd
48	1-Methoxy-4-(2-propenyl) benzene	nd	0.48	nd	nd	nd	nd	4.05
49	Hexanoic acid	nd	nd	nd	nd	0.07	nd	nd
50	3-Methyl-1,2-cyclopentanedione	nd	0.15	nd	nd	0.04	nd	nd
51	(*E*)-3-Octen-2-one	nd	0.1	nd	nd	nd	nd	nd
52	2-Methoxyphenol	nd	nd	0	nd	nd	nd	nd
53	2,6-Dimethylphenol	nd	0	nd	nd	nd	nd	nd

(continued)

Table 4. (*Continued*)

No.	Component	*Kecap manis*						
		A	B	C	D	E	F	G
54	Phenylethyl alcohol	nd	0.1	0.1	0.16	nd	0.1	nd
55	2-Ethylhexanoic acid	nd	nd	nd	nd	0.12	0.1	0.1
56	1-(*1H*-Pyrrol-2-acetyl)	nd	0.1	0.2	0.69	0.1	0.89	0.2
57	Phenol	0.11	0.55	nd	nd	nd	nd	nd
58	4-Hydroxy-2-methylacetophenone	nd	nd	nd	nd	nd	nd	0
59	1,2,4-Trimethylbenzene	nd	nd	nd	0.1	nd	nd	nd
60	Octanoic acid	nd	0.18	0.25	0.34	0.63	0.54	nd
61	4-Methoxybenzaldehyde	nd	0.18	0.25	0.34	0.63	0.54	nd
62	2,3-Dimethylphenol	nd	0.1	nd	nd	nd	nd	nd
63	3-Methylphenol	nd	0.16	nd	nd	nd	nd	nd
64	2-Methylphenol	nd	0.3	nd	nd	nd	nd	nd
65	Sorbic acid	nd	nd	nd	nd	nd	nd	18.4
66	2-Methoxy-4-(2-propenyl) phenol	nd	nd	0	nd	nd	nd	nd
67	A dimethylphenol	nd	0.34	nd	nd	nd	nd	nd
68	2,4,5-Trimethylphenol	nd	0	nd	nd	nd	nd	nd
69	Nonadecanoic acid ethyl ester	nd	nd	nd	0.1	nd	nd	nd
70	Decanoic acid	nd	0.16	0.19	0.91	0.73	0.46	nd
71	Benzoic acid	6.78	0.63	2.26	5.38	0.08	3.49	3.37
72	Benzyl benzoate	0.1	nd	nd	nd	nd	nd	nd
73	Dodecanoic acid	nd	0.15	0.56	3.81	1.64	1.78	0.1
74	Tetradecanoic acid	nd	0.13	0.1	0.55	0.2	0.26	nd
75	Hexadecanoic acid	0.2	nd	0.87	0.36	0.15	0.19	0.91
76	A carboxylic acid	nd	0.1	nd	nd	nd	nd	nd
77	Hexadecanoic acid ethyl ester	nd	0.22	0.1	0.3	nd	0.1	nd

[a]The figure is obtained from average of 2 replicates. nd = not detected.

manis quantitatively increased with time of brine fermentation; these included most pyrazines and acids, whereas the third group was those which quantitatively decreased; these included 2- and 3-methylbutanal, decanoic acid, 2-furancarboxaldehyde and 2-acetylfuran.

The increase of pyrazines during brine fermentation time is interesting to note since this may correlate with the change of amino acids of *moromi* filtrate during brine fermentation time, since pyrazines are formed from reactions between dicarbonyls and alpha amino acids produced via the Strecker degradation. The increase of pyrazines present in *kecap manis* prepared with *moromi* filtrate with increasing time of brine fermentation was supposed to correlate with the increase of total amino acids present in *moromi* filtrate brine fermented with increasing time. However, total amino acids present in *moromi* filtrate increased only up to 1 month brine fermentation time and then decreased (Table 2). The decrease of total amino acids after 1 month brine fermentation time may indicate that the formation of pyrazines had already taken place during brine fermentation, since the amino acids, apart from being used by microorganism grown in *moromi*, were used for pyrazine formation.

Sensory Analysis

It is quite surprising that *kecap manis* prepared from *moromi* filtrate brine fermented for more than 1 month were less preferred than those prepared for 0 and 1 month. It is also noted that less preferred *kecap manis* were produced from *moromi* filtrate prepared with increasing time of brine fermentation (Table 5). It was expected that *kecap manis* prepared

Table 5. Hedonic scale of consumer preference to flavor of *kecap manis* prepared from *moromi* filtrate brine fermented for 0–4 months

Brine fermentation time (month)	Hedonic scale of consumer preference to *kecap manis*[a,b]
0	5.5
1	5.1
1.5	4.4
2	4.5
3	3.5
4	2.8

[a]Average figure of 2 replicates with 20 panelists for each replicate.
[b]Hedonic scale ranging from 7 (like extremely) to 1 (dislike extremely).

from *moromi* filtrate and brine fermented for longer time would result in a more preferred *kecap manis* prepared from *moromi* filtrate (Table 2). In addition, the highest intensity of *gurih* taste (tasty, *umami*) was attained for *kecap manis* prepared from 1 month brine fermented *moromi* (Table 6). Therefore, *gurih* taste which would probably correlate well with glutamic acid content is one of the key factors affecting the consumer's preference. However, the interaction of several taste attributes may influence the consumer preference, since not much differences were observed for *kecap manis* prepared from *moromi* filtrate brine fermented for 0 and 1 month possessed caramel aroma with the highest intensity (Table 7) and was the most preferred by consumers (Table 5). Therefore, caramel and *moromi* aroma may influence the consumer preference of *kecap manis*, where *kecap manis* with the highest intensity of caramel aroma and the lowest intensity of *moromi* aroma are the most preferred.

When assessment of consumer preference to commercial *kecap manis* was based on taste or aroma (not flavor as a single criteria), it is clear that there was a different perception of consumer toward *kecap manis*. Based on the hedonic scale of consumer preference to the taste, commercial *kecap manis* could be divided into 4 groups where *kecap manis* C was most preferred and *kecap manis* G was least preferred (Table 8). However, based on the hedonic scale of consumer preference to aroma, *kecap manis* could only be divided into 2 groups (Table 8). Thus, consumers were more sensitive to taste rather than aroma as far as the consumer acceptance of *kecap manis* is concerned.

Table 6. Quantitative taste description of *kecap manis* prepared from *moromi* filtrate brine fermented for 0–4 months

Brine fermentation time (month)	Score of taste description of *kecap manis*[a,b]					
	Sweet	Sour	Salty	*Gurih*	Bitter	Aftertaste
0	94.7	13.1	35.2	21.7	8.0	7.8
1	96.0	16.6	35.8	27.2	7.3	15.4
1.5	94.6	16.0	33.6	19.0	4.5	9.9
2	94.8	13.6	27.8	18.4	3.9	7.8
3	97.7	19.9	43.6	24.4	9.5	14.8
4	87.1	15.1	43.2	20.2	9.5	12.5

[a]The score was based on intensity of each taste description standard as presented in Table 1.
[b]The figures are obtained from the average of 10 trained panelists.

Table 7. Quantitative aroma description of *kecap manis* prepared from *moromi* filtrate brine fermented for 0–4 months

Brine fermentation time (month)	Score of aroma description of *kecap manis*[a,b]						
	Coconut sugar	Spicy	Caramel	Acidic	Smoky	*Koji*	*Moromi*
0	56.3	6.3	62.9	17.4	5.1	7.7	3.6
1	57.0	7.4	63.0	14.7	4.9	7.3	5.6
1.5	49.3	5.1	44.9	13.4	5.3	2.9	6.3
2	60.0	4.7	45.4	17.1	4.0	4.3	6.7
3	47.3	6.3	44.3	18.7	2.6	10.3	8.4
4	54.7	8.6	41.4	16.6	3.1	11.1	21.4

[a]The score was based on intensity of each aroma description standard as presented in Table 1.
[b]The figures are obtained from the average of 10 trained panelists.

Table 8. Hedonic scale of consumer preference to aroma and taste of commercial *kecap manis*

Kecap manis	Hedonic scale[a,b,c]	
	Taste	Aroma
A	3.8^{2}	4.0^{1}
B	3.5^{2}	3.9^{1}
C	5.0^{1}	4.6^{1}
D	3.0^{3}	4.0^{1}
E	4.1^{2}	4.4^{1}
F	4.1^{2}	4.2^{1}
G	2.7^{4}	3.2^{2}

[a]Average figure of 25 panelists.
[b]Hedonic scale ranging from 7 (like extremely) to 1 (dislike extremely).
[c]Different superscript number indicates significantly different ($p<0.05$).

Kecap manis C which was most preferred by consumers possessed the highest intensity of sweet and *gurih* (tasty, *umami*) taste (Table 9). In addition, according to PCA (Principal Component Analysis) *kecap manis* C can be separately grouped from the others and characterized by having a high intensity of sweet and *gurih* taste (Figure 2). Therefore, sweet and *gurih* taste may play an important role for the preference of consumer to *kecap manis*. Conversely, aroma of *kecap manis* might not be important for affecting the consumer preference since they could not distinguish sensitively the preference to aroma of

Table 9. Quantitative taste description of commercial *kecap manis*

Kecap manis	Score of taste description of *kecap manis*[a,b]					
	Sweet	Sour	Salty	Bitter	*Gurih*	Aftertaste
A	69.8	19.8	54.1	12.7	16.5	6.4
B	62.3	29.6	68.3	35.5	19.2	15.9
C	75.0	14.0	47.4	13.1	32.2	8.4
D	64.8	22.3	51.9	15.4	18.7	7.6
E	66.1	21.9	59.9	27.2	19.7	15.8
F	67.4	17.2	70.5	27.7	19.1	10.1
G	51.3	18.6	57.7	22.7	12.1	14.2

[a]The score was based on intensity of each taste description standard as presented in Table 1.
[b]The figure is average of 2 replicates with 10 trained panelists for each replicate.

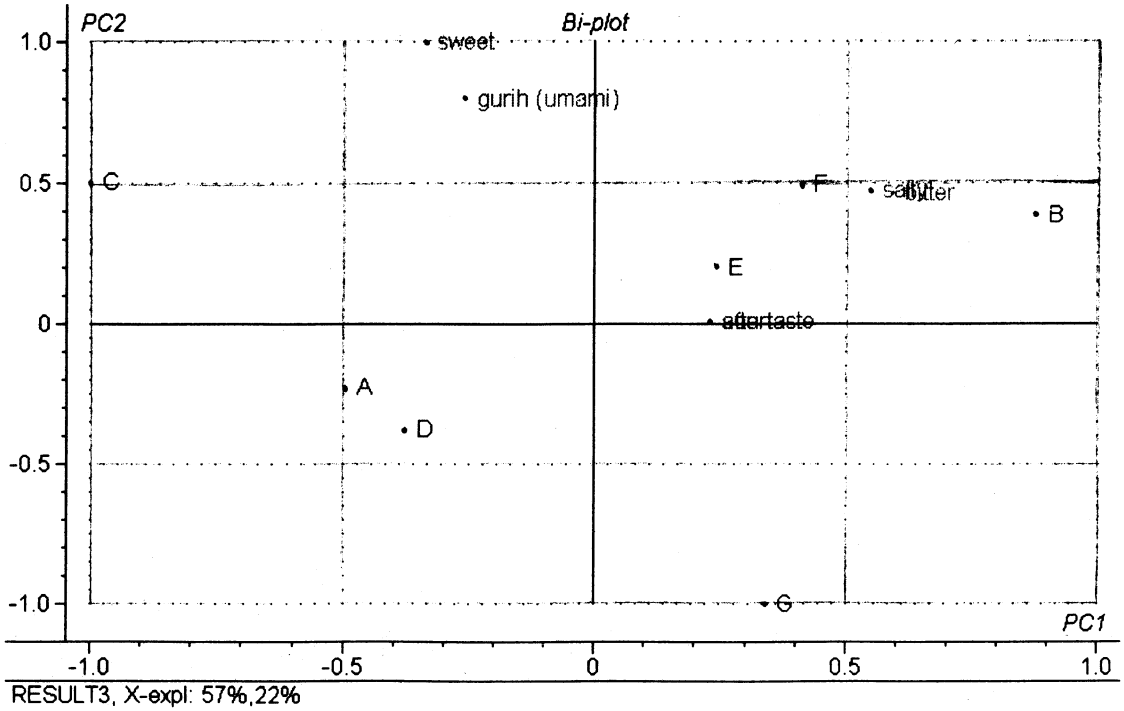

Figure 2. Biplot of PCA of commercial *kecap manis* taste.

kecap manis, unless it was judged dislike slightly by the consumer as was the case for *kecap manis* G (Table 8).

Unlike the results of quantitative aroma description and the consumer preference to flavor of *kecap manis*, samples prepared in the laboratory for different brine fermentation time (Tables 5 and 7) where the least *kecap manis* was the one with the highest intensity of *moromi* aroma, the least preferred of commercial *kecap manis* (*kecap manis* G) was not the one with the highest intensity of *moromi* aroma. Also, it was very difficult to find good correlation between the consumer preference to aroma of commercial *kecap manis* and its quantitative aroma description (Tables 8 and 10). Moreover, PCA of quantitative aroma

Table 10. Quantitative aroma description of commercial *kecap manis*

	Score of aroma description[a,b]								
Kecap manis	Palm sugar	Sugarcane	Coconut sugar	Caramel	Acid	*Koji*	Spice	Smoky	Moromi
A	29.9	33.1	28.6	36.1	10.0	17.2	11.2	11.4	3.0
B	30.4	15.9	24.0	22.3	7.8	7.7	12.7	32.8	11.5
C	15.6	26.7	40.3	24.9	10.5	13.1	13.6	12.2	5.2
D	15.2	28.2	35.9	31.1	19.1	21.1	10.0	11.2	10.3
E	23.9	29.4	55.4	33.5	15.6	18.4	13.2	7.2	7.4
F	29.6	22.8	47.8	36.5	9.7	9.1	11.3	5.7	6.7
G	26.0	28.4	28.3	21.8	12.3	15.2	14.0	9.9	6.3

[a]The score was based on intensity of each aroma description standard as presented in Table 1.
[b]The figure is average of 2 replicates with 10 trained panelists for each replicate.

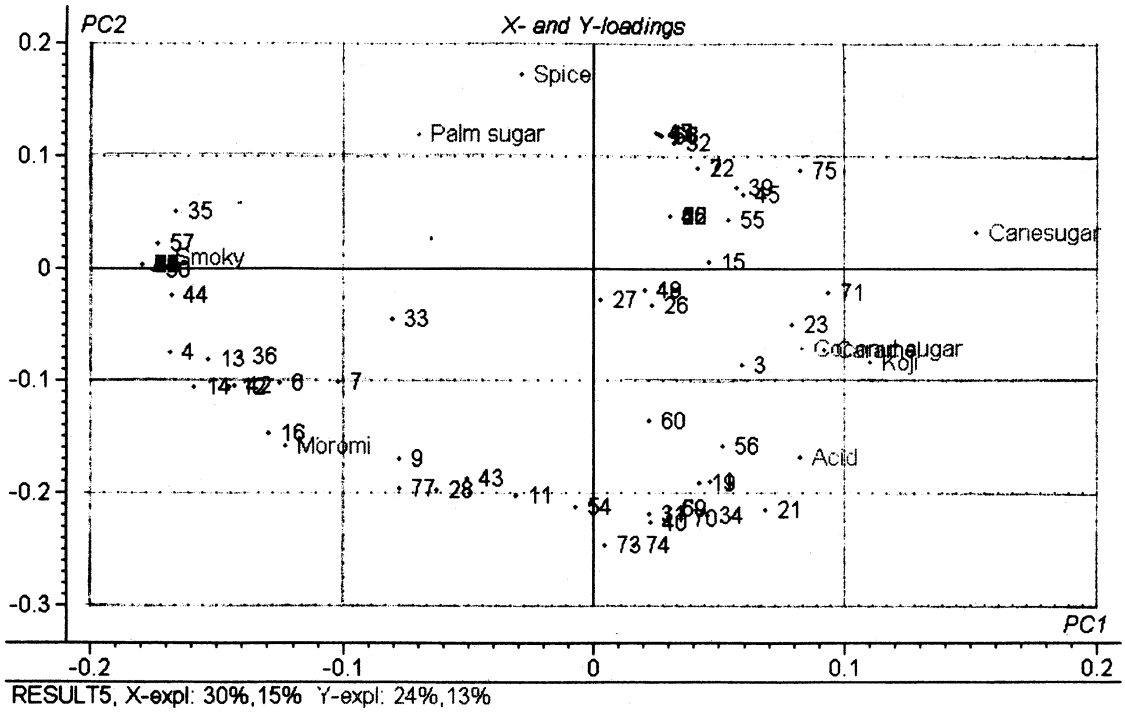

Figure 3. X and Y-loadings of commercial *kecap manis* aroma and volatiles composition.

description data presented in Table 10 showed that *kecap manis* could not be grouped well based on quantitative aroma description, since the sum of variation of PC1 and PC2, PC1 and PC3, or PC1 and PC4 were very small indicating that the relation between x-variables is very low. Therefore, the consumers were not sensitive enough to assess the aroma and they could not classify the aroma description well. In addition, it is clear that the consumer preference to *kecap manis* is more affected by its taste rather than its aroma.

Attempts were made to correlate volatiles composition of commercial *kecap manis* (Table 4) with quantitative aroma description of commercial *kecap manis* (Table 10), in order to make possible prediction of *kecap manis* aroma using its volatiles composition. To achieve this, PLS-2 (Partial Least Square) analysis was performed. However, very low variation (the sum of PC1 and PC3) either for X, or Y variable was obtained (Figure 3). Therefore, volatiles composition of *kecap manis* do not correlate well with its aroma, hence it can not be used for predicting its aroma profile.

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4

VOLATILE COMPOUNDS ISOLATED FROM SA CHA SAUCE

Chao-Yin Tai and Chi-Tang Ho

Department of Food Science
Cook College, Rutgers University
65 Dudley Road, New Brunswick, New Jersey 08901-8520

The volatile compounds of Sa Cha sauce were isolated by simultaneous steam distillation/solvent extraction. The extract possessed a strong Sa Cha sauce flavor. A total of 48 volatile compounds were identified. This group includes compounds derived directly from spices, from the Maillard reaction, and from thermal degradation of flavor precursors. The spice-derived components are the most dominant volatile constituents; 1-methoxy-4-(1-propenyl)-benzene (*trans*-anethole) which comes from star anise, overwhelms the whole volatile profile of Sa Cha sauce. The sulfides and other sulfur compounds, which originate mostly from the *Allium* species, are not only formed in significant quantities but because they have a relatively low odor threshold, also play an important role in the development of the overall flavor of Sa Cha sauce. Pyrazines, which are the products of the Maillard reaction, can be provided by roasted sesame and are also possibly generated through the interaction of dried shrimp, fish, and sugar.

INTRODUCTION

Sa Cha sauce is commonly used in many Taiwanese and southern Chinese cuisines. It is used primarily to marinate meat for barbecue and to stir-fry, and is also a favored dipping sauce for hot-pot meals. The ingredients of Sa Cha sauce generally include dried shrimp and fish, onion, garlic, sesame, oil, sugar, and spices. The ingredients can vary slightly from brand to brand.

Today, many commercially available culinary products such as Sa Cha sauce are actually traditional seasonings, which have been used for generations in Chinese cooking. The complexity of the ingredients used in Sa Cha sauce contributes to the unique flavor enjoyed by most Chinese. From the point of view of flavor chemistry, dried shrimp and dried fish used in some Chinese dishes provide a protein source and some characteristic aromas of dried seafood. Upon addition of sugar, the Maillard reaction system can be created during heating. Onion and garlic are the most prevalent culinary spices from *genus*

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Allium. Garlic, in particular, is an indispensable ingredient in Chinese cooking. Onion and garlic are known to contribute sulfide compounds which usually possess strong pungent and spicy organoleptic characters. In addition to being used in cooking, historically, onion and garlic have been used for medicinal purposes in many cultures (Fenwick and Hanley, 1985; Carson, 1987). The biological activities of the natural compounds in garlic and onion have drawn more attention recently. Sesame is another important ingredient used in Chinese cooking. Sesame oil, sesame sauce, and roasted sesame seeds have been frequently added into Chinese cuisine for their delightful aromas.

Chinese foods have become globally popular. As such, it is important to understand the volatile constituents found in the ingredients that create its unique flavor. In this study we investigate the flavor compounds of Sa Cha sauce. Our goal is to identify the volatile compounds which contribute to the flavor of Sa Cha sauce, and to then trace those compounds back to their origins.

MATERIAL AND METHODS

Volatile Isolation

This procedure was conducted using a Likens-Nickerson apparatus. One hundred grams of Sa Cha sauce was diluted with 800 mL of distilled water and then subjected to steam distillation for two hours. At the same time, the distillate was extracted constantly with 50 mL of methylene chloride. Before extraction, 0.5 mL of 1000 ppm tridecane was added into the Sa Cha sauce solution as the internal standard. Following extraction, the methylene chloride extract was dried over anhydrous sodium sulfate (5 g) and concentrated under a nitrogen flow to 0.3 mL/min.

Gas Chromatographic Analysis

The volatile compounds isolated from Sa Cha sauce were analyzed using a Varian 3400 gas chromatograph (GC) equipped with a fused silica capillary column (60 m x 0.32 mM i.d.; 1μm film thickness, DB-1, J&W Inc.) and a flame ionization detector. One microliter of the extract was injected into the GC with a split ratio of 25:1. The GC was ran with an injector temperature of 270°C, a detector temperature of 300°C, and a helium carrier flow rate of 1 mL/min. The column temperature was programmed as follows: from 40°C to 280°C with 2°C/min increasing rate.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The concentrate was analyzed by GC-MS, using a Hewlett Packard 5790A GC coupled to a Hewlett Packard 5970A MS. The column was the same as that used for gas chromatographic analysis. The mass spectra were obtained by electron ionization at 70 eV.

Identification of the Volatile Compounds

Identification of the volatile compounds was based on GC-MS analysis. The compounds from the isolate were identified by comparing the mass spectral data with those of authentic compounds available in the Wiley 138 computer library or the literature.

RESULTS AND DISCUSSION

The methylene chloride extract, obtained from steam distillation, possessed a strong Sa Cha sauce flavor indicating that the steam distillation method worked successfully to isolate the volatiles. Following GC-MS analysis, a total of 48 volatile compounds were identified from the Sa Cha sauce, including sulfur compounds, pyrazines, benzene-derived compounds, carbonyl compounds, and other plant-derived compounds (Table 1).

Pyrazines

Pyrazines are typical Maillard reaction products which are involved in the interaction between amino acids and reducing sugars. We identified the following pyrazines in the Sa Cha sauce: methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, 2-vinyl-6-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine. All of the these compounds have also been identified in processed sesame (Manley, *et al.*, 1974; Schieberle, 1995; Shahidi *et al.*, 1997). In fact, pyrazines are important volatile constituents of roasted sesame seed (Manley, *et al.*, 1974; Schieberle, 1995). These pyrazines all have been described as having a roasted and nutty aroma. 2,3-Dimethylpyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine are the most abundant pyrazines, ranging from 2400 to 4500 ppb. Mono-, di-, and trimethylpyrazines all possess a relatively higher odor threshold value than the ethyl-substituted pyrazines. The odor threshold of pyrazines can be considerably reduced when one or more methyl groups are substituted by ethyl groups (Gudadagni *et al.*, 1972). 3-Ethyl-2,5-dimethylpyrazine, in which an ethyl group replaced a methyl group of trimethylpyrazine reduces the odor threshold 10 folds (Maga, 1992) and becomes an important compound of nutty aromas. 3-Ethyl-2,5-dimethylpyrazine has a strong roasted nutty odor, and is a major character-impact compound of peanut butter (Joo and Ho, 1997). Roasting is the vital step to develop sesame aromas. In comparison of several sesame processing methods, roasting is the most effective method to consume the amino acids in sesame seed and generate volatile compounds (Shahidi *et al.*, 1997). Another possible source of pyrazines comes from the products of the Maillard reaction which contain dried fish, dried shrimp, and sugar as ingredients.

Sulfur Compounds

Sulfides are the major volatiles found in Sa Cha sauce. They are the decomposition products of thiosulfinates, derived from amino acid flavor precursors such as S-alkyl (or alkenyl)-L-cysteine S-oxides of the *Allium* family including onion, garlic, scallion, leek, shallot, chive, etc. Due to the popularity of the *Allium* family in cooking, a considerable amount of research has been conducted to determine the mechanism for developing the characteristic aroma of the *Allium* species.

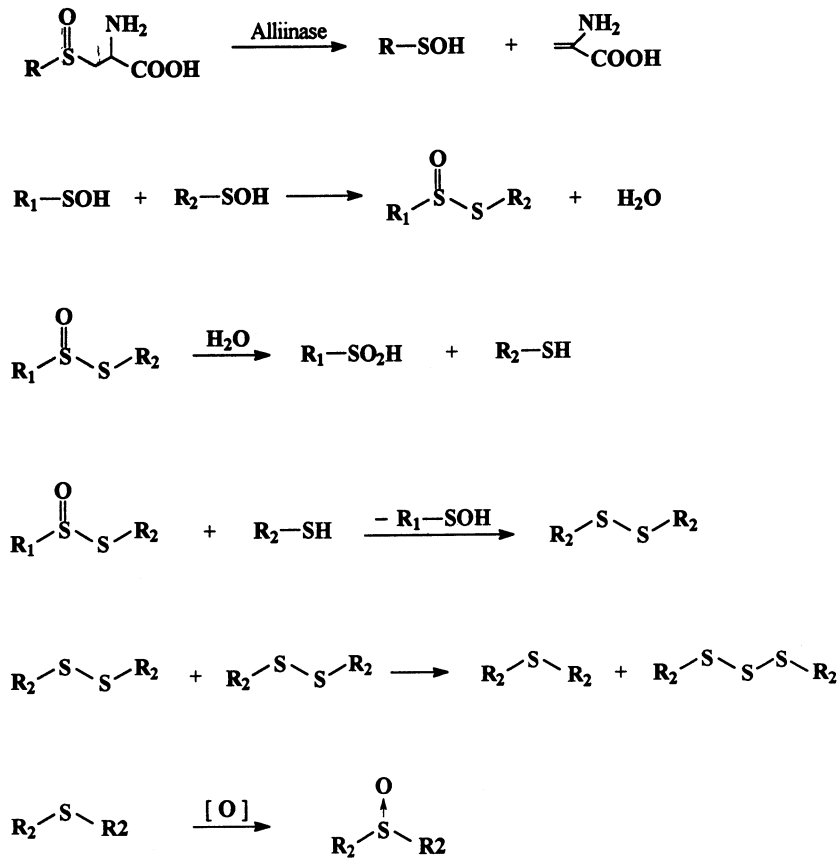
A total of thirteen sulfides were isolated from Sa Cha sauce. All of them have been reported in other research (Carson and Wang, 1961; Broddnitz and Pollock, 1970; Kuo and Ho, 1992a; 1992b; Yu *et al.*, 1993). The sulfides found in the largest amounts were methyl allyl trisulfide, diallyl disulfide, and methyl allyl disulfide. Diallyl sulfide, diallyl disulfide, and diallyl trisulfide are the flavor character-impact compounds of garlic (Nagodawithana, 1995). Even though the propenyl derivatives are not major compounds in Sa Cha sauce, they contribute significantly to the onion aroma (Brodnitz and Pollock, 1970). Methyl propyl disulfide is the flavor character-impact compound of onion as well (Nagodawithana, 1995). Brodnitz *et al.* (1969) indicated that di- and trisulfides were the major factors accountable for onion flavor.

Table 1. Volatile compounds isolated from Sa Cha sauce

Compounds	Possible precursors	Conc. (μg/kg)
Pyrazines		
Methylpyrazine	sesame	148
2,3-dimethylpyrazine	sesame	4471
2,5-dimethylpyrazine	sesame	2369
Trimethylpyrazine	sesame	3542
2-vinyl-6-methylpyrazine	sesame	150
3-ethyl-2,5-dimethylpyrazine	sesame	823
Sulfur compounds		
allyl methyl sulfide	garlic	1716
Dimethyl disulfide	garlic and onion	1013
diallyl sulfide	garlic	680
methyl allyl disulfide	garlic	4471
methyl propyl disulfide	garlic and onion	1032
methyl 1-propenyl disulfide	onion	1124
diallyl disulfide	garlic	8399
allyl propyl disulfide	garlic	891
Dimethyl trisulfide	garlic and onion	1757
methyl allyl trisulfide	garlic	8537
methyl propyl trisulfide	garlic and onion	1774
methyl 1-propenyl trisulfide	onion	451
diallyl trisulfide	garlic	213
methyl allyl sulfoxide	garlic	2342
methyl propyl sulfoxide	garlic and onion	512
allyl mercaptan	garlic	6536
1-propanethiol	garlic and onion	590
2,4-dimethylthiophene	garlic and onion	1057
Benzene derivatives		
Benzaldehyde	herb and spices	218
Phenol	herb and spices	320
Benzeneacetaldehyde	herb and spices	1422
1-(3-methylphenyl)-ethanone	star anise	635
Terpenoids		
dl-limonene	herb and spices	4408
γ-terpinene	herb and spices	264
Linalool	herb and spices	6868
(*E*)-farnesene	herb and spices	438
trans-caryophyllene	herb and spices	4881
(*Z,E*)-α-farnesene	herb and spices	979
α-humulene	herb and spices	650
4-methyl-1-isopropyl-3-cyclohexene	Herb and spices	404
Others		
2-butanone	Sugar	282
2,3-butanedione	Sugar	661
3-methylbutanal	Sesame, dried shrimp, fish	4304
2-methylbutanal	Sesame, dried shrimp, fish	3235
methyl butyrate		114
Dihydro-2-methyl-3(2H)-furanone	Sugar	1371
Hexanal	Oil	178
2-methyl-1-butanol		503

Allyl mercaptan and 1-propanthiol are both possibly the degradation products of thiosulfinates of the *Allium* family. Allyl mercaptan possesses a strong garlic aroma and has a very low odor threshold of 0.037 ppb in the air (Maga, 1976). 1-Propanthiol was described as having a cabbage odor and it too has a low odor threshold of 1.6 ppb in the air (Maga, 1976). Allyl mercaptan is the building block of such sulfides as diallyl sulfide, diallyl disulfide, and diallyl trisulfide. The condensation of allyl mercaptan and 1-propanethiol produces allyl propyl disulfide. However, the formation pathways of the sulfides were proposed through the direct disproportionation rearrangement of the thiosulfinates (Barnard, 1957) instead of the condensation of these elemental moieties. The existence of allyl mercaptan and 1-propanthiol, therefore, indicates the presence of their precursor allyl thiosulfinate and propyl thiosulfinate, respectively. The formation mechanisms of sulfides are postulated in Figure 1.

Both methyl allyl sulfoxide and methyl propyl sulfoxide are the oxidized forms of methyl allyl sulfide and methyl propyl sulfide which originate from the *Allium* family. Since sulfides are at the core of the flavor components in the *Allium* family, it would be in-



R, R_1, R_2 = allyl, methyl, propyl, or 1-propenyl

Figure 1. Mechanisms for the formation of volatile sulfur compounds in garlic and onion.

Table 2. The EI mass spectral data of methyl allyl sulfoxide and methyl propyl sulfoxide

Compounds	MW	Mass spectral data, m/z (relative intensity)
Methyl allyl sulfoxide	104	41 (100), 39 (72), 45 (54), 74 (52), 31 (43), 29 (35), 104 (13), 85 (8)
Methyl propyl sulfoxide	106	42 (100), 47 (95), 41 (73), 31 (59), 43 (58), 39 (41), 76 (40), 106 (25), 61 (13)

teresting to compare the sensory significance of the reduced and oxidized forms of the sulfides. The EI mass spectral data of these two compounds are listed in Table 2. The formation pathways of these two compounds are illustrated in Figure 1.

2,4-Dimethylthiophene is an important onion aroma contributor. It has been identified from onions processed in several different ways, including fresh-cut onion, onion oil, boiled onion, and fried onion (Boelens *et al.*, 1971). Boelens *et al.* (1971) reported that several dimethylthiophenes were produced by heating alkyl propenyl disulfides such as propenyl methyl disulfide and propyl propenyl disulfide. The formation of 2,4-dimethylthiophene in Sa Cha sauce was probably via the degradation of certain disulfides as well.

Benzene Derivative

Benzene derivatives especially 1-methoxy-4-(1-propenyl)-benzene (*trans*-anethole) and 2-methoxy-4-(1-propenyl)-benzene are the most dominant volatile compounds of Sa Cha sauce. It is likely that the star anise contributes the isomer, 1-methoxy-4-(2-propenyl)-benzene (allylanisole), and all these three benzene derivatives are commonly used in Chinese cooking.

Terpenoids

Terpenoids, including dl-limonene, γ-terpinene, linalool, (*E*)-farnesene, (*E,Z*)-α-farnesene, *trans*-caryophyllene, α-humulene, and 4-methyl-1-isopropyl-3-cyclohexene, are present in the culinary herbs and spices used in Sa Cha sauce. Limonene is the flavor character-impact compound of cardamom (Nagodawithana, 1995) and was also identified in roasted sesame (Shahidi *et al.*, 1997); γ-terpinene is the flavor character-impact compound of cumin seed (Nagodawithana, 1995); α-linalool is the flavor character-impact compound of coriander, cinnamon and bay leaves (Nagodawithana, 1995).

Others

3-Methylbutanal, described as having a malty odor (Schieberle, 1995) and 2-methylbutanal are the Strecker aldehydes of leucine and isoleucine, respectively, and are also produced in roasted sesame (Shahidi *et al.*, 1997). Hexanal, a degradation compound from ω-6 fatty acids such as linoleic acid, possesses a green note which is usually considered an off-flavor in foods. 2,3-Butanedione, 2-butanone and dihydro-2-methyl-3(2H)-furanone are derived from sugar degradation. 2,3-Butanedione not only contributes a buttery aroma but also serves as the reactant for the formation of 2,3-dimethylpyrazine and trimethylpyrazine.

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5

FORMATION OF VOLATILE ACIDS DURING FERMENTATION OF FISH SAUCE

Norlita G. Sanceda,[1] Emiko Suzuki,[2] and Tadao Kurata[1]

[1]Institute of Environmental Science for Human Life
Ochanomizu University, Tokyo, Japan
[2]Department of Human Biological Studies
Ochanomizu University, Tokyo, Japan

The formation of volatile acids in fish sauce was investigated. When fish was allowed to spoil prior to salting, very high concentrations of volatile acids were produced. Addition of salt to spoiled fish suppressed the formation of volatile acids but did not eliminate those already formed. It was clarified that volatile acids were formed by atmospheric oxidation of lipid and normal acids were derived from long chain fatty acids. The volatile acids in the aerobically fermented sauce were significantly higher than in the anaerobically fermented sauce which might also suggest atmospheric oxidation of lipids. Increasing the amount of amino acids in the fish mixtures did not provide any clear information about their role in the formation of volatile acids during the fermentation process.

INTRODUCTION

Fish sauce is a clear brown liquid, hydrolysis product of salted fish and possesses a characteristic odor. It is one of the most popular fermented fish products in Southeast Asia and is known by various names according to the country of origin (for example, Patis in the Philippines; Nampla in Thailand; Ngampi-pya-ye in Burma or presently Myanmar; Shottsuru in Japan; Ketjap-ikan in Indonesia; or Nuocmam in Vietnam). It is generally used as a condiment, but it is also an important source of protein in the diets of some social classes and in specific areas in the region. Lafont (1955) reported that fish sauces should not be considered as condiments only. Those with 1% nitrogen or more could be considered as rather better than condiments. Fish sauces contain 20 g /L of nitrogen, of which 16 g/L are in the form of amino acids.

Fish sauce is prepared by salting fish at a ratio of 2:1 or 3:1 depending on the quality of fish used and the salt used in its manufacture. Fish that exhibit some form of deterioration require a ratio of 2:1 while a ratio of 3:1 suffices if the fish is relatively fresh. The fish-salt mixture is then placed in clay jars, wooden or concrete vats. To facilitate extrac-

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tion and increase the yield, weights, usually hard stones, are placed on top of the mixture. The fish sauce manufacturing process is complete when the characteristic flavor, aroma and color develop. The sauce is extracted, filtered, pasteurized and bottled. The first extract may be considered as extra special and sold as such.

STANDARD IDENTITY OF FISH SAUCES IN THE PHILIPPINES

The standard set by Food and Drug Administration (FDA), 1980 for Philippine fish sauce is as follows:

a. Specific gravity, 1.21–1.22
b. Total solids, Not less than 32%
c. Alkalinity of the water soluble ash of one gram of original sample. Not less than 1 and not more than 2 mL of 0.1N acid
d. Protein:
    1. Extra special patis, not less than 12%
    2. Special patis, not less than 10%
    3. Regular patis, Not less than 6%
    4. Patis below standard or patis flavor, below 6% but not lower than 3%.

There are a number of reports on the kinds of volatile compounds in fish sauces. Earlier studies revealed that low-molecular-weight volatile compounds such as methyl ketone (van Veen, 1953), organic acids (Nguyen and Vialard-Goudou, 1953; Truong, 1963; Yanagihara *et al.*, 1963) and carbonyl compounds (Yurkowski, 1965 a,b) have been reported to contribute to the flavor of fermented fish products. Particularly, organic acids were shown to be associated with the aroma of fish sauce (Vialard-Goudou, 1941 and 1942; Nguyen and Vialard-Goudou, 1953; Truong, 1963). Volatile acids which contributed to the aroma of fish sauce would have been produced by bacterial activity (Saisithi *et al.*,1966). However, there was no detailed report on the extent of their role on the aroma quality. Martin and Sulit (1953) reported a characteristic cheese-like odor and flavor in fish paste. Dougan and Howard (1975) reported that the aroma of fish sauce consisted of three notes; "cheesy note" produced by low molecular weight fatty acids, "ammoniacal note" by ammonia and amines" and meaty note", but the meaty odor was more complicated thus was not analyzed. Sanceda *et al.* (1986) reported some volatile compounds in several kinds of fish sauces as summarized in Table 1 and graphically presented in Figures 1–3. Although quantitative analysis was not done in this study, the gas chromatograms suggested that volatile acids were the most abundant among the compounds identified. This data is supported by the results presented in Table 2.

The sauce has a characteristic aroma which often serves as a gauge to measure the quality of fish sauce, since the very salty taste tends to overpower the other flavor constituents. A number of reports revealed that volatile acids were the most abundant group of volatile compounds in fish sauce. Formic, acetic, propionic, and n-butyric acids were identified by Truong Van–Chom (1963) and Saisithi *et al.* (1966) obtained similar results except that they identified iso-butyric acid instead of n-butyric acid. Patis, nuocmam, nampla and shottsuru contained volatile acids with C2 to C10, both straight and branched-chain. Acetic, propionic, iso-and n-butyric, and iso-valeric acids were identified in the acidic fraction of the steam distillate of patis (Sanceda *et al.*, 1983, 1984) and in other samples by Dougan and Howard (1975) and Beddows *et al.* (1979). Propionic and n-Butyric acids were predominant in patis (Sanceda *et al.*, 1984), both are believed to have unpleasant odor but the

Table 1. Concentrations of volatile acids in fresh and spoiled fish (%)[a]

	Incubation period, days		
Acids	0	3	4
Fresh fish			
Acetic	0.10 ± 0.02	0.12 ± 0.01	0.13 ± 0.01
Propionic	0.07 ± 0.02	0.08 ± 0.03	0.08 ± 0.02
Isobutyric	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.03
Butyric	0.09 ± 0.03	0.14 ± 0.02	0.18 ± 0.04
Isovaleric	0.14 ± 0.03	0.14 ± 0.02	0.15 ± 0.02
Valeric	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
		4	
	3	with salt	without salt
Spoiled fish			
Acetic	0.51 ± 0.01[b]	0.90 ± 0.02[b]	1.78 ± 0.01[b,c]
Propionic	0.43 ± 0.03[b]	0.87 ± 0.03[b]	1.02 ± 0.01[b,c]
Isobutyric	0.65 ± 0.03[b]	0.70 ± 0.02[b]	0.97 ± 0.02[b,c]
Butyric	2.00 ± 0.05[b]	2.58 ± 0.05[b]	3.79 ± 0.05[b,c]
Isovaleric	0.50 ± 0.02[b]	0.61 ± 0.01[b]	0.91 ± 0.03[b,c]
Valeric	0.68 ± 0.01[b]	0.73 ± 0.01[b]	1.03 ± 0.03[b,c]

[a]Values are means of three replicates.
Fresh fish were stored at 0°C for one day before purchase and left to spoil at room temperature. Fresh fish and spoiled fish: salt ratio was 3:1.
Values of acids in the 3 and 4 days incubated fresh fish are not significantly different from the control (0 day).
[b]Values of the acids in the spoiled fish are significantly different at $p<0.05$ with their respective acids in the fresh fish in the corresponding incubation period.
[c]Values of the acids in the spoiled fish without salt incubated for 4 days are significantly different at $p<0.05$ from those incubated with salt.

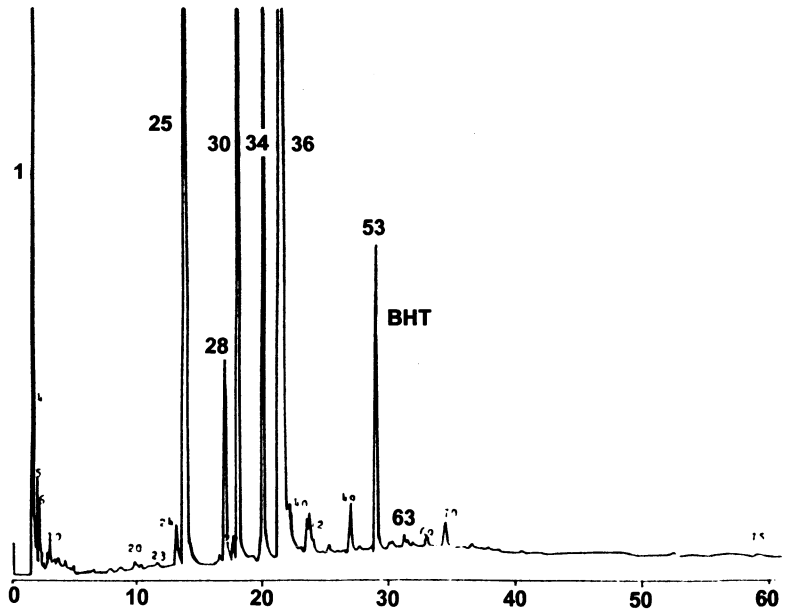

Figure 1. Gas chromatogram of the whole volatile distillate of Shottsuru.

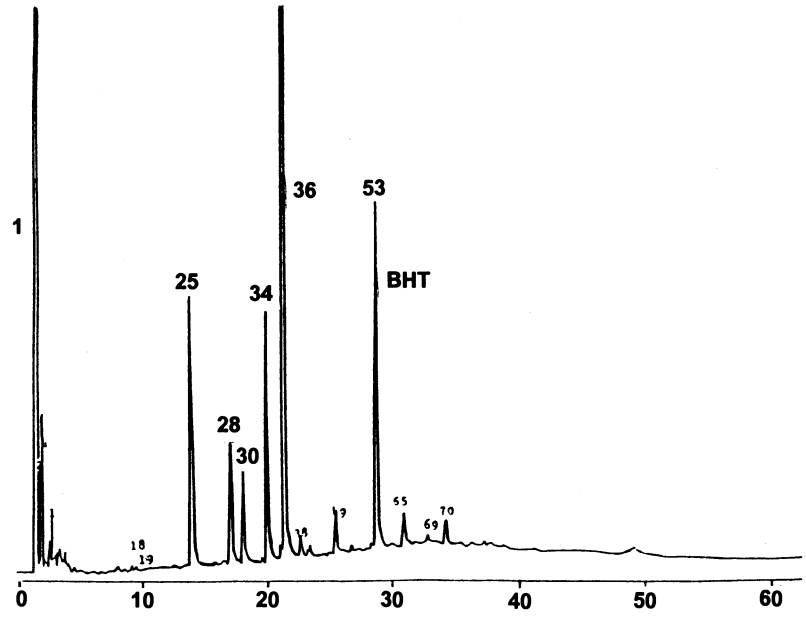

Figure 2. Gas chromatogram of the whole volatile distillate of Nampla.

odor was not defined when blended with other components, while Beddows *et al.* (1979) reported acetic and n-butyric acids as the most abundant and McIver *et al.* (1982) reported acetic and propionic acids as being present in the highest amounts.

In a previous study, Sanceda *et al.* (1983) showed that the yield of the neutral fraction was the highest followed by the acidic and basic fractions while that of the phenolic

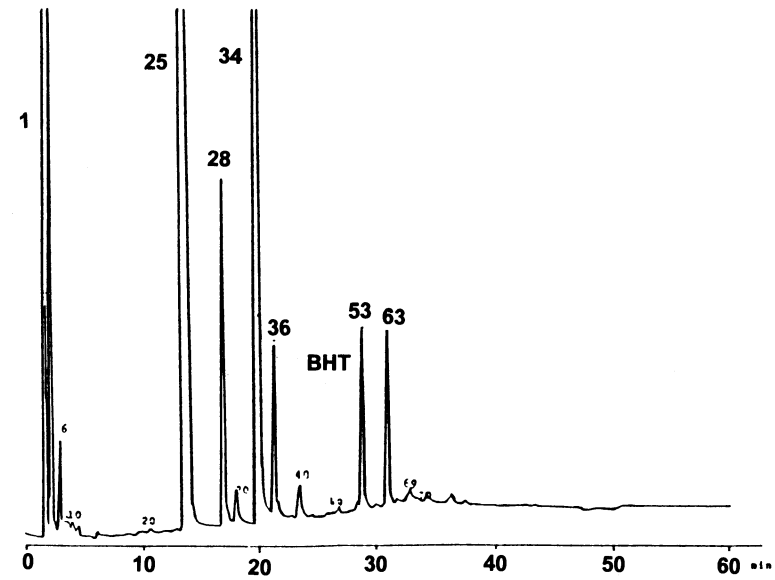

Figure 3. Gas chromatogram of the whole volatile distillate of Noucmam.

Table 2. Volatile acids in an aerobically and anaerobically fermented fish sauces (%)[a]

	Incubation period, month			
	Aerobic		Anaerobic	
Acids	2.5	12	2.5	12
Acetic	0.54 ± 0.01	1.04 ± 0.01	0.31 ± 0.04[b]	0.04 ± 0.02[b,c]
Propionic	1.57 ± 0.02	2.57 ± 0.05	0.19 ± 0.03[b]	0.02 ± 0.00[b,c]
Isobutyric	0.09 ± 0.03	0.19 ± 0.01	0.06 ± 0.01[b]	0.05 ± 0.01[b,c]
Butyric	1.84 ± 0.05	3.11 ± 0.09	0.46 ± 0.06[b]	0.14 ± 0.01[b,c]
Isovaleric	2.11 ± 0.02	2.91 ± 0.04	1.36 ± 0.05[b]	0.98 ± 0.05[b,c]
Valeric	0.11 ± 0.03	0.21 ± 0.03	0.02 ± 0.01[b]	0.01 ± 0.01[b,c]
Isohexanoic	0.08 ± 0.03	0.17 ± 0.03	0.05 ± 0.01[b]	0.05 ± 0.01[b,c]
n-Hexanoic	0.11 ± 0.01	0.20 ± 0.01	0.09 ± 0.07[b]	0.03 ± 0.01[b,c]
n-Heptanoic	0.01 ± 0.01	0.01 ± 0.01	tr	nd[a]

[a]Values are means of three replicates.
[b]Values in the anaerobically fermented sauces are significantly different at $p<0.05$ from the aerobically fermented ones in their corresponding incubation period.
[c]Values in the 12 mo anaerobically fermented sauces are significantly different at $p<0.05$ from the 2.5 mo fermented ones.
tr: Values are less than 0.01%
ND: Not detected

fraction was the lowest (Table 3). Several conflicting findings on the formation and development of these compounds have been reported. For instance, Nguyen -An-Cu and Vialard-Goudou (1953) identified acetic and n-butyric acids and suggested that lactic acid digesting bacteria could be involved. The findings of Dougan and Howard (1975) on the determination of individual volatile fatty acids showed that appreciable amounts of straight chain acids were more likely to have been formed by atmospheric oxidation of fish lipids than by any other mechanism ever considered. However, Beddows *et al.* (1980) reported that it seems unlikely that acetic and n-butyric acids could be derived from oxidation of lipids in the manufacture of fish sauce since the quantity of lipid present in the fish was insufficient to account for the amount of volatile fatty acids (VFA). It was found that when fresh fish was mixed with salt and fermented, (no spoilage prior to salting), very little VFA were formed (Beddows *et al.*, 1979). Inclusion of antibiotics also prevented the formation of VFA suggesting microbial involvement in the production of VFA (Beddows *et al.*, 1979; Uyenko *et al.*, 1952). Saisithi *et al.* (1966) and Beddows *et al.* (1980) isolated bacterial species that were able to produce VFA when inoculated on hydrolyzed rockfish (Seabastodes species or Stolephorus species). Propionic, n-butyric, and n-valeric acids appeared to be derived from amino acids via bacterial actions using U-14C labeled protein hydrolysates. An obvious difference in the volatile fatty acids profile in fish sauce fermented in the absence of oxygen (anaerobic fermentation) was reported (Sanceda *et al.*, 1992). Preliminary studies on the volatile fatty acids in aerobically fermented fish sauce showed that volatile acids were quantitatively greater and qualitatively superior to that of the anaerobically fermented fish sauce (Sanceda *et al.*, 1991) and the product was sensorially acceptable (Sanceda *et al.*, 1994). Though several reports have been made on the formation of volatile acids in fish sauce, several conflicting issues remain unresolved.

This study aimed to provide more information on the formation of volatile acids during fermentation process in the manufacture of fish sauce.

Table 3. Volatile acids in the linoleic acid-added fish liquid (%)[a]

Acids	Incubation period, days		
	2	7	30
Control			
Acetic	tr	0.13 ± 0.02	0.74 ± 0.03
Propionic	tr	0.10 ± 0.01	0.58 ± 0.02
Isobutyric	tr	tr	0.14 ± 0.02
Butyric	0.21 ± 0.02	0.30 ± 0.02	0.81 ± 0.04[d]
Isovaleric	0.31 ± 0.01	1.91 ± 0.05	3.24 ± 0.06[d]
Valeric	0.24 ± 0.01	0.18 ± 0.03	0.24 ± 0.01
Isohexanoic	nd	tr	0.09 ± 0.01
n-Hexanoic	nd	tr	tr
Linoleic acid added[b]			
Acetic	0.10 ± 0.01	0.22 ± 0.01	0.96 ± 0.04[d,e]
Propionic	tr	0.15 ± 0.01	0.64 ± 0.01[d,e]
Isobutyric	0.07 ± 0.01	0.07 ± 0.01[c]	0.15 ± 0.02[d,e]
Butyric	0.20 ± 0.01[c]	0.31 ± 0.02[c]	1.50 ± 0.02[d,e]
Isovaleric	0.53 ± 0.03[c]	1.99 ± 0.05[c]	4.03 ± 0.03[d,e]
Valeric	0.20 ± 0.01[c]	0.24 ± 0.02	0.24 ± 0.02
Isohexanoic	nd	tr	tr
Hexanoic	nd	0.01 ± 0.01	0.01 ± 0.01

[a]Values are means of three replicates.
[b]Linoleic acid was added before incubation.
[c]Values in the linoleic acid-added liquid are not significantly different from the control in their corresponding.
[d]Values in the 1 month linoleic acid-added and control are significantly different at $p<0.05$ from the 48 hr incubation in the control.
[e]Values in the 1 month linoleic acid-added liquid are significantly different at $p<0.05$ from the 1 month control.
tr: Values are less than 0.01%.
ND: Not detected.

MATERIALS AND METHODS

Materials

Fish, about 13 cm long, of sardine family (*Sardinops melanostictus*) were used in all mixtures. Linoleic and, penicillin, azide were purchased from Waco Chemical Ind. Ltd., Osaka, Japan.

Methods. "Spoilage" Experiment

Uneviscerated iwashi, a sardine family fish, were cut 3–4 cm in length and were allowed to spoil. On day 3, a part of the spoiled fish was sampled out and analyzed. The remaining part was divided into two, one half was added with salt and the other half was without salt and both samples were further incubated for another day and the volatile acid contents were analyzed qualitatively and quantitatively.

Small Scale Preparation (Aerobic and Anaerobic Fermentation)

The same kind of fish were sliced and mixed with salt at a ratio of 3:1. Two kinds of mixtures were prepared, one in the absence of air (anaerobic) and the other in the presence

of air (aerobic). In the anaerobic preparation, the mixture was placed in layers in an air tight glass flask with weights placed on top. The container was first loosely closed and nitrogen gas was purged into the container at a rate of 200 ml/min for about one hour to remove air and the container was sealed. In the aerobic fermentation, the mixture was placed in layers using similar type of container, weights placed on top and loosely closed. Both containers were kept in an incubator at about 31 ± 1°C until the liquid was analyzed.

Use of a Long Chain Fatty Acid

A 10% linoleic acid was added to the whole fresh fish-salt mixture and incubated for a specified time. The volatile acids were compared with the control.

Use of Acidic and Basic Amino Acids

Acidic and basic amino acids were individually added to the fish mixtures before incubation.

Use of Antibacterial Agents

One fish mixture was added with 2% benzylpenicillin Potassium with a potency of 1,430 units/mg from Wako Chemical Ind. Ltd, Tokyo, Japan, and incubated aerobically. On the other hand, two fish mixtures were added with 5% sodium azide, from the same company, and were incubated aerobically and anaerobically.

Collection of Volatile Acids

Volatile acids were collected using a steam-distillation procedure under reduced pressure (SDRP). The procedure is detailed in a previous contribution (Sanceda *et al.*, 1990).

Gas Chromatographic Conditions (GC)

Gas chromatography was accomplished using a Shimadzu 9A model gas chromatograph (Gasukuro Kogyo Inc., Kyoto, Japan) equipped with a flame ionization detector. Separation of volatile compounds was achieved using a 0.25 mm i.d. × 50 m fused silica column coated with Carbowax 20M. The column temperature was programmed from 60°C held for 4 min, increased to 180°C at a rate of 2°C/min. The injection port was kept at 200°C. Carrier gas was nitrogen and helium with a flow rate of 1.0mL/min with a split ratio of 1:40. A multifunction data processor (C-R4A Chromatopac, Shimadzu, Kyoto, Japan), connected to the GC, was used for relative quantitative calculations. For complete identification of the volatile acids, co-chromatography using authentic compounds was employed.

Statistical Analysis

Test of significance for the analytical study was done using a Students t-Test .

RESULTS AND DISCUSSION

Our analysis on a day old fish stored at 0°C before use showed that volatile acids were already formed even in the presence of salt. Previous work Beddows *et al.*, (1979)

Table 4. Volatile acids in the specific single amino acids-added fish liquid[a] incubated for 48 hr

	Volatile acids (%)			
	Propionic	iso-Butyric	n-Butyric	iso-Valeric
Fresh fish and amino acids				
Control	0.21 ± 0.01	0.12 ± 0.02	0.73 ± 0.02	0.28 ± 0.01
Asp	0.11 ± 0.02	0.09 ± 0.02	0.70 ± 0.02	0.29 ± 0.01
Glu	0.13 ± 0.01	0.09 ± 0.01	0.60 ± 0.03	0.14 ± 0.02
His	0.12 ± 0.02	0.05 ± 0.02	0.58 ± 0.03	0.20 ± 0.02
Lys	0.10 ± 0.02	0.05 ± 0.02	0.57 ± 0.02	0.19 ± 0.01
Arg	0.10 ± 0.01	0.07 ± 0.03	0.49 ± 0.02	0.17 ± 0.03
Spoiled fish + amino acids				
Control	0.52 ± 0.01[b]	0.74 ± 0.02[b]	1.70 ± 0.05[b]	0.75 ± 0.04[b]
Asp	0.53 ± 0.01[b]	0.73 ± 0.01[b]	1.69 ± 0.03[b]	0.74 ± 0.02[b]
Glu	0.52 ± 0.01[b]	0.75 ± 0.05[b]	1.71 ± 0.04[b]	0.70 ± 0.01[b]
His	0.51 ± 0.02[b]	0.67 ± 0.03[b]	1.69 ± 0.04[b]	0.70 ± 0.01[b]
Lys	0.51 ± 0.01[b]	0.73 ± 0.04[b]	1.50 ± 0.03[b]	0.74 ± 0.02[b]
Arg	0.49 ± 0.02[b]	0.64 ± 0.02[b]	1.68 ± 0.02[b]	0.70 ± 0.03[b]

[a]Values are means of three replicates. Values of acids in the amino acids added fresh fish and spoiled fish are not significantly different from the control.

[b]Values of volatile acids in theamino acids added spoiled fish acids are significantly different from amino acids added the fresh fish.

suggested that bacterial action occurred prior to salting and volatile fatty acids were produced when fish were allowed to spoil Beddows *et al.*, (1980). In this study, acetic, propionic, iso-and n-butyric, iso-and n-valeric acids were detected during the 0–4 days incubation (Table 4.) Although not shown, long straight chain carbon acids such as hexanoic, heptanoic, octanoic, nonanoic and decanoic acids were also detected in trace amounts. These long chain carbon acids have not been reported before. After 4 days incubation, the amount of the short chain volatile acids in the fresh fish mixtures increased but not significantly. When fish were allowed to spoil for 3 days, the level of volatile acids increased drastically and the increase continued as incubation time progressed. The acids in both the salted and non-salted spoiled fish increased with incubation time, but the rate of increase in the non-salted sample was much faster than in the salted one. Though the presence of salt suppressed the formation of volatile acids in the samples, the amount of volatile acids slightly increased after one day incubation suggesting that enzymes in the inner part of the fish body, especially in the intestine, are still active. The role of NaCl as a prooxidant or as an antioxdant depending upon its concentration and/ or the food system composition has been reported. Kanner *et al.* (1991) suggested that catalytic effect of NaCl was due to its capability to displace iron ions binding macromolecules, therefore enhancing their activity toward lipid peroxidation. Salt is a bacteriostatic agent for many bacteria including pathogenic and spoilage bacteria. Micrococcus, Streptococcus and Pediococcus which were reported to be important halotolerant species in fermented foods were viable up to 40 days fermentation (Sands and Crisan, 1974). Addition of salt during manufacturing process improves the flavor of the sauce and also helps ease the filtering process as fish liquefies. Although we could not analyze the bacteria present in the sauce, our results suggest that bacterial action might be involved in the production of volatile acids, in agreement with the results of Beddows *et al.* (1980).

The amounts of volatile acids were higher in the aerobically fermented sauces than those of the anaerobically fermented ones in both the 2.5 and 12 months fermentation (Ta-

Table 5. Changes in the concentrations of volatile acids in fresh fish added with specific single amino acids

Amino acids added[a]	Volatile acids (%)[b]			
	Pro	iso-But	n-But	iso-Val
Control	0.12	0.25	0.73	0.28
Ala	0.11	0.21	0.70	0.29
Val	0.09	0.27	0.68	0.25
Leu	1.13	0.24	0.74	0.29
Ile	0.11	0.22	0.69	0.28
Ser	0.08	0.20	0.65	0.26
Thr	0.09	0.24	0.70	0.28
Asp	0.10	0.27	0.68	0.27
Glu	0.11	0.21	0.73	0.29
Asn	0.13	0.19	0.75	0.31
Gln	0.12	0.27	0.75	0.27
His	0.10	0.19	0.70	0.26
Lys	0.13	0.27	0.74	0.29
Arg	0.09	0.19	0.69	0.25
Cys	0.10	0.20	0.67	0.25
Met	0.08	0.21	0.68	0.25
Phe	0.11	0.23	0.69	0.30
Tyr	0.08	0.19	0.69	0.24

Pro: propionic; iso-But: isobutyric; -but: -butyric; iso-Val: valeric

[a]2% Amino acids were mixed with salt before adding to fresh fish at a ratio of 3 parts fish: 1 part salt.

[b]Values are average of two replicates.

ble 5). While the volatile acids in the aerobically fermented sauce increased as fermentation time progressed, those in the anaerobically fermented ones decreased and the decrease was parallel to the fermentation period. If bacteria present in the mixture thrive under aerobic condition and fed on oxygen, then we can conclude that volatile acids have been formed by bacterial action. It may also be assumed that volatile acids might have been produced by atmospheric oxidation of fat. The rate of oxidation in fermented foods may be affected by many factors. Access to oxygen is one factor. The low oxygen pressure or its absence in the anaerobically fermented sauces during fermentation might have brought about a low content of volatile acids compared to the aerobically fermented sauces. Marcus and Frederickson (1968) reported that termination reactions in the course of lipid oxidation could be different and produce a variety of oxidative products under different oxygen concentrations. It has also been theorized that halide ions may activate the myeloperoxide-H_2O_2-halide system associated with blood components in fish (Kanner and Kinsella, 1983), thus initiating lipid oxidation.

Beddows *et al.* (1980) reported that it is unlikely that acetic and n-butyric acids are derived from oxidation of lipids. In this study, addition of linoleic acid to fish mixture before incubation resulted in an increase in volatile acids and the increase continued as fermentation progressed (Table 6). The amounts of acids were higher in the linoleic acid-added samples than in the control. The formation of the normal volatile acids in the sauce might have been derived from the oxidation of long chain fatty acids. Also, a slight increase in the amounts of isovaleric and isohexanoic acids was observed, however, these branched acids are unlikely to be formed from long chain fatty acids but are theoretically believed to be derived from amino acids. It has been reported that certain volatile acids

Table 6. Changes in the concentrations of volatile acids in spoiled fish[a] added with specific single amino acids

Amino acids added[b]	Volatile acids (%)[c]			
	Pro	iso-But	n-But	iso-Val
Control	0.52	0.70	1.70	0.73
Ala	0.45	0.69	1.69	0.72
Val	0.49	0.73	1.71	0.69
Leu	0.54	0.70	2.05	0.70
Ile	0.49	0.68	1.90	0.69
Ser	0.47	0.67	1.65	0.73
Thr	0.50	0.67	1.68	0.74
Asp	0.53	0.65	1.69	0.70
Glu	0.52	0.71	2.01	0.75
Asn	0.55	0.75	1.71	0.76
Gln	0.54	0.70	1.73	0.71
His	0.51	0.67	1.69	0.70
Lys	0.51	0.73	1.50	0.74
Arg	0.46	0.64	1.68	0.71
Cys	0.51	0.70	1.70	0.76
Met	0.50	0.71	1.78	0.74
Phe	0.54	0.73	1.72	0.75
Tyr	0.50	0.70	1.73	0.68

Pro: propionic; iso-But: isobutyric; n-But:n-butyric; iso-Val:valeric

[a]Fish were left to spoil for 1 day at room temperature before adding amino acids.

[b]2% Amino acids were mixed with salt before adding to spoiled fish at a ratio of 3 parts fish: 1 part salt.

[c]Values are average of two replicates.

Table 7. Changes in the concentration of volatile acids in fresh and spoiled fish mixtures with added specific mixtures of amino acids incubated for 48 hr

Added amino acids[a]	Volatile acids (%)[b]							
	Fresh fish				Spoiled fish[c]			
	Pro	iso-But	n-But	iso-Val	Pro	iso-But	n-But	iso-Val
Control	0.09	0.21	0.61	0.25	0.45	0.62	1.40	0.70
Leu+Ala	0.07	0.20	0.24	0.24	0.44	0.61	1.41	0.72
Leu+Val	0.07	0.25	0.25	0.25	0.46	0.61	1.38	0.69
Leu+Gly	0.09	0.21	0.23	0.23	0.45	0.59	1.51	0.72
Leu+Met	0.09	0.19	0.25	0.25	0.45	0.62	1.21	0.68
Leu+Pro	0.08	0.21	0.25	0.25	0.44	0.59	1.40	0.72

Pro: propionic; iso-But:isobutyric; -But:n-butyric; isoVal:valeric

[a]2% Amino acids were mixed with salt before adding to fresh and spoiled: salt ratio (3:1).

[b]Values are average of two replicates.

[c]Fish were left to spoil for 1 day at room temperature before adding amino acids.

can be produced by Stickland reaction on specific amino acids with some Clostridia sp. (Nisman, 1954). It was further demonstrated that n-butyric acid could not be produced by this reaction due to the absence of an amino acid with an appropriate carbon skeleton. Our result showed that addition of basic and acidic amino acids, individually, to fresh fish or spoiled fish mixtures showed no change in the volatile acids contents after 48 h of fermentation (Table 7). Similarly, addition of almost all types of amino acids individually to fresh (Table 8) or spoiled fish mixtures (Table 9) had a similar effect. Moreover, addition of aliphatic amino acids, in combinations, did not bring any change in the content of volatile

Table 8. Changes in the concentrations of volatile acids in fresh fish added with specific single amino acids

Amino acids added[a]	Volatile acids (%)[b]			
	Pro	iso-But	n-But	iso-Val
Control	0.12	0.25	0.73	0.28
Ala	0.11	0.21	0.70	0.29
Val	0.09	0.27	0.68	0.25
Leu	0.13	0.24	0.74	0.29
Ile	0.11	0.22	0.69	0.28
Ser	0.08	0.20	0.65	0.26
Thr	0.09	0.24	0.70	0.28
Asp	0.10	0.27	0.68	0.27
Glu	0.11	0.21	0.73	0.29
Asn	0.13	0.19	0.75	0.31
Gln	0.12	0.27	0.75	0.27
His	0.10	0.19	0.70	0.26
Lys	0.13	0.27	0.74	0.29
Arg	0.09	0.19	0.69	0.25
Cys	0.10	0.20	0.67	0.25
Met	0.08	0.21	0.68	0.25
Phe	0.11	0.23	0.69	0.30
Tyr	0.08	0.19	0.69	0.24

Pro:propionic; iso-But: isobutyric; n-But: n-butyric; iso-Val:valeric

[a]2% Amino acids were mixed with salt before adding to fresh fish at a ratio of 3 parts fish: 1 part salt.

[b]Values are average of two replicates.

Table 9. Changes in the concentrations of volatile acids in spoiled fish[a] added with specific single amino acids

Amino acids added[b]	Volatile acids (%)[c]			
	Pro	iso-But	n-But	iso-Val
Control	0.52	0.70	1.70	0.73
Ala	0.45	0.69	1.69	0.72
Val	0.49	0.73	1.71	0.69
Leu	0.54	0.70	2.05	0.70
Ile	0.49	0.68	1.90	0.69
Ser	0.47	0.67	1.65	0.73
Thr	0.50	0.67	1.68	0.74
Asp	0.53	0.65	1.69	0.70
Glu	0.52	0.71	2.01	0.75
Asn	0.55	0.75	1.71	0.76
Gln	0.54	0.70	1.73	0.71
His	0.51	0.67	1.69	0.70
Lys	0.51	0.73	1.50	0.74
Arg	0.46	0.64	1.68	0.71
Cys	0.51	0.70	1.70	0.76
Met	0.50	0.71	1.78	0.74
Phe	0.54	0.73	1.72	0.75
Tyr	0.50	0.70	1.73	0.68

Pro:propionic; iso-But: isobutyric; n-But: n-butyric; iso-Val:valeric

[a]Fish were left to spoil for 1 day at room temperature before adding amino acids.

[b]2% Amino acids were mixed with salt before adding to spoiled fish at a ratio of 3 parts fish: 1 part salt.

[c]Values are average of two replicates.

Table 10. Changes in the concentration of volatile acids in fresh and spoiled fish mixtures with added specific mixtures of amino acids incubated for 48 hr

Added amino acids[a]	Volatile acids (%)[b]							
	Fresh fish				Spoiled fish[c]			
	Pro	iso-But	n-But	iso-Val	Pro	iso-But	n-But	iso-Val
Control	0.09	0.21	0.61	0.25	0.45	0.62	1.40	0.70
Leu+Ala	0.07	0.20	0.59	0.24	0.44	0.61	1.41	0.72
Leu+Val	0.07	0.25	0.59	0.25	0.46	0.61	1.38	0.69
Leu+Gly	0.09	0.21	0.16	0.23	0.45	0.59	1.51	0.72
Leu+Met	0.09	0.19	0.62	0.25	0.45	0.62	1.21	0.68
Leu+Pro	0.08	0.21	0.60	0.25	0.44	0.59	1.40	0.72

Pro: propionic; iso-But:isobutyric; n-But:n-butyric; isoVal:valeric

[a]2% Amino acids were mixed with salt before adding to fresh and spoiled:salt ratio (3:1).

[b]Values are average of two replicates.

[c]Fish were left to spoil for 1 day at room temperature before adding amino acids.

acids (Table 10). The volatile acids in the amino acid-added spoiled fish were significantly higher than those in the amino acid-added fresh fish but these differences were not due to the increased amounts of amino acids, but to spoilage. Based on these results, it was difficult to explain the role of amino acids on the formation of volatile acids during fermentation in the manufacture of fish sauce.

Addition of bacteriostatic agents to fish mixtures before incubation showed that volatile acids were still formed (data not shown) however, our data were so scattered that we could not derive any clear information from them.

CONCLUSION

Fish spoilage increased the amount of volatile acids in fish liquid during fermentation. Contents of volatile acids in the aerobically fermented fish sauces were significantly higher than those in the anaerobically fermented ones. When linoleic acid was added to the fish mixture prior to incubation, the amount of volatile acids increased, thus suggesting that volatile acids were derived from long chain fatty acids by atmospheric oxidation of lipids, however, increasing the amount of amino acids in the fish mixtures did not clarify their role in the formation of volatile acids during the fermentation process. The findings in this study might have, therefore helped clarify some controversial points in the formation of volatile acids during fermentation process in the manufacture of fish sauce.

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6

FLAVOR CHEMISTRY OF SELECTED CONDIMENTS AND SPICES USED IN CHINESE FOODS

Chi-Tang Ho, Jiangang Li, and May-Chien Kuo

Department of Food Science, Cook College, Rutgers University
65 Dudley Road, New Brunswick, New Jersey 08901-8520

The rich and delicate flavors are the main reason that Chinese cuisine is held in high esteem worldwide. Condiments and spices are heavily used in most Chinese foods. This review summarizes the flavor chemistry of some condiments and spices such as soy sauce, garlic, scallions, shallots, ginger, star anise and black mushroom. The possible interactions of condiments and spices with other ingredients in the cooking of Chinese dishes are emphasized.

INTRODUCTION

The Chinese have developed one of the most sophisticated cuisines in the world. Every day a billion people help to make Chinese food better and better. While it is often said that a common written language holds the Chinese civilization together, chopsticks, perhaps, play a stronger role as 'cultural cement' than the Chinese writing brush (Chen *et al.*, 1983).

The rich and delicate flavors are the main reason that Chinese cuisine is so well liked and popular. Seasonings and spices are heavily used in most Chinese foods.

Climate has a considerable influence on the seasonings favored in any particular geographical area. The people of Sichuan and Hunan, situated in the Yangtze River Basin, use chili peppers heavily in their cooking; in the Yellow River Basin areas, large quantities of scallions and garlic are consumed; and in the coastal provinces of Guandong (Canton), Fujian, and Taiwan, ginger root is preferred.

We have reviewed the flavor chemistry of Chinese foods in 1989 (Ho *et al.*, 1989), the attempt is made here to revise and update that article, particularly, the flavor chemistry of some condiments and spices commonly used in Chinese foods. Special effort has been made to discuss the possible interactions of condiments or spices with other ingredients in the cooking of Chinese dishes.

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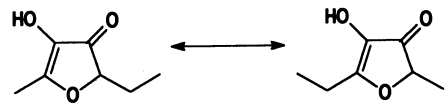
SOY SAUCE FLAVOR

Soy sauce is the most popular condiment used in Chinese cuisine. Many Chinese consider their food "naked" without the presence of soy sauce. In the production of genuine fermented soy sauce, heat-treated soybean and wheat are cultured with *Aspergillus oryzae* or *sojae* and then mixed with salt water to make a mash. The mash is fermented with lactobacilli and yeast, and then is well aged to make soy sauce (Yokotsuka, 1981; Chen, 1989; Nunomura and Sasaki, 1993). Soy sauce contains free amino acids and small peptides which give significant taste and enhance the flavor of foods. It has also lots of aroma compounds which provide a flavorful tone to foods.

Cyclic Dicarbonyl Compounds Identified in Soy Sauce

The main character-impact compounds identified in soy sauce are the tautomers of 4-hydroxy-2-ethyl-5-methyl-3(2H)-furanone (1) and 4-hydroxy-2-methyl-5-ethyl-3(2H)-furanone (2) (HEMF) (Scheme 1), which existed in the ratio of 3:2 (Nunomura *et al.*, 1976). HEMF has an intense sweet odor reminiscent of that of soy sauce. The concentration of HEMF in soy sauce ranges from 50–100 ppm. The odor value of HEMF in soy sauce is calculated at more than 1,250,000, which makes it undoubtedly the most odor-active compound of soy sauce. It is not surprising that the content of HEMF was found to correlate positively with the quality of soy sauce (Nunomura *et al.*, 1980, Lee and Kwok, 1987). HEMF is formed by the action of yeasts (*Zygosaccharomyces rouxii* and *Candida*) during the aging of soy sauce (Sasaki *et al.*, 1991; Nunomura and Sasaki, 1993). The pentose-phosphate cycle has been shown to be the key step for yeasts to produce HEMF and that HEMF is probably derived from sedoheptulose 7-phosphate (Sasaki, 1993).

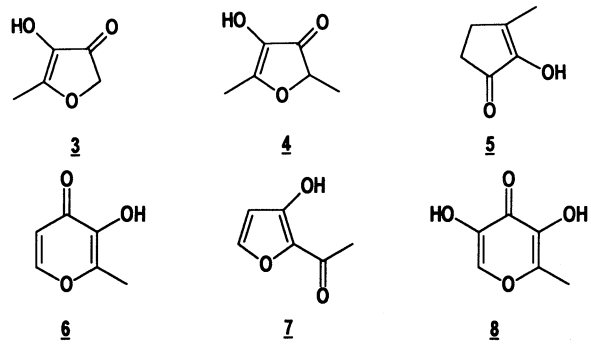


1 2

Scheme 1

Another major compound identified in soy sauce which has a similar structure to HEMF is 4-hydroxy-5-methyl-3(2H)-furanone (HMF) (3) (Scheme 2) (Nunomura *et al.*, 1979). This compound was previously isolated as cooked beef flavor components by Tonsbeek *et al.* (1968). HMF exhibits a heavier note, a cooked beef-like aroma and also an odor reminiscent of soy sauce. Considering the HMF has a very low odor threshold value and that it is one of the major flavor components in soy sauce, HMF is also considered to be one of the important constituents of the characteristic soy sauce flavor (Nunomura *et al.*, 1979).

Besides HEMF, other cyclic dicarbonyl compounds such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (4), cyclotene (5), maltol (6), isomaltol (7), and 5-hydroxymaltol (8) (Scheme 2) found in soy sauce also contribute a typical caramel-like flavor to soy sauce (Nunomura *et al.*, 1980). The molecular condition for caramel-like tonalities is a planar enol-carbonyl structure leading to configurations in a cyclic dicarbonyl compounds with strong hydrogen bonds (Ohloff, 1994). These compounds have been generally considered to be very important as flavor components of foods (Ohloff, 1981).



Scheme 2

Stability of 4-Hydroxy-3(2H)-Furanones

Nunomura *et al.* (1979) reported that HEMF is relatively stable in soy sauce, but it is unstable in alkali or when it is exposed to air. With headspace in a soy sauce container, the concentration of HEMF decreased drastically with time (Nunomura and Sasaki, 1992, 1993). Oxidative products of HEMF have been studied by Nunomura and Sasaki (1992). Under basic conditions, HEMF changes to the odorless compound, 4,4,5-trihydroxy-2-ethyl (or methyl)-5-methyl (or ethyl)-3-tetrahydrofuranone. Autooxidation of HEMF give α-keto acids such as 2-oxobutanoic acid and 2-oxopropanoic acid, aldehydes such as propanal and acetaldehyde; and carboxylic acids such as acetic and propanoic acids (Nunomura and Sasaki, 1992).

The thermal decomposition of HDMF has been reported (Shu *et al.*, 1985a). When the aqueous solution of HDMF was heated at 160°C for 0.5 hr, the major decomposition products identified were 2,3-pentanedione, 2-hydroxy-3-pentanone, and 3-hydroxy-2-pentanone. It implies that during degradation the ring structure of HDMF opens first to an intermediate, which then undergoes a retrodegradation process to produce a group of primary products which react in an intermolecular fashion to form secondary products. Figure 1 shows the mechanism for the formation of 2,3-pentanedione. In addition to acyclic carbonyls, three 3(2H)-furanones [2,4,5-trimethyl- (9), 2-ethyl-5-methyl- (10), and 2,4-dimethyl-5-ethyl-3(2H)-furanone (11)] (Figure 1) were also identified in thermal decomposition products of HDMF. These 3(2H)-furanones could also be derived from 2,3-pentanedione (Shu, 1984).

The most interesting property of 4-hydroxy-3(2H)-furanones is their reactivity toward sulfur-containing compounds such as hydrogen sulfide, cysteine, and cystine, all of which are important constituents of meat. The possible interaction between 4-hydroxy-3(2H)-furanones in soy sauce and sulfur-containing compounds during cooking of Chinese foods may generate compounds significant to their flavor van den Ouweland and Peer (1975) investigated the possibility that HMF, a furanone discovered in beef broth, could act as an intermediate in forming additional meat flavor. They prepared a model system consisting of HMF and hydrogen sulfide in an aqueous medium heated at 100°C for 4 hr. The overall aroma generated from this reaction was described as resembling roasted meat. Complex mixtures of compounds including mercapto-substituted furan and thiophene derivatives were identified (van den Ouweland and Peer, 1975).

In another model system, Shu *et al.* (1985b) treated a mixture of cystine and HDMF, and discovered two interesting new compounds, 2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone (12) and 2,5-dimethyl-2-hydroxy-3(2H)-thiophenone (13). These two com-

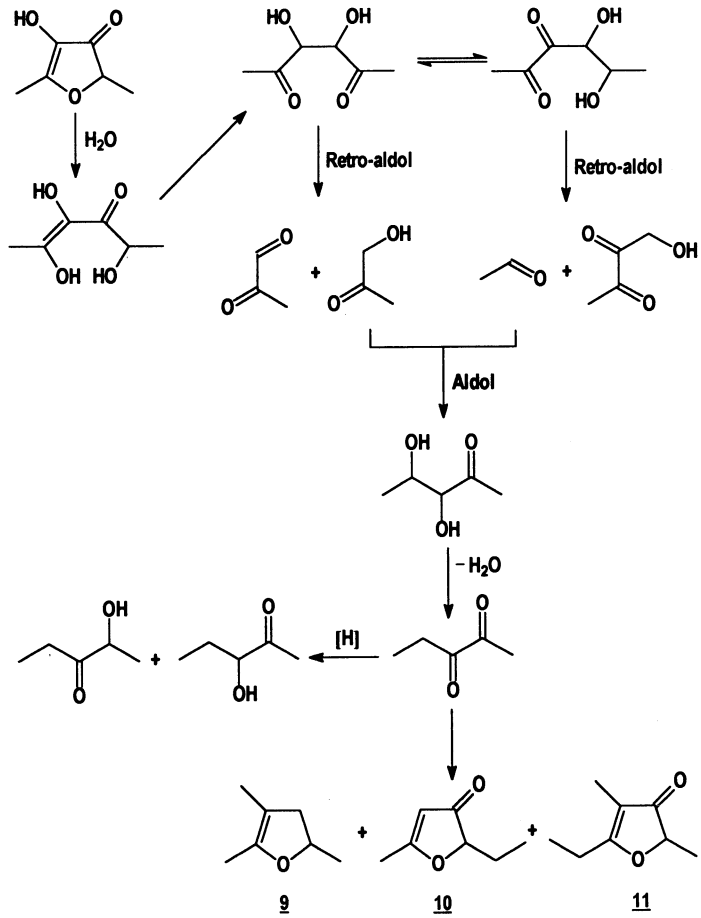


Figure 1. Mechanism for the formation of 2,3-pentanedione and 3(2H)-furanones from thermal degradation of HDMF.

pounds, together with 2,5-dimethyl-4-hydroxy-3(2H)-thiophene (14) (Figure 2) and 2,4-hexanedione, accounted for almost 40% of the volatiles in this model system. The first compound had a pot-roasted character in both aroma and taste; the second was described as roasted onion in aroma and taste. The relationship of the three thiophenones identified in the reaction of HDMF and cystine can be rationalized as shown in Figure 2.

In the reaction of cysteine and HDMF, 2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone and 2,5-dimethyl-2,-hydroxy-3(2H)-thiophenone were identified only as minor products. Two thiophenes, 3-methyl-2-(2-oxopropyl)-thiophene (15) and 2-methyl-3-propionylthiophene (16) (Scheme 3), along with 2,4-hexanedione appeared to be the major products from the reaction of cysteine and HDMF (Shu et al, 1986). The volatile compounds generated from the reaction of glutathione, a cysteine-containing tripeptide with HDMF were compared with those generated from the reaction of cysteine and HDMF (Zheng *et al.*, 1997). The results showed that cysteine is more reactive than glutathione. In addition to the amount of sulfur-containing compounds, other heterocyclic compounds such as pyrazines and thiazoles formed in the reaction of cysteine and HDMF were more, in number and amount, than what was formed in the reaction of glutathione and HDMF.

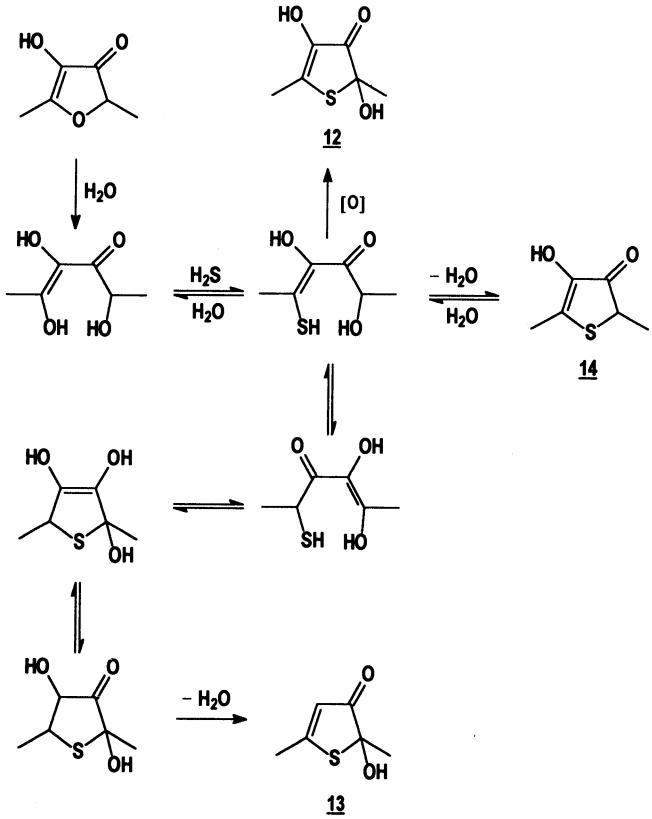


Figure 2. Relationship of the three thiophenes identified in the reaction of HDMF and cystine.

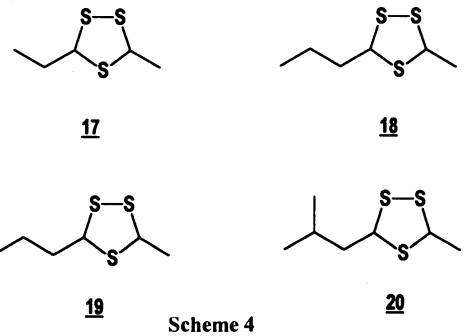
15 **Scheme 3** 16

Traditional Chinese stewed pork is prepared by cooking the pork with sugar, soy sauce, and star anise for several hours. Chou and Wu (1983) compared the volatile components of pork stewed with soy sauce to those of soy sauce used for the cooking of pork. It was interesting to find that both HDMF and HEMF, which were abundant in the volatiles of soy sauce, were not present in the volatiles of soy sauce-stewed pork. It is possible that HEMF and HDMF in soy sauce react with the constituents of pork such as cysteine or cystine during the cooking of stewed pork.

Effect of Soy Sauce on the Flavor of Stewed Pork

In the studies of Chou and Wu (1983), several alkyl-substituted trithiolanes such as *syn*- and *anti*-3-methyl-5-ethyl-1,2,4-trithiolanes (17), *syn*- and *anti*-3-methyl-5-propyl-

1,2,4-trithiolanes (18), *syn*- and *anti*-3-methyl-5-butyl-1,2,4-trithiolanes (19), *syn*- and *anti*-3-methyl-5-isobutyl-1,2,4-trithiolanes (20) (Scheme 4) were identified in the headspace volatile components of Chinese stewed pork. Although 3-methyl-5-ethyl-1,2,4-trithiolanes and 3-methyl-5-isobutyl-1,2,4-trithiolanes have recently been found in the volatiles of pork (Werkhoff *et al.*, 1993), in the study of Chou and Wu (1983), neither soy sauce nor cooked pork contained these trithiolanes. Apparently, they were more favorably generated when pork was stewed with soy sauce. The sensory properties of 3-methyl-5-ethyl-1,2,4-trithiolane has been described as herbaceous, oniony, allium-like, leek-like, nutty, roasted peanut and Shiitake-like. 3-Methyl-5-isobutyl-1,2,5-trithiolane has an interesting oniony, roasty, allium-like and meaty sensory properties (Werkhoff *et al.*, 1993). Figure 3 shows the mechanism for the formation of trithiolane from the reaction of aldehydes with hydrogen sulfide as proposed by Takken *et al.* (1976).



Scheme 4

The amount of soy sauce used in the preparation of stewed pork also showed a significant effect on the quantity of some volatile compounds generated. Table 1 shows the relative percentages of 3,5-dimethyl-1,2,4-trithiolane and 2,4,6-trimethylperhydro-1,3,5-dithiazine (thialdine) in stewed pork samples prepared with different amounts of soy sauce. The amounts of *syn*- and *anti*-3,5-dimethyl-1,2,4-trithiolanes increased with increasing amounts of soy sauce. Soy sauce may provide acetaldehyde, the necessary intermediate for the formation of 3,5-dimethyl-1,2,4-trithiolanes. There is no simple explanation for the lesser amount of thialdine generated in the stewed pork sample cooked with the higher concentration of soy sauce.

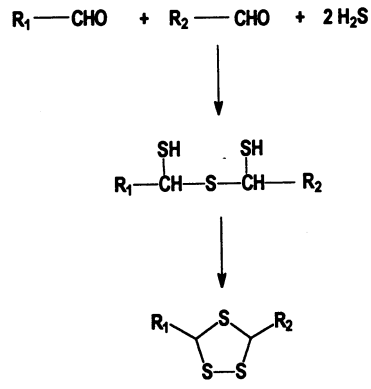


Figure 3. Mechanism for the formation of 3,5-dialkyl-1,2,4-trithiolanes.

Table 1. Effect of soy sauce on the formation of trithiolane and thialdine in stewed pork (Chou and Wu, 1983)

	Relative percentage[a]			
Compound	A	B	C	D
anti-3,5-Dimethyl-1,2,4-trithiolane	0.7	1.8	4.3	5.3
syn-3,5-Dimethyl-1,2,4-trithiolane	1.3	2.2	3.3	5.5
Thialdine	42.9	44.9	41.1	27.2

[a]Cooking conditions:
A: pork cooked with 2.5% soy sauce and 27.5% water.
B: pork cooked with 5.0% soy sauce and 25.0% water.
C: pork cooked with 7.5% soy sauce and 22.5% water.
D: pork cooked with 10.0% soy sauce and 20.0% water.

Another striking difference between the volatiles of pork and stewed pork samples is the presence and absence of unsaturated aldehydes. Many unsaturated aldehydes including 2,4-decadienal, 2-undecenal, 2-dodecenal, and 2-tridecenal identified in the volatiles of cooked pork were not found in the volatiles of stewed pork (Chou and Wu, 1983). It is possible that soy sauce contains reactive substances that can react with unsaturated aldehydes during the stewing of pork. Considering the sensory significance of unsaturated aldehydes, soy sauce definitely modified the flavor profile of the cooked pork.

Role of Pyrazines Identified in Soy Sauce

The pyrazines constitute a very important class of flavor compounds. They are generally associated with pleasant and desirable sensory properties. Nunomura *et al.* (1978) identified 24 pyrazines in the basic fractions of soy sauce flavor components. They are considered to occur during three kinds of processing: heat treatment of the raw material, aging of the mash, and pasteurization of the liquid part of the mash. It was found that during pasteurization, the quantity of alkylpyrazines increased markedly. Major pyrazines identified in soy sauce were 2-methylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-methyl-6-vinylpyrazine, and 2-acetyl-3-methylpyrazine (Chou and Wu, 1983). Organoleptically, the pyrazines are considered to be indispensable to soy sauce flavor.

ROLE OF GARLIC, SCALLION, AND SHALLOT IN CHINESE FOOD PREPARATION

Various species of genus *Allium* have been used as flavoring vegetables in a wide range of Chinese dishes. Among them, garlic (*Allium sativum*), scallions (*Allium fistulosum L. var. caespitosum*), and shallots (*Allium cepa L., Aggregatum g.*) are most important.

Garlic and scallions, and also ginger, are used in almost every stir-fried Chinese dish. Stir-frying is a unique Chinese technique that has been used regularly for at least a thousand year. In stir-frying, the wok is preheated on high heat until very hot, then a small amount of oil is poured in. The oil is swished around to coat the surface of the wok. This prevents the ingredients from sticking to the wok and helps to heat the food evenly. Garlic, scallions, or ginger is then put in and stirred to generate flavor. Other food ingredients are then dropped in and cooked. In stir-frying, garlic, scallions, or ginger is exposed to a high temperature for a short period of time. Thermally generated flavor from these vegetables plays a key role in the flavor of Chinese foods prepared by this process.

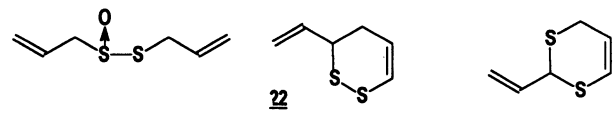
Garlic, scallions, and shallots are characterized by the presence of a large amount of sulfur-containing compounds. The volatile sulfur compounds are produced by enzymic splitting of the nonvolatile *S*-alk(en)ylcysteine sulfoxide after destruction of the tissue. The chemistry and biological properties of onions and garlic have been extensively reviewed (Whitaker, 1976; Carson, 1987; Block, 1992; Yu and Ho, 1993; Lawson, 1996; Reuter *et al.*, 1996, Block *et al.*, 1997). This section discusses only the flavor changes of these vegetables during heat treatment.

Garlic

Northern Chinese delight in eating the garlic plant raw. They eat all parts of plant: the tender leaves, or 'garlic sprouts'; the white and green of the stem and mature leaves; of course, the bulb; and even the garlic blossoms.

It is well known that the most important precursor for the flavor of garlic is allicin (allyl 2-propenethiosulfinate) (21) which is formed by the action of alliinase on alliin. Allicin, which contributes the pungent flavor of garlic, is very unstable. It decomposes into other sulfur compounds when garlic is crushed and then heated. The sulfur compounds thus formed contribute to the heated flavor of the cooked garlic.

It was found that in a GC column, allicin will decompose into 3-vinyl-[4H]-1,2-dithiin (22), 2-vinyl-[4H]-1,3-dithiin (23) (Scheme 5), and a few trace compounds (Table 2) (FIRDI, 1988).



21 22 23

Scheme 5

The major compounds of heated garlic oil have been identified as diallyl trisulfide, diallyl disulfide, methyl allyl trisulfide, methyl allyl disulfide, diallyl sulfide, methyl allyl sulfide, dimethyl sulfide, 2-vinyl-[4H]-1,3-dithiin, and 3-vinyl-[4H]-1,2-dithiin. Most of these sulfur-containing compounds come from the decomposition and rearrangement of allicin at high temperatures (Yu and Ho, 1993). The mechanisms for the formations of volatile sulfides in garlic samples are presented in Figure 4. Stir-frying of chopped and smashed garlic in hot (near the smoke point) soybean oil for 1 min in a Chinese wok retained about 16% of the sulfides in the oil, but no allicin remained (Lawson, 1993).

Several studies have been carried out on the volatile compounds formed upon cooking garlic by a variety of methods, such as oil-frying, baking, boiling and microwaving (Yu *et al.*, 1993, 1994a,b,c). In all cases, 40–60 volatile compounds were identified, with

Table 2. Volatile compounds from GC analysis of allicin

Compound	Area (%)
2-Propen-1-ol	1.08
Diallyl disulfide	2.06
Methyl allyl trisulfide	0.21
3-Vinyl-[4H]-1,2-dithiin	26.16
Diallyl trisulfide	2.65
2-Vinyl-[4H]-1,3-dithiin	63.46

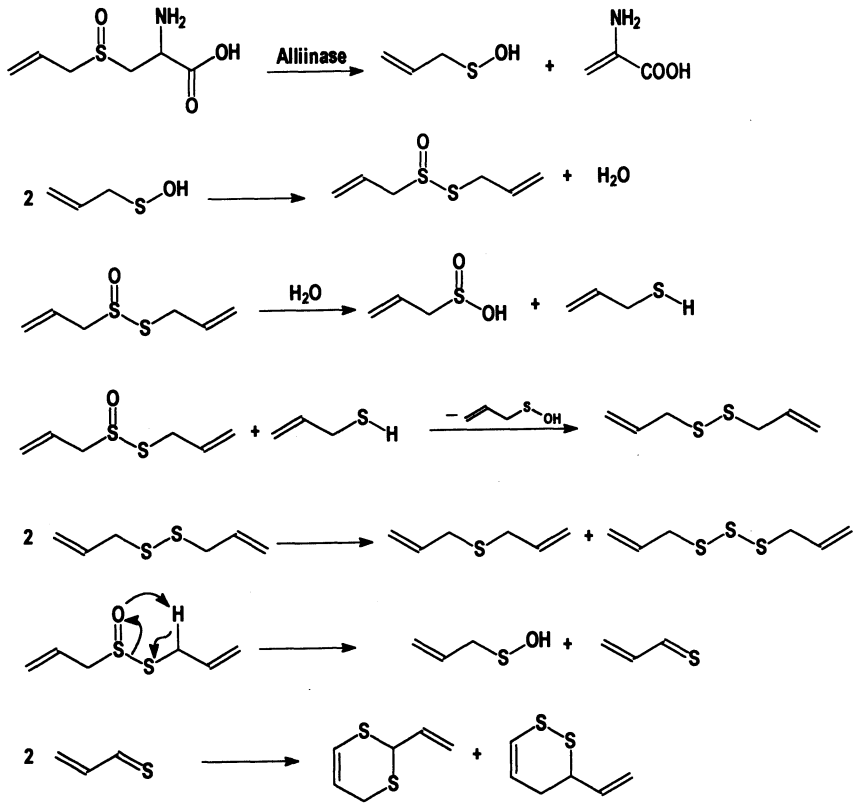


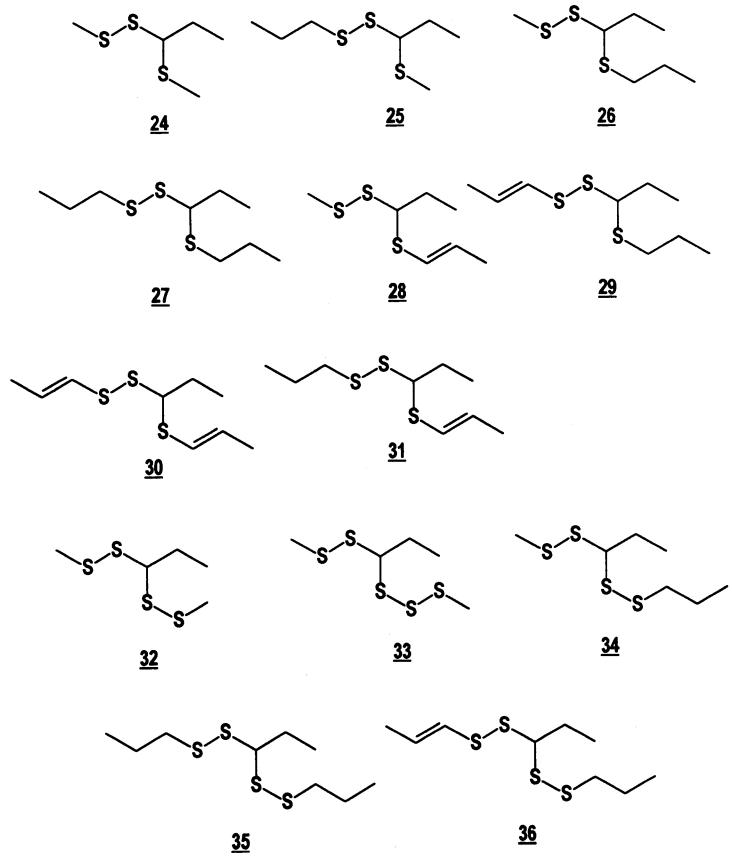
Figure 4. Proposed mechanism for the formation of sulur compounds in garlic.

16 found in boiled garlic. Baking and microwaving garlic slices produced the highest amount of volatiles while boiling and baking whole clove produced the least. Diallyl disulfide was found to be the dominant volatile formed in all these studies. Diallyl trisulfide was abundant only in the baked and microwaved garlic slices.

Scallions

Northern Chinese are every bit as fond of scallions as they are of garlic. Scallions turn up in everything from egg fried rice, to miso soup, to stir-fried greens. Because of its great versatility, the ancient Chinese referred to the scallion as 'the vegetable that gets along with everyone.'

The major volatile compounds in the methylene chloride extracts of scallion were found to be propyl 1-propenyl disulfide, dipropyl disulfide, dimethyl thiosulfinate, and dimethyl thiosulfonate. After steam treatment, methyl propyl trisulfide and methyl 1-propenyl trisulfide become most abundant (Kuo *et al.*, 1990). Scheme 6 shows the structures of several novel polysulfides (24–36) identified in the volatiles of heated scallions. Increased amounts of *syn*- and *anti*-3,5-diethyl-1,2,4-trithiolanes were also identified in the sample of heat-treated scallions (Kuo *et al.*, 1990). 1-Propenyl 1′-alkyl(di)thiopropyl disulfides (29,30,36) may be the precursors of these trithiolanes identified (Figure 5).



Scheme 6

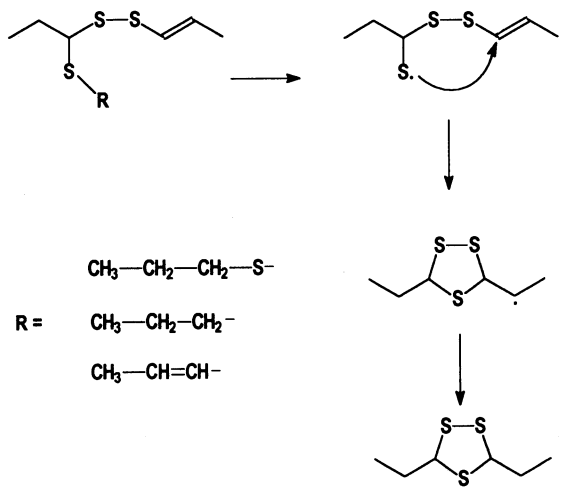


Figure 5. Possible mechanism for the formation of 1,2,4-trithiolanes in heated scallions.

Table 3. Identification of decomposition products from the mixture of propyl 1-propenyl disulfide (PPD) and dipropyl disulfide heated at 150°C for 1 hr

Compound	GC area %
2.5-Dimethylthiophene	0.01
2,4-Dimethylthiophene	0.07
3,4-Dimethylthiophene	1.80
Dipropyl disulfide and propyl *cis*-1-propenyl disulfide	50.39
Dispropyl *trans*-1-propenyl disulfide	34.58
Dipropyl trisulfide	4.97
Propyl 1-propenyl trisulfide (*cis*- & *trans*-)	0.40
5,6-Dimethyl-2,37-trithiabicyclo[2.2.1]heptane	0.01
1-(Propylthio)propyl propyl disulfide	0.39
1-(1-Propenylthio)propyl propyl disulfide	0.10
1-(1-Propylthio)propyl 1-propenyl disulfide	0.02
1-(Propenylthio)propyl 1-propenyl disulfide	0.32
Propyl 3,4-dimethylthienyl disulfide	0.08
6-Ethyl-4,5,7,8-tetrathiaundecane	0.10
6-Ethyl-4,5,7,8-tetrathia-2-undecene	0.02

(From Kuo and Ho, 1991)

Thermal decomposition of a mixture of propyl 1-propenyl disulfide and dipropyl disulfide at 150°C for 1 hr was conducted by Kuo (1991) to simulate the heating of scallion. The major products were reported to be dipropyl trisulfide and 3,4-dimethylthiophene. In addition, several novel polysulfides identified in distilled green onion oils were detected as minor products of the thermal decomposition of propyl 1-propenyl disulfide and dipropyl disulfide (see Table 3).

Thermal interaction of propyl 1-propenyl disulfide, dipropyl disulfide and dimethyl disulfide were also conducted by Kuo (1991). Methyl propyl disulfide (4.14%) was the predominant product, followed by dipropyl trisulfide (2.58%) and methyl 1-propenyl disulfide (1.43%) from the interaction of propyl 1-propenyl disulfide/dipropyl disulfide and dimethyl disulfide. Dimethyl trisulfide, methyl propyl trisulfide (*E* and *Z*), propyl 1-propenyl trisulfides (*E* and *Z*) and dimethyl thiophenes (3 isomers) were also found to be minor volatile compounds.

Shallots

Shallots are usually used as seasoning after being deep-oil fried. They add a subtle but pleasant flavor to soups and braised dishes.

The volatile oils from raw, baked, and deep-fat-fried shallots have been studied (Wu *et al.*, 1982). Approximately 0.03, 0.012, and 0.005% (v/w) of volatile oils were obtained, respectively, from raw, baked, and deep-fat-fried shallots. In the heating process, either by baking or deep-frying, the amounts of 2,4-dimethylthiophene, 3,4-dimethylthiophene, methyl 1-propenyl trisulfide, and propyl 1-propenyl trisulfide increased while the amount of saturated alkyl (dimethyl, methyl propyl, and dipropyl) trisulfides and unsaturated alkyl disulfides decreased. Methyl propyl trisulfide had the highest percentage content in all three volatile oils of shallots. Methyl propenyl disulfide and propyl propenyl disulfide had lesser amounts but their changes upon heating were conspicuous (Wu *et al.*, 1982). Boelens and Brandsma (1972) have proposed a free-radical mechanism for the formation of 2,4-dimethylthiophene from alkyl propenyl disulfide. 3,4-Dimethylthiophene can be formed by the same mechanism.

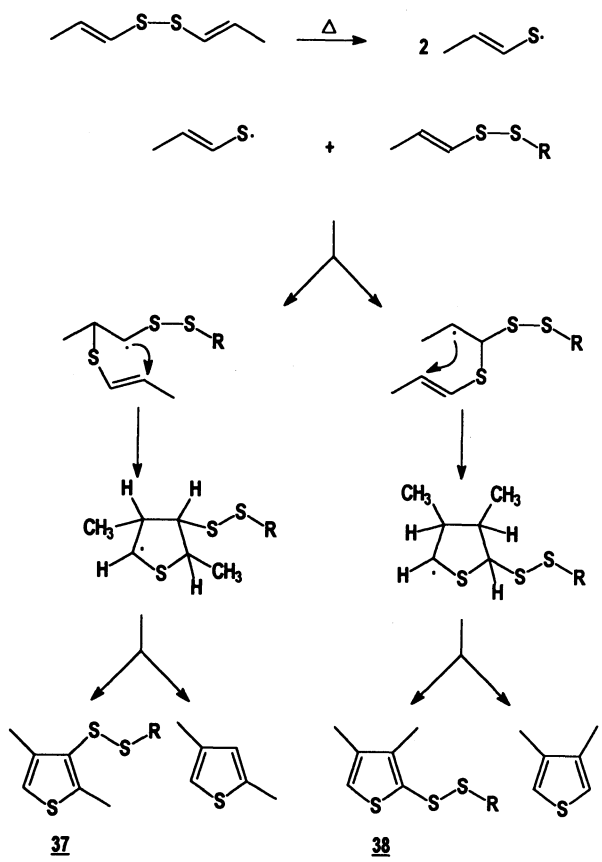


Figure 6. Possible mechanism for the formation of alkylthiophenes in heated shallots and scallions.

Figure 6 shows a possible mechanism for the formation of 2,4- and 3,4-dimethylthiophenes. This mechanism is further supported by recent identification of two alkyl dimethylthienyl disulfides (37, 38) in the volatiles of thermally processed scallions (Kuo *et al.*, 1990). Boelens *et al.* (1971) described the aroma of 2,4- and 3,4-dimethylthiophenes as being 'distinctly that of fried onions.' They should contribute significantly to the flavor of deep-fried shallots.

Frying temperature was found to have a significant effect on qualitative and quantitative changes of the volatile compounds formed from shallots (Chou and Wu, 1985). Table 4 shows the effect of temperature on the sulfur-containing compounds of fried shallots. Higher temperatures obviously favored the formation of sulfur-containing compounds.

Interaction of *Allium* Components with Lipids

When green onions are used as flavoring material in Chinese cuisine prepared by stir-fry, they are often heated in hot cooking oil to generate aroma prior to the cooking of vegetables or meats. Because of the thermally labile nature of the flavor compounds of the *Alliums*, considerable changes in the volatile composition may occur as a result of cooking and processing. In addition, the interaction of *Alliums* flavor compounds with other food components during cooking or processing are of interest in pursuit of the generation of food flavors by thermal reaction.

Table 4. Effect of temperature on the sulfur compounds of shallots which have been fried for 3 minutes (Wu *et al.*, 1982)

Compound	Amount at each temperature, °C(relative peak area)			
	120	140	160	180
Methyl propyl sulfide	—	—	1.7	9.9
Ethyl butyl sulfide	t[a]	2.2	26.7	42.6
Propyl propenyl sulfide	t	1.7	5.9	12.8
cis-Methyl propenyl disulfide	2.1	t	11.8	68.5
Methyl propyl disulfide + *trans*-methyl propenyl disulfide	t	2.1	58.5	504.9
Dipropyl disulfide + *trans*-dipropenyl disulfide	t	t	1.0	1.4
cis-Dipropenyl disulfide	t	1.4	1.8	5.1
Dimethyl trisulfide	1.2	2.2	26.7	42.6
Methyl propyl trisulfide	t	2.1	6.2	18.4
Dimethyl tetrasulfide	t	t	t	t
2,4-Dimethylthiophene	t	t	3.3	4.8
3,4-Dimethylthiophene	t	t	3.4	52.9
syn-3,5-Dimethyl-1,24-trithiolane	t	2.8	3.0	4.6
anti-3,5-Dimethyl-1,24-trithiolane	1.6	2.9	4.2	6.7

[a]t = trace.

Model systems containing 2,4-decadienal (2,4-D) and flavor components of *Alliums* were studied by examining the generation of sulfur-containing volatile constituents from the thermal interaction of 2,4-D with propanethiol. 2,4-D is one of the major oxidative degradation products of linoleic acid, the main component of vegetable oils such as soybean oil or corn oil. Propanethiol is a major volatile component of green onion flavor. Table 5 shows the volatile compounds generated from the thermal interaction of 2,4-decadienal with propanethiol. Interaction products of hydrogen sulfides or propanethiol with saturated or unsaturated aldehydes were observed (Kuo, 1991).

A model system has been used to study the interaction of alliin, the flavor precursors in garlic, with 2,4-decadienal (Yu *et al.*, 1994a). The volatile compounds generated may be divided into three groups: those generated from thermal degradation of alliin, those generated from the thermal degradation of 2,4-decadienal, and those generated from the interactions of 2,4-decadienal and alliin. Ten volatile compounds, namely allyl caproate, 2-butylthiophene, 4-(3-hydroxy-1-propenyl)phenol, 2-pentylthiophene, 2-pentylpyridine, methylpentylthiophene, 2-hexylthiophene, 2-hexanoylthiophene, 2-pentylbenzaldehyde, and 5-formyl-2-pentylthiophene, are thought to be generated from the interaction of 2,4-decadienal with alliin.

Similarly, a model system study has been conducted to examine the interaction of diallyl disulfide, a garlic component with 2,4-decadienal (Hsu et. al., 1993). S-allyl thiohexanoate was identified as an interaction product; Figure 7 shows the mechanism for its formation.

FLAVORS OF GINGER AND STAR ANISE

Table 6 shows a recent statistical survey of spices used in 84 Chinese dishes (FIRDI, 1988). Ginger and chives are the most frequently used spices. In this contribution, only ginger and star anise were selected for discussion.

Table 5. Volatile compounds generated from the thermal interaction of 2,4-decadienal with propanethiol

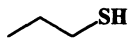
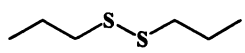
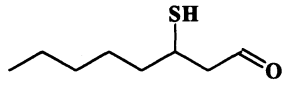
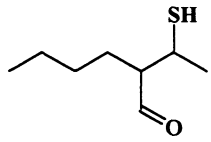
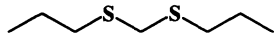
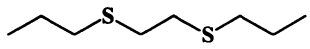
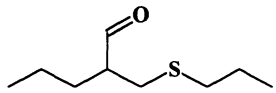
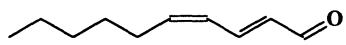
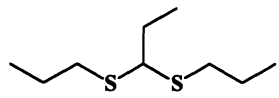
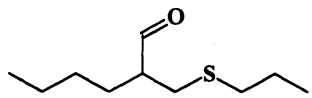
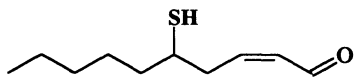
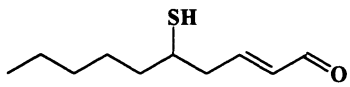
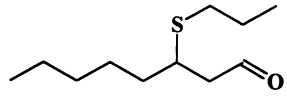
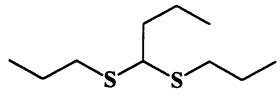
Compound	GC area %
Propanethiol	45.01
	
Dipropyl disulfide	22.23
	
3-Mercaptooctanal	3.83
	
2-Mercapto-3-formyl-heptane	0.03
	
4,6-Dithianonane	1.85
	
4,7-Dithiadecane	0.01
	
1-(Propylthio)-2-formylpentane	0.03
	
2,4-Decadienal	0.01
	
1,1-Bis(propylthio)-propane	0.06
	
1-(Propylthio)-2-formylhexane	0.21
	
5-Mercapto-2-decenal	0.12
	
5-Mercapto-2-decenal	0.01
	
3-(Propylthio)octanal	0.01

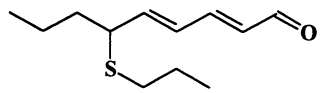
Table 5. (*continued*)



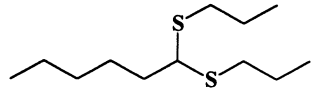
1,1-Bis(propylthio)-butane 0.06



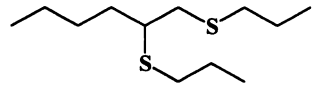
6-(Propylthio)-2,4-decadienal 0.01



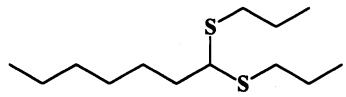
1,1-Bis(propylthio)-hexane 1.95



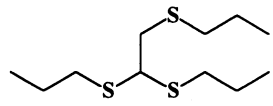
1,2-Di(propylthio)-hexane 0.56



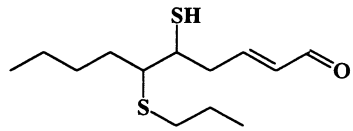
1,1-Bis(propylthio)-heptane 2.51



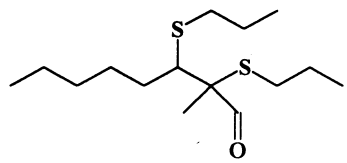
1,1,2-Tri(propylthio)-ethane 0.03



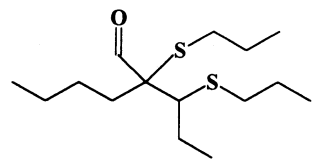
5-Mercapto-6-propylthio-2-decenal 0.06



2-Formyl-2,3-di-(propylthio)octane 0.25



4-Formyl-3,4-di-(propylthio)octane 0.23



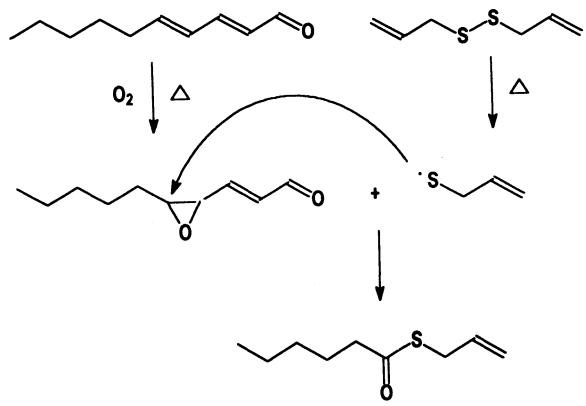


Figure 7. Proposal mechanism for the formation of S-allyl thiohexanoate.

Ginger

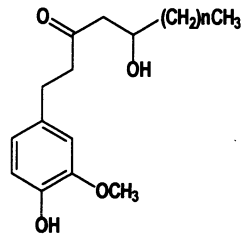
Ginger (*Zingiber officinale Roscoe*) has had a long, illustrated role in Chinese cuisine (Simoons, 1991). The earliest historical written record of the use of ginger dates back to the Confucian age (551–479 B.C.) (Lawreuce, 1984). The rhizome is the edible part of ginger. According to the time of harvesting, it can be classified into older rhizome (dry ginger) and younger rhizome (green ginger). Dry ginger is used primarily for flavoring and seasoning. Tender and mild green ginger can be used as a vegetable in itself, as when it is stir-fried with beef; or it can be pickled in brine, vinegar, or soy sauce for use as a condiment. Ginger is prized for its ability to cover up unpleasant or undesired fishy odors and tastes in seafood.

Pungency and aroma are the two most important characteristics of ginger or ginger products. Gingerol compounds, the dominant principles in rhizomes of ginger, are thermally labile due to the presence of a β-hydroxy keto functional group in their structures. Two homologous series of gingerol compounds — 6-, 8-, 10-, 12-, 14-gingerols (39–43) and methyl-6- (44), methyl-8- (45), methyl-10- (46), and methyl-12-gingerols (47) (Scheme 7) — were identified in the liquid CO_2 extract of ginger (Chen *et al.*, 1986a).

Table 6. Common spices used in chinese dishes (FIRDI, 1988)

Spice	Frequency of use (% of dishes)
Ginger	28.8
Chive	28.8
Garlic	13.1
Pepper	7.5
Capsicum	11.3
Sichuan pepper	3.8
Cinnamon	2.5
Star anise	4.4

Note. Based on a sample of 84 dishes.



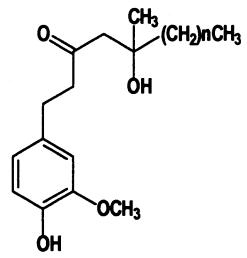
39 6-Gingerol, n=4

40 8-Gingerol, n= 6

41 10-Gingerol, n= 8

42 12-Gingerol, n=10

43 14-Gingerol, n=12



44 Methyl-6-Gingerol, n= 4

45 Methyl-8-Gingerol, n= 6

46 Methyl-10-Gingerol, n= 8

47 Methyl-12-Gingerol, n=10

Scheme 7

An old Chinese saying implies: 'The older the ginger, the more pungent the taste.' It was reported in the study of Chen *et al.* (1986b) that the amount of gingerols in older ginger was approximately twice the amount in green ginger. This also explains why older ginger is suitable for flavoring and seasoning purposes, while green ginger is for making pickled ginger.

High temperatures cause retro-aldolization of gingerols to produce aldehydes (C_6, C_8, C_{10}, C_{12}, and C_{14}) or methyl ketones (2-heptanone, 2-nonanone, 2-undecanone, and 2-tridecanone) (Chen *et al.*, 1987). Dehydration of gingerols at high temperatures generates shogaols (Chen *et al.*, 1986c). Figure 8 shows the thermal transformation of gingerols. It is clear that thermal treatment of ginger, such as cooking, will change both the taste and flavor of ginger.

The major sesquiterpene, α-zingiberene (48) could undergo oxidative conversion and for α-curcumene (49) (Figure 9) (Chen *et al.*, 1986d, Chen *et al.*, 1988). Such reaction will lead to the change of ginger flavor during cooking.

Wu et al (1990) studied the free and glycosidically bound aromas from ginger. Many glycosidically bound monoterpene alcohols were identified. It is interesting that several aroma compounds such as 2,6-dimethyl-3,7-octadiene-1,6-diol, 2,6-dimethyl-2,6-octadiene-1,8-diol, 2-hydroxy-1,8-cineole, p-mentha-1,5-dien-8-ol, p-mentha-1,5-dien-7-ol, p-mentha-2,8-dien-1-ol, p-mentha-1,8-dien-7-ol, and p-cymenol, were identified only in the glycosidically bound form in ginger.

Star Anise

Today star anise is cultivated in various countries, but early in this century China enjoyed a virtual world monopoly in star anise production (Simoons, 1991). It was mentioned previously that in preparing Chinese stewed meat, the commonly adopted practice is to place the proper amount of star anise, soy sauce, sugar, and meat together, and then stewing the resulting mixture for several hours. Soy sauce and star anise are two indispensable ingredients in the formation of stewed meat flavor.

The most abundant volatile component in star anise is *trans*-anethol (50), which constitutes over 80% of the volatile oil (Chen *et al.*, 1986). The thermal oxidation of *trans*-anethol has been reported (FIRDI, 1988). When *trans*-anethol was heated in water,

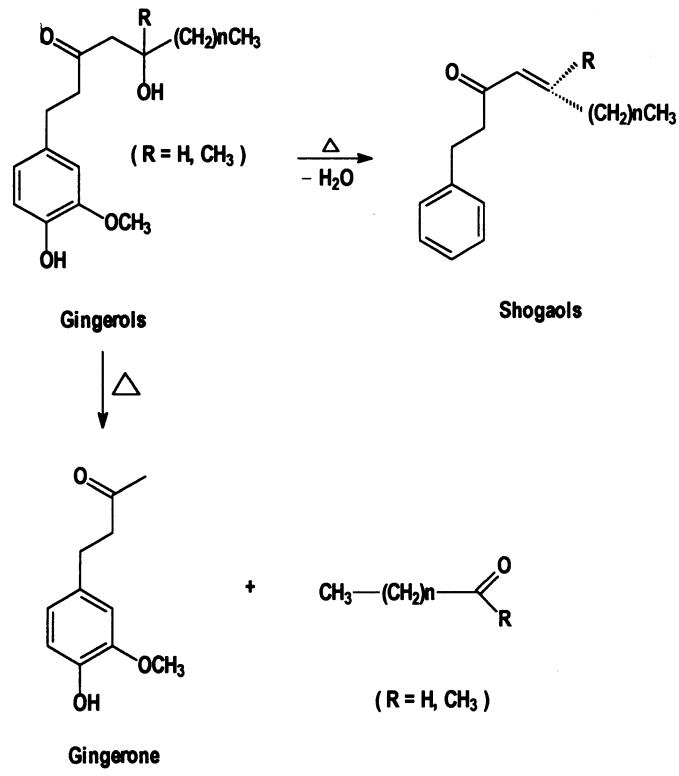


Figure 8. Thermal transformation of gingerols.

anisaldehyde (51), anisketone (52), ethyl *p*-methoxyphenyl ketone (53), and *cis*-anethol (54) were obtained (Figure 10). During the cooking of stewed meat, anisaldehyde may react with the sulfur-containing constituents of meat such as cysteine or cystine to form components of sensory significant (FIRDI, 1988).

FLAVOR OF BLACK MUSHROOM

The most common mushroom in China is the black mushroom (*Lentinus edodes Sing*), known as *Donggu* (winter mushroom) by the Chinese and often known to Westerners under the Japanese name, *Shiitake*. Due to the characteristic aroma of dried black mushroom, it is used extensively in poultry, meat, and other vegetable dishes. The flavor chemistry and medicinal properties of black mushroom has recently been reviewed (Mizuno, 1995).

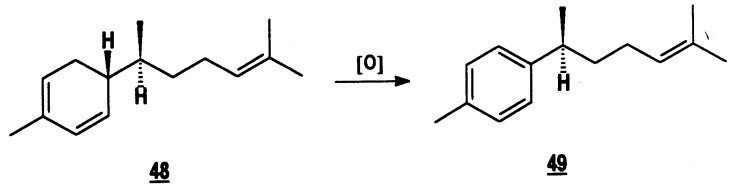


Figure 9. Thermal transformation of zingibrene.

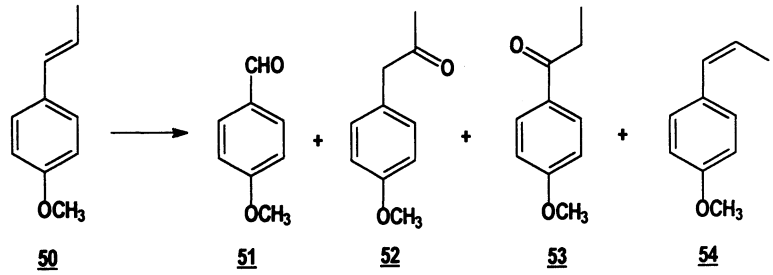
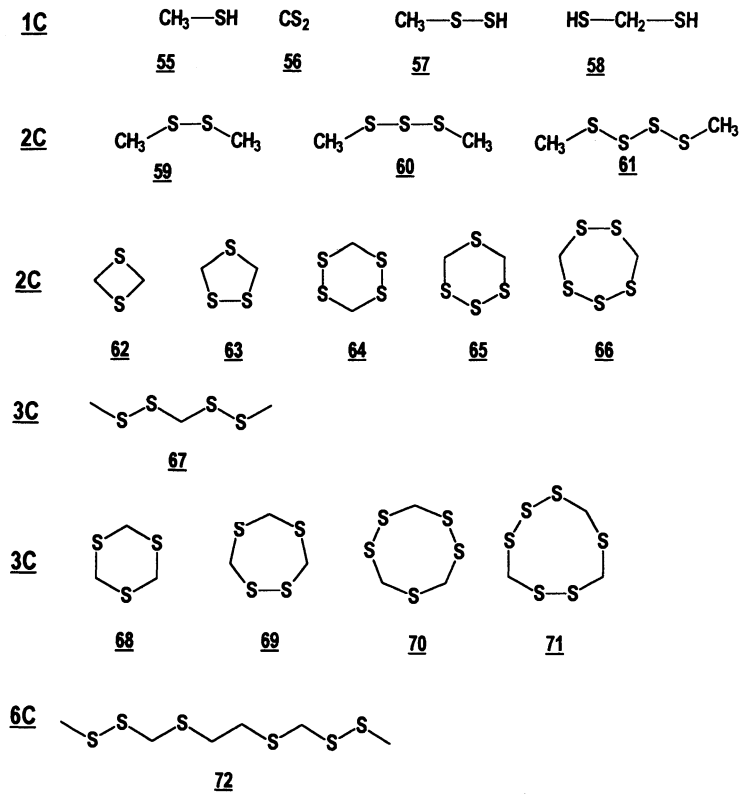


Figure 10. Thermal oxidative decomposition products of trans-anethol.

The fresh black mushroom exhibits only a slight odor, but upon drying and/or crushing, a distinct sulfurous aroma gradually develops. Lenthionine (1,2,3,5,6-pentathiepane) (66), a cyclic polysulfide known to possess the characteristic aroma of black mushroom, was first identified in dry mushroom (Morita and Kobayashi, 1966). A total of 18 acyclic and cyclic sulfur-containing compounds has been identified from the extract of the homogenate of fresh black mushroom. Their structures are shown in Scheme 8 (compounds 55–72). Cyclic polysulfides such as lenthionine, 1,2,4,5-tetrathiane, 1,2,3,5-tetrathiane, and 1,24-trithiolane were the major compounds identified (Chen and Ho, 1986).



Scheme 8

It is known the cyclic polysulfides are susceptible to thermal decomposition. Wada *et al.* (1967) reported that lenthionine will decompose completely if heated at 100°C in a 10% alcohol solution for 1 h (pH>5.0). In a typical dish of "steamed chicken with black mushroom soup," black mushrooms are cooked with chicken in boiling water for 2–3 hr. Under such conditions, it is possible that polysulfides such as the lenthionine of the black mushrooms will decompose and react with the constituents of chicken, such as hydrogen sulfide and carbonyls, to form compounds of significance to the flavor of the resulting soup.

CONCLUSION

The rich and delicate flavors are the main reason that Chinese cuisines are held in high esteem worldwide. Condiments and spices are heavily used in most Chinese foods. The volatile and nonvolatile components of these condiments and spices may react with lipids, proteins, sugars and other minor compounds of foods during the cooking of Chinese dishes and result in the development of complicated flavors.

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7

CHARACTER-IMPACT AROMA COMPONENTS OF CORIANDER (*CORIANDRUM SATIVUM* L.) HERB

K. R. Cadwallader, R. Surakarnkul, S.-P. Yang, and T. E. Webb

Department of Food Science and Technology
Mississippi Agricultural and Forestry Experiment Station
Mississippi State University
Box 9805, Mississippi State, Mississippi 39762

Volatile components were isolated from freshly harvested and market samples of coriander herb (cilantro) by direct solvent extraction with dichloromethane and analyzed by gas chromatography (GC)-mass spectrometry, GC-olfactometry, and aroma extract dilution analysis (AEDA). Enzyme decompositon of volatiles was minimized by conducting extractions at reduced temperature and in the presence of saturated sodium chloride. Volatile components of both samples were composed mainly of (E)-2-alkenals and alkanals, with (E)-2-decenal and (E)-2-dodecenal, and (E)-2-tetradecenal being the most abundant compounds. Results of AEDA revealed that (Z)-3-Hexenal (green/cut-grass) and an unknown odorant (rancid/sour/old cut-grass) had the greatest impact on the aroma of fresh-picked cilantro; however, in the market sample (Z)-3-hexenal was not detected and the unknown was at low odor intensity. (E)-2-Alkenals from C9-C14 and decanal were predominant odorants in both samples. These odorant provided mainly green/cut-grass and fatty/waxy aroma notes. Three unknown odorants, having fresh/swimming pool-like notes, were also found a high intensity in both samples.

INTRODUCTION

Coriandrum sativum L., a member of the Umbelliferae family, is cultivated worldwide to produce both coriander spice (fruit) and fresh leaves (herb). Coriander herb, more commonly referred to as cilantro or Chinese parsley, is an important culinary herb and an ingredient of many ethnic foods. A considerable amount of research has been conducted on the essential oil of coriander spice; however, comparatively few studies have focused on the volatile constituents of coriander herb.

Carlblom (1936) performed the first study on coriander herb composition and reported aldehydes (≈95%) as the major volatile components. Decanal was the most abun-

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dant aldehyde, with 2-decenal and 8-methyl-2-nonenal also being identified. Later studies confirmed the presence of these compounds in coriander herb. Although these reports differ from one another with respect to the relative abundances of these compounds, most agree in that a series of saturated aldehydes and 2-alkenals are the major volatiles. Scratz and Qadry (1966) reported 2-tridecenal and decanal to be the predominant leaf volatiles during the early stages of coriander development. MacLeod and Islam (1976) and Potter and Fagerson (1990) employed simultaneous steam distillation-solvent extraction (SDE) for the isolation of the herb oil constituents. Both studies found alkanals and alkenals to be the major constituents, but differed markedly in the actual composition reported. MacLeod and Islam (1976) found 7-dodecenal (≈21%) as the major component and did not detect any 2-alkenals; however, 7-dodecenal was not identified by Potter and Fagerson (1990) who reported (E)-2-decenal (≈46%) as predominant. Elsewhere, Lawrence (1986) reported alkanals and 2-alkenals as major constituents of coriander plants during ontogenesis. Similar results were reported by Mookherjee *et al.* (1989). Smallfield *et al.* (1993) studied the effects of postharvest treatment on the composition of coriander herb oil isolated by steam distillation and solvent extraction. Alkanals and alkenals were identified, with (*E*)-2-decenal being the major volatile constituent. The relative levels of aldehydes were found to decrease during storage of chopped herb, while levels of alcohols increased. Recently, Potter (1996) reported coriander leaf oil to contain mainly aldehydes (C_{10}-C_{16}) with (*E*)-2-alkenals predominanting. Considerable quantitative differences were observed between two commercial samples that were examined, as well as during ontogenesis for plants propagated in growth chambers.

The above review demonstrates the considerable confusion that exists over the volatile composition of coriander herb. While it is clear that alkanals and 2-alkenals are major constituents, the role of these compounds in the aroma of coriander herb has not been addressed. Furthermore, previous researchers relied mainly on distillation methods for isolation of volatiles, but such techniques could lead to artifact formation or loss of thermally labile constituents. The objectives of the present study were 1) to develop a solvent extraction technique to isolate the volatile components of coriander herb with minimal compositional changes and 2) to identify potent odor-active components in the extracts by aroma extract dilution analysis and GC-MS.

MATERIALS AND METHODS

Materials

Coriander plants (hereafter referred to as cilantro) were obtained from two sources. Sample A was cultivated locally (Starkville, MS) by a commercial produce and herb grower. Sample B was obtained from a local grocery store and originated from California. Both varieties are unknown. In the case of sample A, plants were harvested by uprooting and extracting within 1 h. For sample B, extraction was within 1 h of purchase.

Reference standards listed in Table I were obtained from the following commercial sources: nos. 2, 3, 5, 7, 12, 13, 16, 18, 20, 22, and 24 (Adrich Chemical Co., St. Louis, MO); nos. 6, 9, and 11 (Alfa, Ward Hill, MA); nos. 1 and 26 (Bedokian Research Inc., Danbury, CT); and no. 10 (Polyscience, Niles, IL). 3-Heptanol (internal standard), methanol, and sodium chloride were purchased from Aldrich Chemical Co. Dichloromethane (Aldrich Chemical Co.) was redistilled prior to use.

Isolation of Volatiles

Fresh leaves (10 g) were cut from plants with stainless steel scissors. Leaves plus 10 μL of an internal standard solution (3-heptanol, 3.07 μg/μL in methanol) and 10 g of sodium chloride were transferred to a 250-mL glass centrifuge bottle. The bottle was immersed in liquid nitrogen and the frozen contents ground into a fine powder with a pestle. After warming the ground herb to 0°C in an ice-water bath, ice-cold dichloromethane (50 mL) was added and the contents blended using a hand-held mixer (Bio Homogenizer, Biospec Products, Inc., Bartlesville, OK). The mixture was filtered through no. 40 filter paper (Whatman) and the filtrate was stored at -20°C in order to freeze-out excess water. Extract was concentrated to 10 mL under a gentle stream of nitrogen, dried by passage through 2 g of anhydrous sodium sulfate and then stored at -20°C prior to analysis. Three extracts were prepared for each sample.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS system consisted of an HP 5890 Series II GC/HP 5972 mass selective detector (MSD, Hewlett-Packard, Co., Palo Alto, CA). Separations were performed on fused silica capillary columns (DB-WAX or DB-5ms, 60 m length x 0.25 mm i.d. × 0.25 μm film thickness (d_f); J&W Scientific, Folson, CA). Extracts (3 μL) were injected in the splitless mode (200°C injector temperature; 30 s valve-delay). The carrier gas was helium at a linear velocity of 25 cm/s (at 40°C). Oven temperature was programmed from 40°C to 200°C at a rate of 3°C/min with initial and final hold times of 5 and 60 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 280C; ionization energy, 70 eV; mass range, 33–350 a.m.u.; EM voltage (Atune + 200 V); scan rate, 2.2 scans/s.

Compounds were identified by comparison or their mass spectra, retention indices (van den Dool and Kratz, 1962), and odor properties with those of authentic reference standards.

Quantitation

MS response factors relative to the internal standard were used quantify selected positively identified compounds. Extractions were performed as previously described except that 10 mL of deodorized water spiked with various amounts of each standard was substituted for the cilantro sample.

Aroma Extract Dilution Analysis

GC-olfactometry was conducted on a Varian 3300 (or 3400) GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector (FID) and sniffing port. Serial dilutions (1:3) were prepared in dichloromethane. Each dilution (3 μL) was analyzed using a capillary column (DB-WAX or DB-5ms, 30 m length × 0.32 mm i.d. x 0.25 μm d_f; J&W Scientific). Column effluent was split 1:1 between FID and sniffing port using deactivated fused silica capillary columns (1 m length × 0.25 mm i.d.). FID and sniffing port were maintained at a temperature of 200°C. Sniffing port was supplied with humidified air (30 mL/min). GC conditions were the same as for GC-MS except that oven temperature was programmed from 40°C to 200°C at a rate of 6°C/min (or 10°C/min for DB-WAX) with initial and final hold times of 5 and 30 min, respectively. Further details of AEDA have been previously described (Baek *et al.*, 1997).

RESULTS AND DISCUSSION

Volatile Composition of Cilantro

In our initial attempts at the isolation of cilantro volatiles we observed that sample preparation and timing of extraction have a dramatic effect on extract composition and variability of results. To minimize enzyme decomposition of volatiles during tissue disruption it was necessary to maintain the fresh leaves in the presence of saturated sodium chloride and under liquid nitrogen. Furthermore, we found it necessary to conduct solvent extraction at ≈0°C. Without the use of these measures, aldehyde content rapidly decreased with an increase in corresponding alcohols (data not shown). This phenomenon was reported by Smallfield *et al.* (1993) for chopped cilantro and they suggested it might be due to the presence of a nonspecific oxidoreductase. By employing the above measures it was possible to minimize enzymatic reduction of aldehydes and obtain extracts with reasonably high reproducibility (Table 1). The incorporation of saturated salt during extraction also served to retard enzyme action, such as lipoxygenase (Buttery *et al.*, 1994).

Volatile composition data for two cilantro samples are given in Table 1. The fresh cilantro (sample A) had both a greater number and higher abundance of volatile constituents than the market sample (sample B). The alkenals (E)-2-decenal (no. 9) and (E)-2-dodecenal (no. 18), and (E)-2-tetradecenal (no. 26) were in highest abundance in both samples. Other alkenals found in both samples included (E)-2-undecenal (no. 13), (E)-2-tridecenal (no. 22), (E)-2-pentadecenal (no. 27), and (E)-2-hexadecenal (no. 28). Sample A contained several alkenals not detected in sample B (e.g. nos 1, 2, 5, 6). (Z)-3-hexenal (no. 1) had not been previously reported in cilantro. Decanal (no. 7) was the most abundant saturated aldehyde in both samples, followed by undecanal (no. 12) and dodecanal (no. 18). Nonanal and tetradecanal were only detected in sample A, while tridecanal was found only in sample B. In addition to aldehydes, two alcohols, namely decanol (no. 10) and (E)-2-decen-1-ol (no. 11) were found at low levels in both samples.

Our quantitative results agree with those of Potter and Fagerson (1990) who found (E)-2-decenal , (E)-2-dodecenal and (E)-2-tetradecenal to be the major aldehydes in cilantro at the blooming stage. In a later study, Potter (1996) reported substantial differences in (E)-2-alkenal contents between two market cilantro samples. Unfortunately, neither of these studies reported actual concentrations of these compounds, but instead estimated their levels as percentages of the total area of all peaks detected. The present study is the first to report concentration estimates of the major volatile constituents of cilantro.

Aroma-Active Components of Cilantro

The aroma of fresh-cut cilantro has a typically pungent citrusy, soapy, and chlorine-like character. The two samples examined in the present study were considered to be typical of fresh cilantro; however, the aroma of sample A was notably stronger had a distinctive green and cut-leaf note that was lacking in sample B. The extracts prepared from both samples were regarded by us as having the same aroma characteristics as the original samples and were, therefore, suitable for aroma extract dilution analysis (AEDA).

AEDA was conducted on two types of GC columns. Flavor dilution (FD) chromatograms determined on DB-WAX and DB-5ms columns are presented in Figures 1 and 2, respectively. The DB-WAX column yielded a total of 10 detected dorants for sample A, while only 8 odorants were detected for sample B. All odorants detected in sample B were detected in sample A. Two odorants, (Z)-3-hexenal (green/cut-grass, no. 1) and an un-

Table 1. Volatile components of cilantro

No.[a]	Compound	Retention index[b]		Concentration (μg/g)[c]		Odor Description[f]
		DB-5ms	DB-Wax	Sample A[d]	Sample Be	
1	(Z)-3-hexenal	801	1137	2.02 (± 0.94)	—[g]	green, cut-grass
2	(E)-2-hexenal	854	1210	0.38 (± 0.16)	—	
3	nonanal	1107	1388	0.13 (± 0.0.03)	—	
4	unknown	1148				stale, old cut-grass
5	(E)-2-nonenal	1163	1528	0.47 (± 0.10)	—	stale, dry hay
6	(E)-4-decenal	1196	1540	tr[h]	—	green, citrus peel
7	decanal	1210	1494	10.6 (± 2.0)	5.56 (± 1.01)	green, citrus peel
8	unknown	1249	1617			rancid, sour, old cut-grass
9	(E)-2-decenal	1265	1639	59.2 (± 12.6)	2.41 (±1.17)	green, cut-grass, lettuce
10	decanol	1271	1765	0.39 (± 0.12)	0.0595 (±0.003)	
11	(E)-2-decen-1-ol	1274	1820	2.23 (± 0.58)	—	
12	undecanal	1310	1599	0.23 (± 0.05)	0.35 (± 0.01)	
13	(E)-2-undecenal	1369	1744	3.96 (± 0.68)	0.57 (±0.15)	fresh, green, waxy
14	unknown	1379				green leaf
15	unknown	1398	1807			fresh, swimming pool area
16	dodecanal	1413	1705	0.38 (± 0.07)	1.03 (±0.09)	green
17	unknown	1453				green, waxy
18	(E)-2-dodecenal	1476	1855	22.3 (± 3.2)	9.10 (±0.55)	green, waxy
19	unknown	1503				fresh, swimming pool area
20	tridecanal	1510	1810	—	0.059 (±0.01)	
21	unknown	1544				fatty, cheesy, waxy
22	(E)-2-tridecenal	1574	1962	2.99 (± 0.56)	1.09 (±0.0.04)	fatty, cheesy, waxy, floral
23	unknown	1604				fresh, swimming pool area
24	tetradecanal	1616	1917	0.18 (± 0.03)	—	
25	unknown	1665				fish bowl
26	(E)-2-tetradecenal	1685	2072	44.9 (± 8.4)	14.2 (±0.16)	fatty, waxy, cheesy
27	(E)-2-pentadecenal[i]	1784	2179	6.49 (± 1.0)	4.65 (±0.24)	
28	(E)-2-hexadecenal[i]	1883	2288	4.86 (± 0.66)	1.93 (±0.10)	

[a]Numbers correspond to those in Figures 1 and 2. [b]Retention indices calculated from GC-O results. [c]Average concentration expressed on wet weight basis. Numbers in parantheses are standard deviations (n=3). [d]Sample A was grown locally. [e]Sample B was obtained from local grocery store. [f]Odor description assigned by panelists during GC-O. [g]Compound not detected. [h]Compound present at trace level. [i]Compound tentatively identified based on mass spectrum. Quantitation based on standard curve data of compound no. 26.

known compound (rancid/sour/old cut-grass, no. 8), had the highest $\log_3$FD-factors in sample A. Compound no. 1 was not detected in sample B, while 8 was detected at a low $\log_3$FD-factor. The occurrence of (Z)-3-hexenal in sample A was probably responsible its intense green and cut-leaf note. This odorant has a low odor detection threshold of 0.25 ppb (Buttery *et al.*, 1987). Odorants having moderately high $\log_3$FD-factors in both samples were decanal (green/citrus peel, no. 5), (E)-2-decenal (green/cut-grass/lettuce, no. 9), (E)-2-undecenal (fresh/green/waxy, no. 13), (E)-2-dodecenal (green/waxy, no. 18), and (E)-2-tridecenal (fatty/cheesy/ waxy/floral, no. 22). (E)-2-Tetradecenal (fatty/waxy/ cheesy, no. 26) and an unknown (fresh/swimming pool area, no. 15) were detected with low $\log_3$FD-factors in both samples. (E)-2-Nonenal was only detected in sample A at a moderate $\log_3$FD-factor.

The results of AEDA obtained on the DB-5ms column were superior those obtained on the DB-WAX column since more odorants were detected in each of the two samples. A total of 18 and 12 odorants were detected in samples A and B, respectively. Eleven of these odorants were common to both samples. As was previously observed for the DB-WAX column, (Z)-3-hexenal was only detected in sample A and had the highest $\log_3$FD-

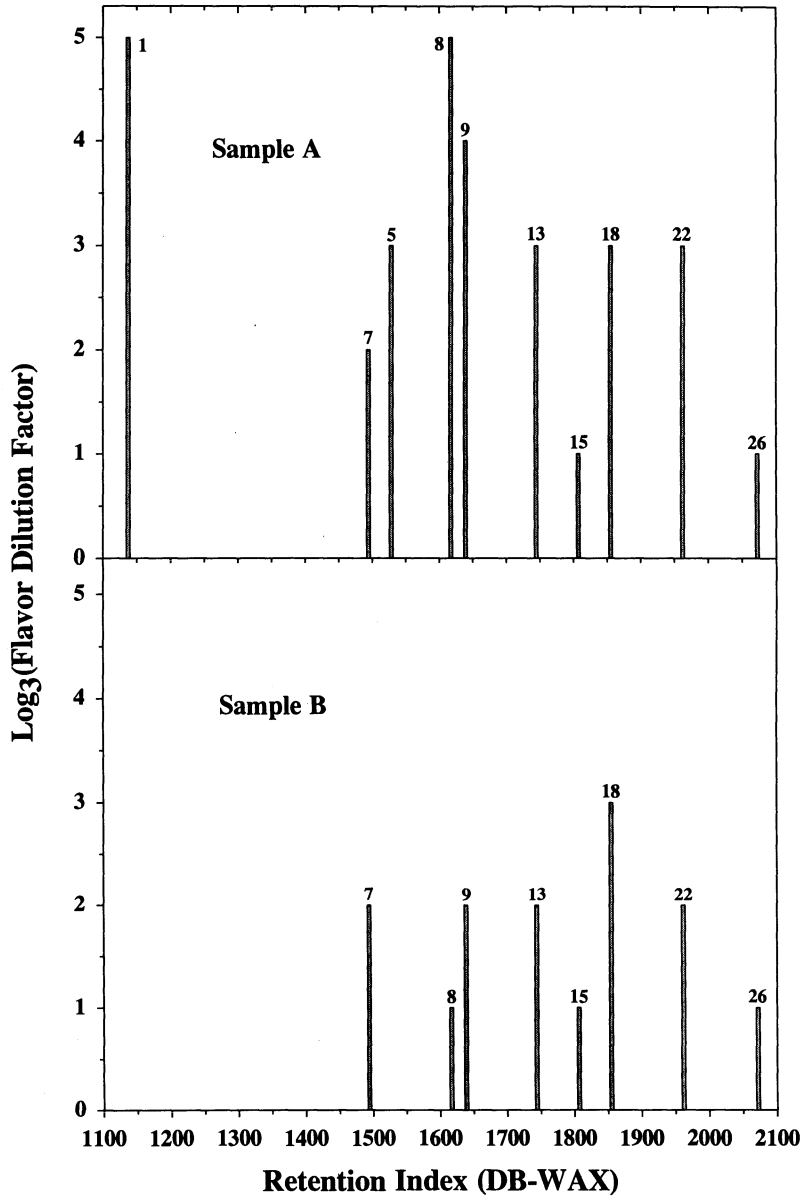

Figure 1. Flavor dilution chromatograms for cilantro samples A and B determined on DB-WAX capillary column. Numbers correspond to those in Table 1 and Figure 2.

factor in this sample. Two unknowns (no. 8 and 15) had the second highest log_3FD-factors in sample A followed by the 2-alkenals (nos. 13, 18, and 22) and four odorants with moderately high log_3FD-factors (nos. 5, 7, 9, 26). Compounds nos. 15 and 18 had the highest log_3FD-factors in sample B followed by nos. 7, 8, 13, 22, and 26. Several odorants (nos. 19, 21, 23) had low log_3FD-factors in both samples. Compounds nos. 4, 6, 14, 17 and 25 were detected with low log_3FD-factors in sample A only, while no. 16 was exclusively detected in sample B.

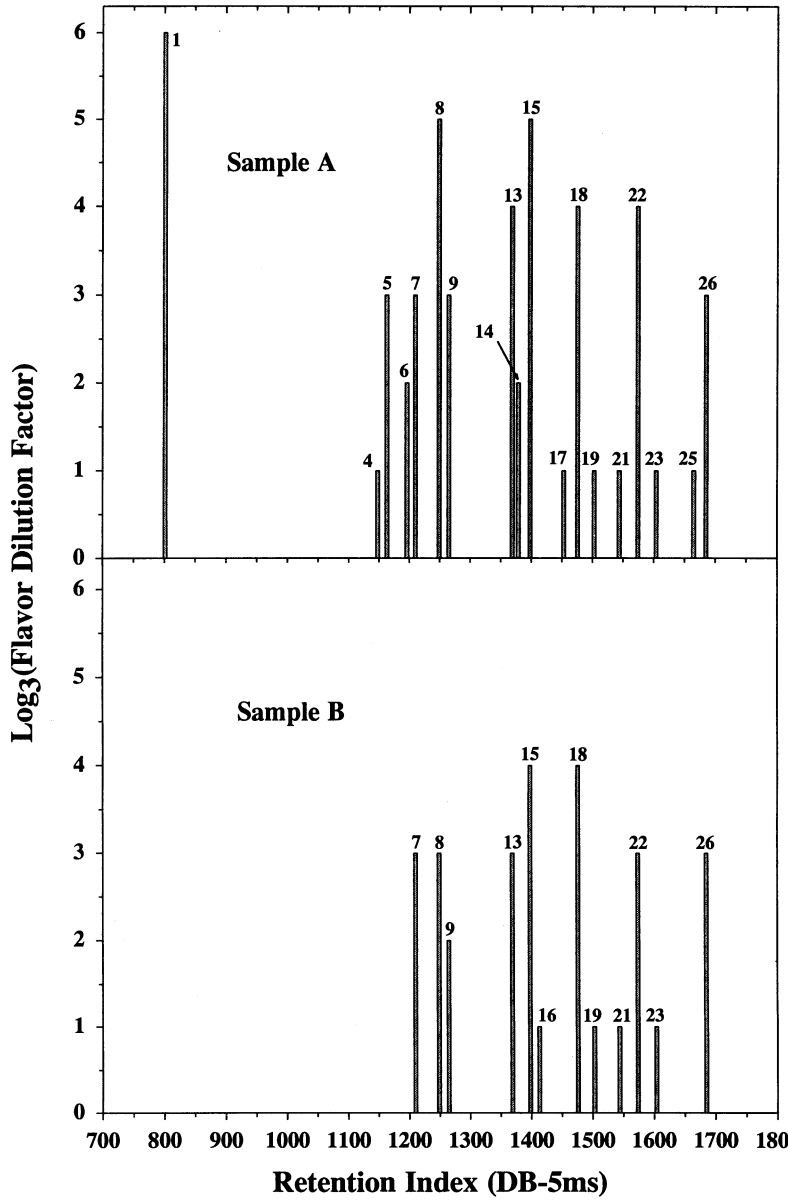

Figure 2. Flavor dilution chromatograms for cilantro samples A and B determined on DB-5ms capillary column. Numbers correspond to those in Table 1 and Figure 1.

Due to its intense green/cut-grass aroma note, (Z)-3-exenal probably has the greatest impact on the aroma of fresh-picked cilantro. However, this compound may not be an essential component of cilantro aroma since its absence from sample B did not alter the "cilantro-like" character of this sample. A similar observation could be made for compound no. 8, which was predominant in sample A but of low intensity in sample B. The (E)-2-alkenals from C9-C14 would appear to be the most important components of cilantro aroma. We are uncertain as to which of these compounds is most important as the levels of these compounds could differ greatly for different cilantro samples. In addition to

the (E)-2-alkenals, three unknown odorants (nos. 15, 19, and 23), which were described as having fresh/swimming pool area-like aroma notes, may influence cilantro aroma. These odorants provide a fresh chlorine-like note that can be readily detected in fresh-cut cilantro. Further work is in progress to elucidate the structures of these compounds.

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8

WASABI, JAPANESE HORSERADISH, AND HORSERADISH

Relationship between Stability and Antimicrobial Properties of Their Isothiocyanates

Hideki Masuda,[1] Yasuhiro Harada,[1] Toshio Inoue,[1] Noriaki Kishimoto,[2] and Tatsuo Tano[2]

[1]Material R&D Laboratories, Ogawa and Co., Ltd.
1-2, Taiheidai, Shoo-cho, Katsuta-gun, Okayama 709-43, Japan
[2]Faculty of Food Culture, Kurashiki Sakuyo University
3515, Nagao, Tamashima, Kurashiki-city, Okayama 710-02, Japan

Wasabi, Japanese horseradish, and horseradish are used as spices mainly due to their strong pungency and antimicrobial action. When the cell membrane of wasabi or horseradish is broken, the isothiocyanates are formed by the myrosinase-assisted hydrolysis of thioglucosides. However, the isothiocyanates are unstable when in contact with water. The stability of 14 isothiocyanates in aqueous methanol was more affected by temperature than by pH. Furthermore, the antimicrobial properties of these isothiocyanates against four species of bacteria and two species of fungi followed a reverse trend in comparison with their stability. However, the inhibitory effects of these isothiocyanates were not the same for growing cells of *S. mutans*.

INTRODUCTION

It is well known that the thioglucosides involved in *cruciferous vegetables* are hydrolyzed by the action of myrosinase to form volatile compounds, including isothiocyanates, thiocyanates and nitriles, among others (Nijssen *et al.*, 1996; Harbone and Baxter, 1993; Kjaer *et al.*, 1953; Mazza, 1984; Kumagai *et al.*, 1994). Among the volatile compounds, isothiocyanates are recognized as being responsible for the characteristic odor and pungency of such vegetables (Ina *et al.*, 1981; Masuda *et al.*, 1996). In general, the isothiocyanates are unstable when allowed to stand in contact with water. Allyl isothiocyanate was gradually decomposed in aqueous solution and produced a garlic-like odor (Kawakishi and Namiki, 1969). In addition, the degradation of the essential oils of wasabi and horseradish in aqueous methanol solution has been studied (Ina *et al.*, 1981; Ina *et al.*,

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Kluwer Academic / Plenum Publishers, New York, 1999.

1981). However, the stability of each isothiocyanate in wasabi and horseradish under various conditions has not yet been reported.

Wasabi and horseradish are well known to exert antimicrobial actions. The inhibitory effects of the essential oil obtained from wasabi and allyl isothiocyanate, the main component in wasabi, against many species of bacteria using minimum inhibitory concentration (MIC) values have been reported (Inoue *et al.*, 1983). The inhibitory effects of allyl isothiocyanate against different species of bacteria were also studied (Kanemaru and Miyamoto, 1990; Tokuoka and Isshiki, 1994). Furthermore, the antimicrobial action of methyl-, ethyl-, benzyl-, and 2-phenethyl isothiocyanate, involved in horseradish, against some bacteria and fungi (Forter, 1940; Kleese and Lukoschek, 1955; Kojima and Ogawa, 1971), as well as a series of alkyl- and aryl isothiocyanates against some fungi was reported (Drobinica *et al.*, 1967a,b; Mckay *et al.*, 1959; Lien *et al.*, 1968). However, the antimicrobial action of ω-alkenyl- and ω-methylthioalkyl isothiocyanates, the characteristic odor compounds in wasabi and horseradish, respectively, has not been studied.

S. mutans is known as the primary causative agent of dental caries in humans (Hamada *et al.*, 1984). The water-insoluble glucan, which is a highly adherent substance, has been reported to be synthesized from *S. mutans* and sucrose (Koga *et al.*, 1986). It has become apparent that *S. mutans* is fixed on the surface of a tooth through the glucan and produces organic acids by metabolism. Benzyl isothiocyanate was found to inhibit the growth and the acid product ability of *S. mutans* (Al-Bagieh and Weinberg, 1988). In addition, allyl isothiocyanate has been reported as an antimicrobial agent against carcinogenic streptococci (Haas, 1976). However, a detailed study of the other isothiocyanates except for benzyl- and allyl isothiocyanate has not yet been made.

This study focuses on the evaluation of the stability and the antimicrobial properties of the isothiocyanates in wasabi and horseradish against four species of bacteria and two species of fungi. Furthermore, the relationship between the stability and antimicrobial properties is presented. The relationship between the stability of the isothiocyanates and the inhibitory effects of selected isothiocyanates on sucrose dependent adherence by growing cells of *S. mutans* is also reported.

EXPERIMENTAL

Four ω-alkenyl isothiocyanates, except allyl isothiocyanate, were prepared by isomerization of their corresponding ω-alkenyl thiocyanates (Masuda *et al.*, 1990). Five ω-alkenyl isothiocyanates were converted to their corresponding ω-methylthioalkyl isothiocyanates (Harada *et al.*, 1995). The other isothiocyanates were purchased from commercial sources and purified by vacuum distillation.

The stability of the isothiocyanates was determined as follows. Each isothiocyanate (0.02 mmol) was introduced into 1.4 ml of methanol and 0.3ml of water; the pH was adjusted to 2, 4, 7, and 9 with concentrated hydrochloric acid, acetic acid, distilled water, and sodium carbonate, respectively. The solution was allowed to stand at different temperatures (3, 25, and 50°C). Each solution was extracted with 3 × 50 ml of dichloromethane and concentrated using a rotary evaporator (35°C/300 mm Hg). The residual amounts of isothiocyanates were determined by GC. A Hitachi G-5000 fitted with an FID was used. A DB-1 (30m × 0.25mm i.d.) fused-silica capillary column was employed. The operating conditions were as follows: initial oven temperature, 60°C, then to 250°C at 3°C/min and held for 30 min; injector temperature, 250°C; carrier gas, 0.5 ml/min N_2.

Peak areas were obtained using a Hitachi D-2500 Chromato-Integrator. To estimate the concentrations of the components diphenyl oxide, was an internal standard.

The antimicrobial action of the isothiocyanates was determined by a dilution method using a microplate with 96 wells (Beppu *et al.*, 1992). The following bacteria were used: *Bacillus subtilis* IFO 3134, *Staphyrococcus aureus* IFO 12732, *Escherichia coli* IFO 3301, and *Pseudomonas aeruginosa* IFO 3080 which were obtained from the Institute for Fermentation, Osaka (Japan). The antimicrobial action was tested in the following medium: peptone (5g), yeast extract (1.5g), meat extract (1.5g), sodium chloride (3.5g), glucose (1g), disodium hydrogenphosphate (3g), potassium dihydrogen phosphate (1.32g), and distilled water (1000 ml). The following fungi were used: *Candida albicans* IFO 1385 and *Aspergillus niger* IFO 4414, both obtained from the Institute for Fermentation, Osaka (Japan). The antifungal action was tested in Saburo broth (Difco Laboratories) (Beppu *et al.*, 1992). For testing strains, except for *B. subtilis* and *A. niger*, each isothiocyanate in 80% aqueous methanol solution (36 μl) was added to the well in the first file contained with 324 μ l of liquid culture (1×10^6 cells/ml) and then serially diluted. For *B. subtilis* and *A. niger*, the same sample solution was added to the well in the first file containing culture broth and spores (*B. subtilis*: 1×10^6 spores/ml, *A. niger*. 1×10^4 spores/ml). The microplates, except for *C. albicans* and *A. niger*, were incubated at 35°C for 6h. The transmittance at 630nm for each well was measured using a Microplate Reader (Iwaki Co., Ltd, Japan). *C. albicans* and *A. niger* were incubated at 25°C for 22h and for 15h, respectively. The MIC of samples was determined at the highest diluted concentration that completely inhibited the cell growth, using serial dilution.

The anticarcinogenic action of the isothiocyanates was determined by a dilution method using a test tube (Kawakishi, 1996). The following bacterium was used: *S. mutans* IFO 13955 which was obtained from the Institute for Fermentation, Osaka (Japan). *S. mutans* IFO 13955, which was cultured in Brain Heart Infusion (BHI) broth (Nissui Seiyaku Co., Ltd., Japan) and each isothiocyanate in 80% aqueous dimethyl sulfoxide (0.05ml) or 80% aqueous ethanol solution (0.05 ml) were added to the test tube containing 4.94 ml of BHI broth and 1% sucrose (1×10^6 CFU/ml). The test tube was allowed to stand at an angle of 30° for 24–48h at 37°C. After the BHI broth was removed, the residue was dried and the weight of the residue was determined. Sucrose dependent adherence of *S. mutans* was represented as the relative percentage compared with the adherence in the absence of isothiocyanate.

RESULTS AND DISCUSSION

The stability of alkyl isothiocyanates (isopropyl- (**1**), *sec*-butyl- (**3**), and isobutyl isothiocyanate (**4**)), ω-alkenyl isothiocyanates (allyl- (**2**), 3-butenyl- (**5**), 4-pentenyl- (**6**), 5-hexenyl- (**7**), and 6-heptenyl isothiocyanate (**8**)), ω-methylthioalkyl isothiocyanates (3-methylthiopropyl- (**9**), 5-methylthiopentyl- (**12**), 6-methylthiohexyl- (**13**), and 7-methylthioheptyl isothiocyanate (**14**)), and aryl isothiocyanates (benzyl- (**10**) and 2-phenethyl isothiocyanate (**11**)) allowed to stand for 7 days at pH 7 and 50°C are shown in Figures 1 and 2. Figures 1 and 2 exhibit a similar behavior at a variety of pHs and temperatures. The stability of isothiocyanates was found to be more affected by temperature than pH. In general, alkyl isothiocyanates **1, 3,** and **4** had a higher stability than ω-alkenyl- **5, 6, 7,** and **8**, ω-methylthioalkyl- **9, 12, 13,** and **14**, and aryl isothiocyanates **10** and **11**. Interestingly, the stability of ω-alkenyl- **5, 6, 7,** and **8**, ω-methylthioalkyl- **9, 12, 13,** and **14**, and aryl isothiocyanates **10** and **11** increased in proportion to the length of the carbon

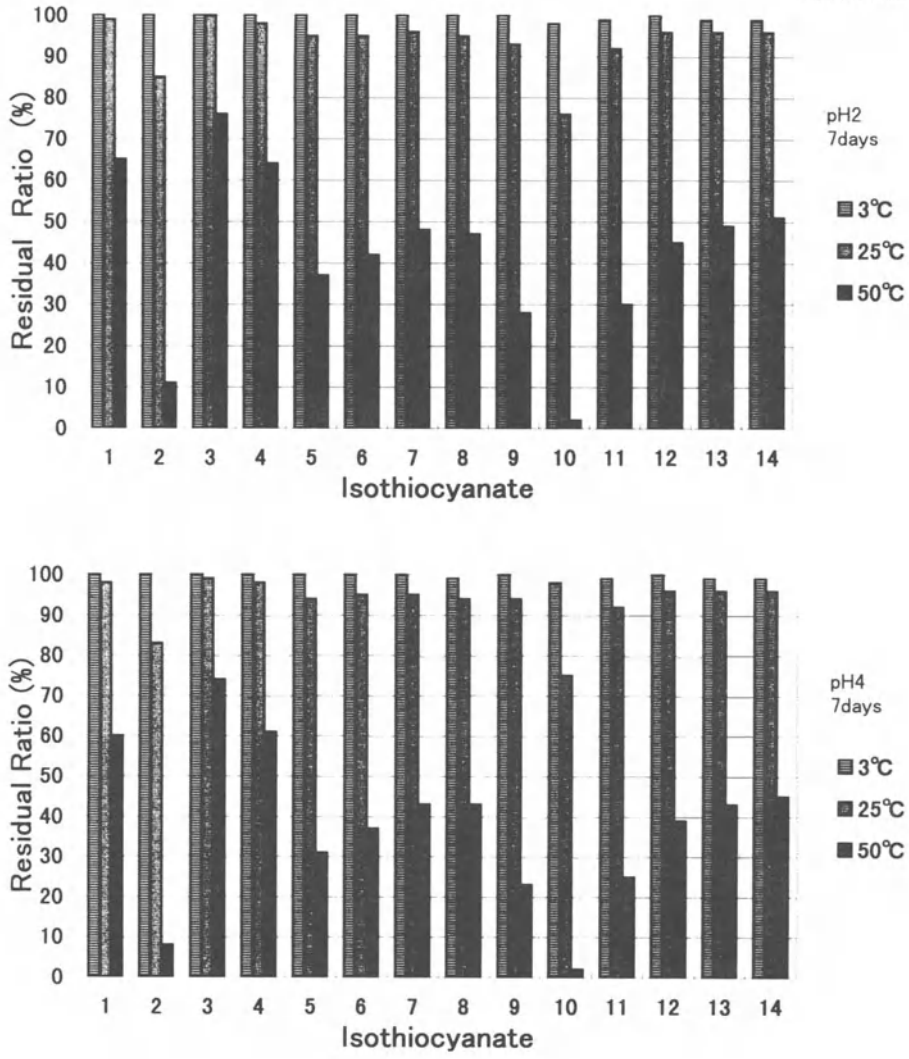

Figure 1. Residual ratios of the isothiocyanates in aqueous methanol solution at 3, 25, and 50°C allowed to stand for 7 days at pH 2 (top) and pH 4 (bottom).

chain. Above all, **10**, one of the characteristic impact odor compounds in horseradish, was found to have a lower stability than **2**, the main volatile component in wasabi and horseradish.

The inhibitory effects of isothiocyanates against *B. subtilis*, *S. aureus, E. coli, P. aeruginosa, C. albicans, and A. niger* are shown in Figure 3. The isothiocyanates have been found to have different MIC values against each bacterium and fungus. The lower the MIC value, the higher the antimicrobial action. Consequently, ω-alkenyl- **5, 6, 7,** and **8**, ω-methylthioalkyl- **9, 12, 13,** and **14**, and aryl isothiocyanates **10** and **11**, in general, possessed a higher antimicrobial activity than alkyl isothiocyanates **1, 3,** and **4**. Especially, ω-methylthioalkyl isothiocyanates **9, 12, 13,** and **14** exhibited a highly effective antimicrobial action against *B. subtilis*, *S. aureus, C. albicans, and A. niger*. The ω-alkenyl isothiocyanates **5, 6, 7,** and **8** also showed a significant antimicrobial action against *S. aureus, P. aeruginosa, C. albicans, and A. niger*. In addition, interestingly, the MIC values have been

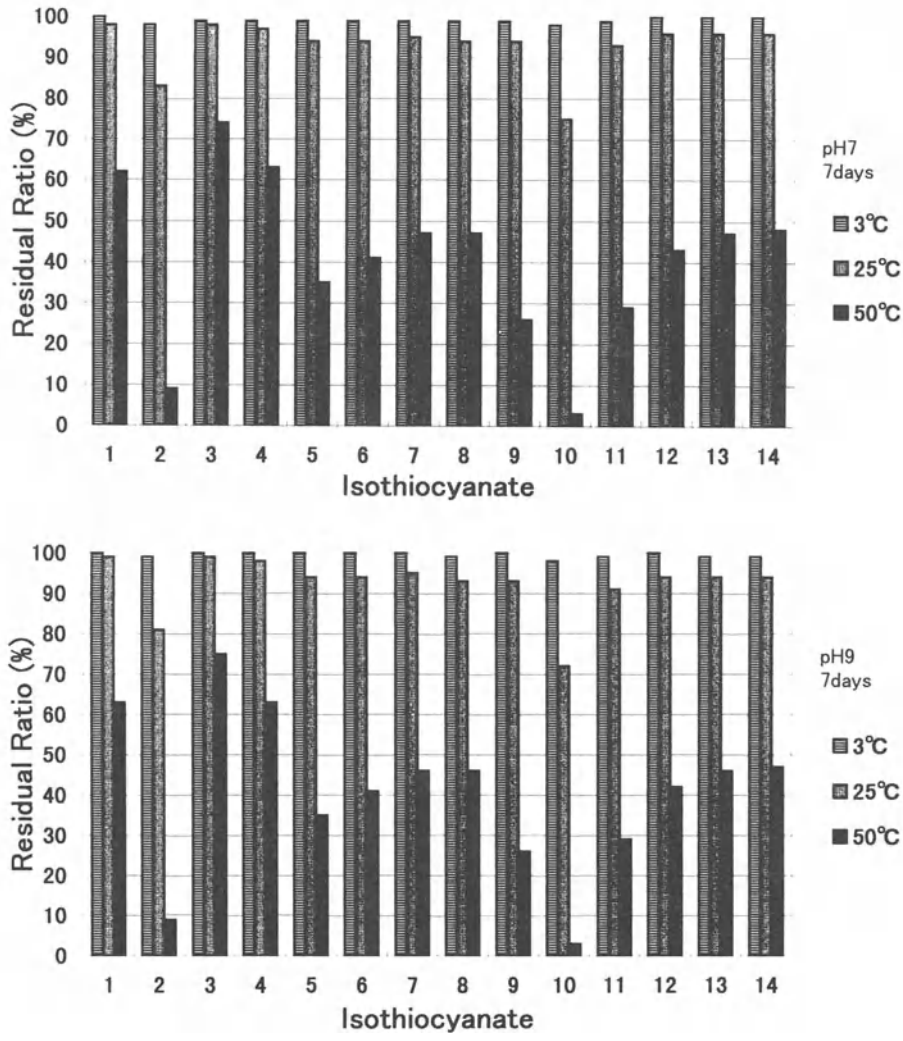

Figure 2. Residual ratios of the isothiocyanates in aqueous methanol solution at 3, 25, and 50°C allowed to stand for 7 days at pH 7 (top) and pH 9 (bottom).

found to show similar behavioral tendencies when comparing the residual ratios (Figures 1–3). Hence, the antimicrobial action of ω-alkenyl- **5, 6, 7,** and **8**, ω-methylthioalkyl- **9, 12, 13,** and **14**, and aryl isothiocyanates **10** and **11**, in general, decreased in proportion to the length of the carbon chain.

The inhibitory effects of the isothiocyanates on sucrose dependent adherence by growing cells of *S. mutans* are shown in Figure 4 (top). The lower the sucrose dependent adherence of *S. mutans*, the higher the caries inhibitory effects. In general, ω-alkenyl-, ω-methylthioalkyl-, and aryl isothiocyanates except for **2** and **5** had higher caries inhibitory effects. The relationships between the concentration of ω-alkenyl- **5, 6, 7,** and **8**, ω-methylthioalkyl- **9, 12, 13, 14,** and **15** (4-methylthiobutyl isothiocyanate), and aryl isothiocyanates **10** and **11** and the sucrose dependent adherence of *S. mutans* are indicated in Figures 4 (bottom), 5 (top), and 5 (bottom), respectively. Interestingly, in all the runs,

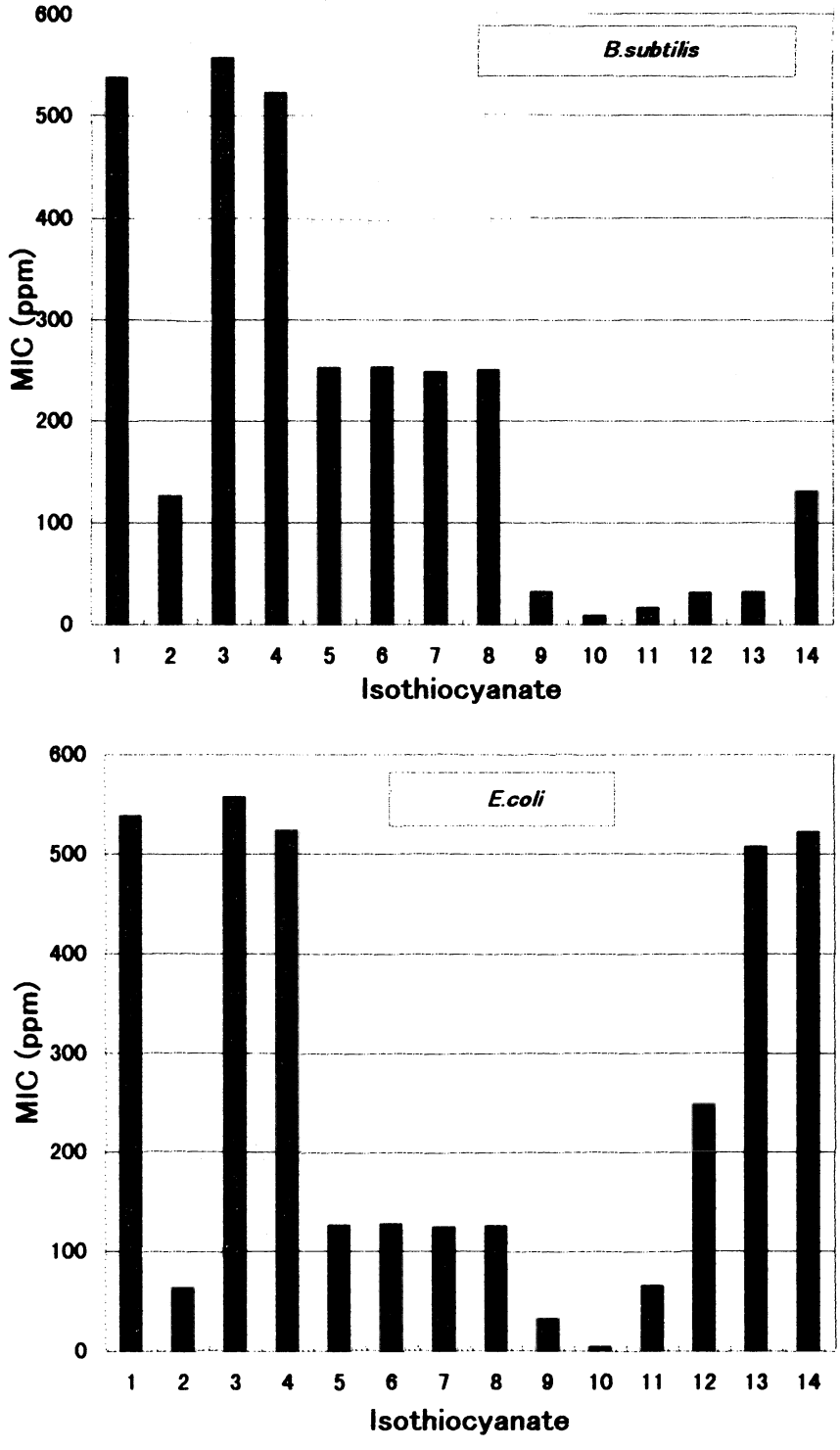

Figure 3 (Part 1). MIC values of the isothiocyanates against *B. subtilis* (top) and *E. coli* (bottom).

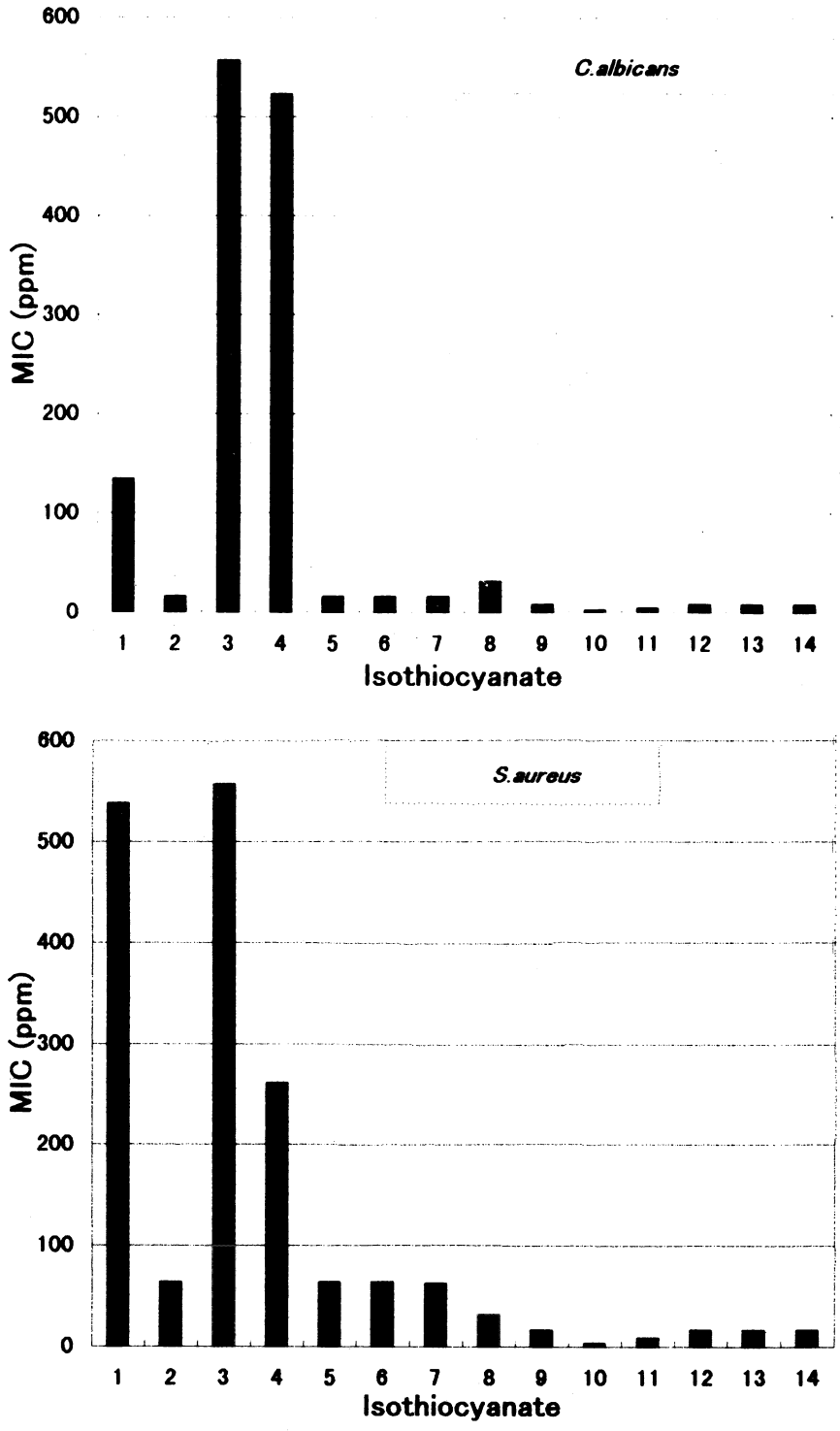

Figure 3 (Part 2). MIC values of the isothiocyanates against *C. albicans* (top) and *S. aureus* (bottom).

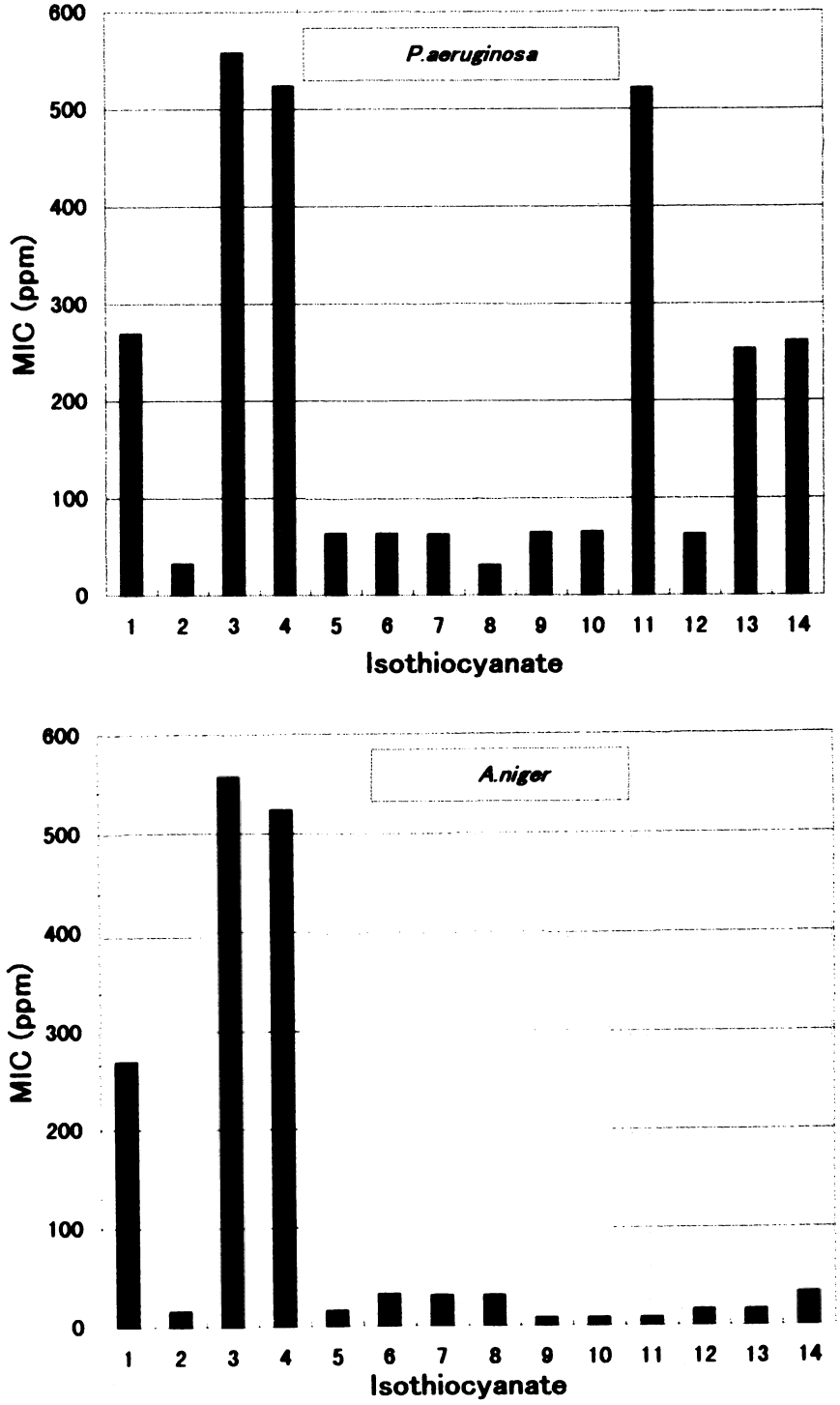

Figure 3 (Part 3). MIC values of the isothiocyanates against *P. aeruginosa* (top) and *A. niger* (bottom).

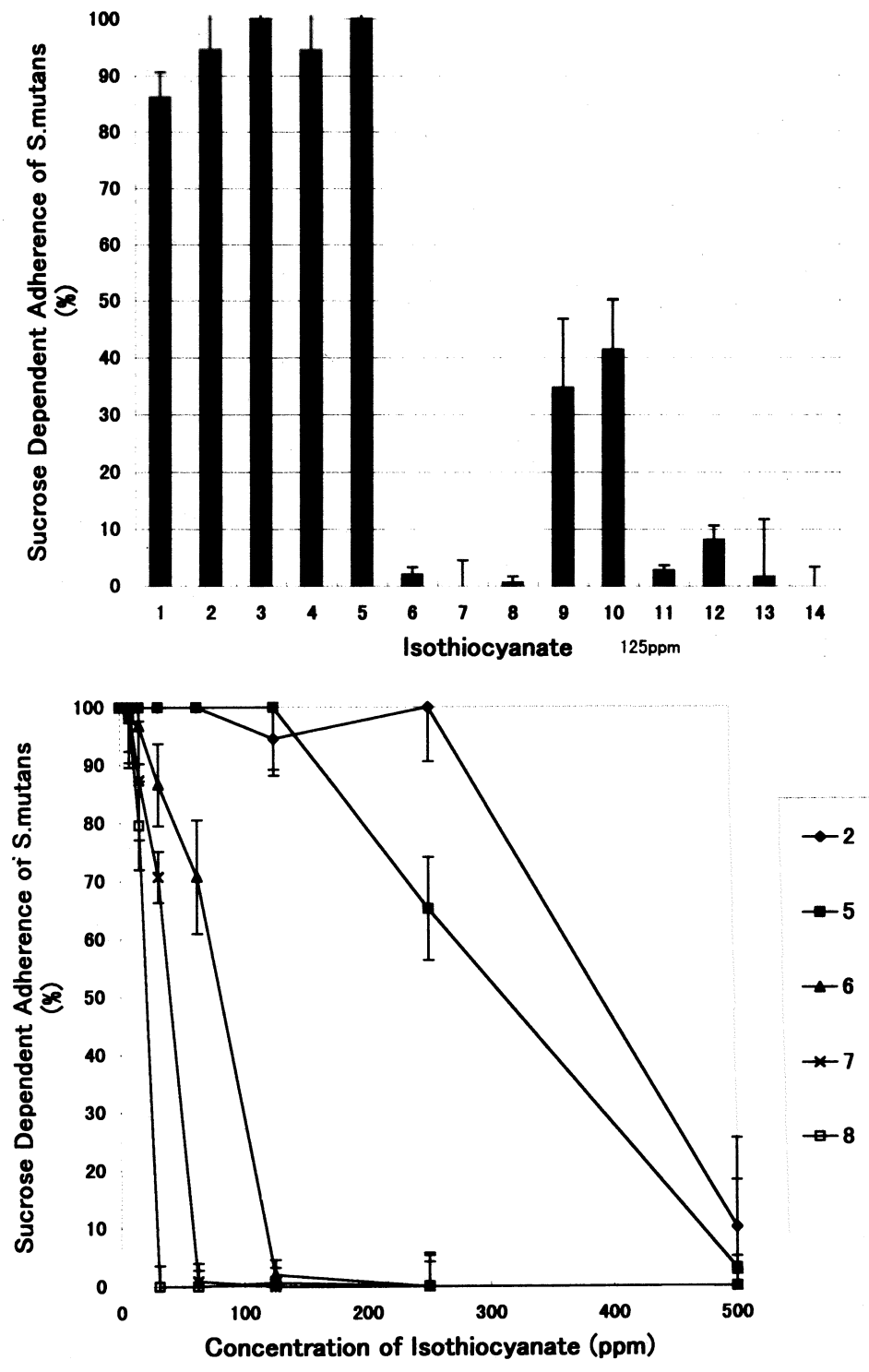

Figure 4. Sucrose dependent adherence of *S. mutans* vs. 125 ppm of each isothiocyanate (top). Sucrose dependent adherence of *S. mutans* vs. the concentrations of ω-alkenyl isothiocyanates (bottom).

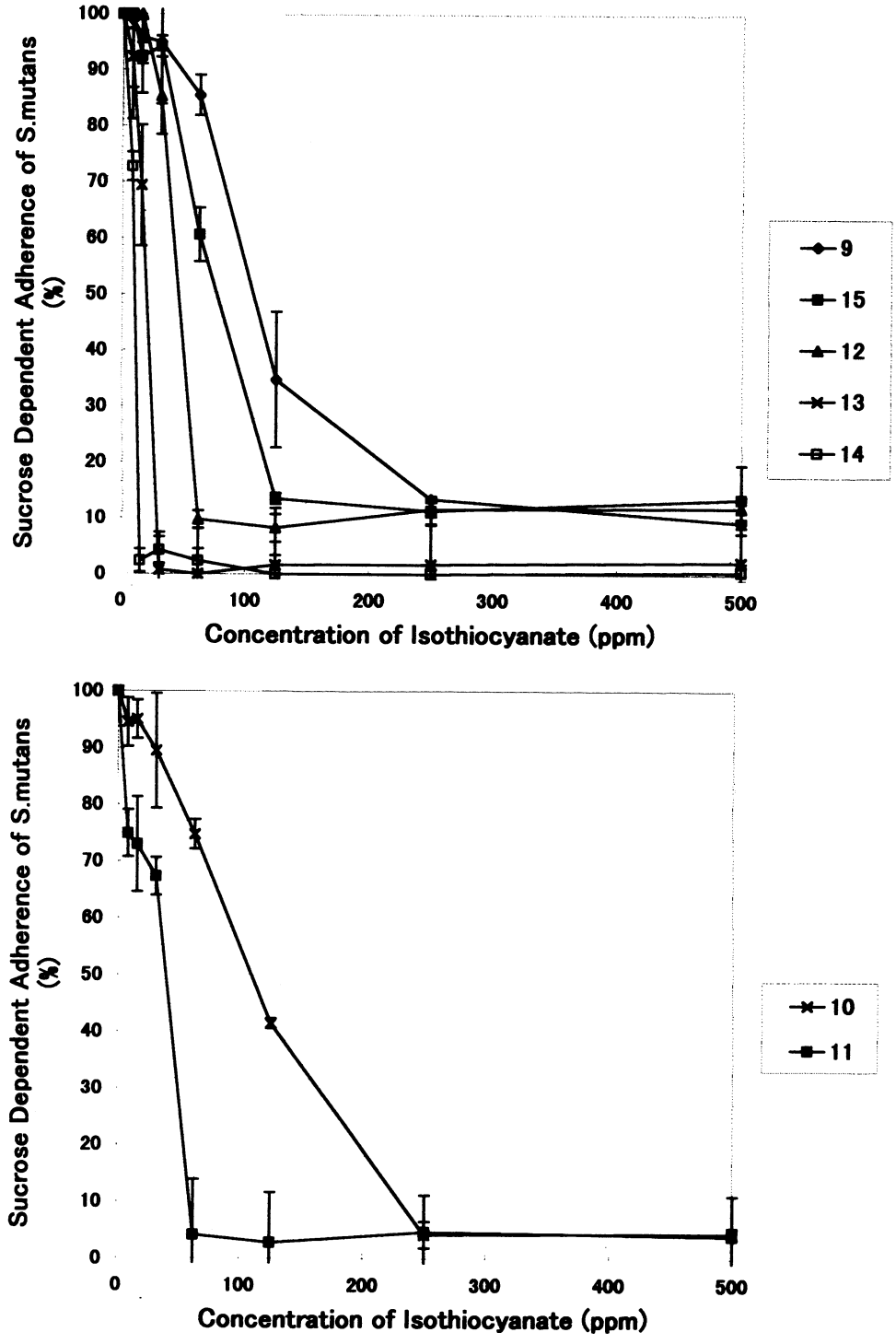

Figure 5. Sucrose dependent adherence of *S. mutans* vs. the concentrations of ω-methylthioalkyl isothiocyanates (top). Sucrose dependent adherence of *S. mutans* vs. the concentrations of aryl isothiocyanates (bottom).

the longer the length of each carbon chain, the lower the sucrose dependent adherence of *S. mutans*, that is, the higher the caries inhibitory effects. Consequently, it has been suggested that the active species of ω-alkenyl-, the ω-methylthioalkyl-, and aryl isothiocyanates in aqueous solution against *B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans, and A. niger* are different from those of *S. mutans*.

CONCLUSIONS

The stability of the isothiocyanates in aqueous methanol solution has been found to be more significantly affected by temperature than by pH. The stability of ω-alkenyl-, ω-methylthioalkyl-, and aryl isothiocyanates increased in proportion to the length of their carbon chain.

The antimicrobial properties of ω-alkenyl-, ω-methylthioalkyl-, and aryl isothiocyanates against *B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans, and A. niger*, in general, showed a reverse tendency in comparison with their stability in an aqueous methanol solution. Furthermore, the antimicrobial action of the above-mentioned isothiocyanates, in general, decreased in proportion to the length of the carbon chain.

The inhibitory effects of ω-alkenyl-, ω-methylthioalkyl-, and aryl isothiocyanates on sucrose dependent adherence by growing cells of *S. mutans*, that is, the caries inhibitory effects, increased in proportion to the length of the carbon chain.

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9

FLAVOR CHARACTERISTICS AND STEREOCHEMISTRY OF THE VOLATILE CONSTITUENTS OF GREATER GALANGAL (*ALPINIA GALANGA* WILLD.)

Kikue Kubota,[1] Yuki Someya,[1] Yoshiko Kurobayashi,[2] and Akio Kobayashi[1]

[1]Department of Nutrition and Food Science
Ochanomizu University, 2-1-1 Ohtsuka
Bunkyo-ku, Tokyo 112, Japan
[2]Technical Research Center
T. Hasegawa Co. Ltd., 335 Kariyado
Nakahara-ku, Kawasaki, Kanagawa 211, Japan

The rhizomes of greater galangal (*Alpinia galanga* Willd. L.) are widely used as a spice throughout south-east Asian countries. A flavor concentrate was prepared by steam distillation under reduced pressure, and the odor character and some potent odorants of the oxygenated compound fraction that presented the characteristic odor of galanga were investigated by GC-MS and aroma extract dilution analysis. Monoterpenoids of linalool and geranyl acetate, 1,8-cineole and acetoxy-1,8-cineoles, and some C_6-C_3 compounds of the derivatives of eugenol and chavicol were determined as important odor components. In addition, the optical purity and odor difference between the enantiomers were determined by GC and GC eluate sniffing, using a cyclodextrin column. 1′-Acetoxychavicol acetate was present in the optically active *S*-configuration in the essential oil of the rhizomes. Only the (*S*)-enantiomer possessed the characteristic cool, woody and ginger-like odor, in contrast of no odor of the (*R*)-enantiomer which played an important role in the odor of the fresh rhizomes, because this compound was degraded during cooking. Among the eight isomers of acetoxy-1,8-cineoles, (1*R*, 4*S*, 6*R*)-1,3,3-trimethyl-2-oxabicyclo[2,2,2]oct-6-yl acetate (*trans*-2-acetoxy-1,8-cineole) which presented a woody and galanga-like odor was the most important odor constituent in the rhizomes of greater galangal.

INTRODUCTION

Greater galangal (*Alpinia galanga* Willd.) belonging to the Zingiberaceae family is widely cultivated in India, China and south-east Asia. The rhizomes and ripening fruits

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have long been used in traditional medicine, and such biological activities as antiulcer (Mitsui *et al.*, 1976), antimicrobiological (Janssen and Scheffer, 1985), inhibition of xanthin oxidase (Noro *et al.*, 1988) and antitumorigenic (Itokawa *et al.*, 1987, Kondo *et al.*, 1993) are well known. In addition, the fresh rhizomes exhibit a refreshing, woody, minty, floral and aromatic odor with a hot taste, and both fresh and dried rhizomes are widely used as one of the essential spices for curry, soup and seafood dishes throughout countries of south-east Asia. These ethnic foods have recently become very popular in Japan, so Japanese people have many chances to taste this spice. Although the constituents of the essential oil have previously been studied (Charls *et al.*, 1992, Pooter *et al.*, 1985, Scheffer *et al.*, 1981), there has been little work done on the oxygenated compounds, except for 1,8-cineole. This is probably because the odor of greater galangal is mild in comparison to ginger, and the biological activity attracted people's attention first. We investigated the oxygenated fraction of the essential oil of fresh rhizomes in detail and found that 1′-acetoxychavicol acetate, which is known as the main biologically active substance in greater galangal, was also an important odor component of the fresh rhizomes, while some 1,8-cineole-related compounds also played an important role in its odor characteristics (Mori *et al.*, 1995). In this contribution we describe the composition of the potent oxygenated volatile compounds, a determination of the absolute configuration of the main components, and the flavor diversity of greater galangal as a spice.

MATERIALS

Fresh rhizomes of greater galangal were purchased from a local market; the supply was transported from Thailand once or twice a week. After washing and having the bad parts removed, samples were used in the experiments.

POTENT ODOR COMPOUNDS IN FRESH RHIZOMES

Preliminary experiments showed that the essential oil of greater galangal prepared by steam distillation exhibited a more floral and sweeter odor than that of fresh rhizomes. We prepared the volatile concentrate by steam distillation under reduced pressure (20 Torr at 40°C) to investigate the odor characteristics of fresh rhizomes (Mori *et al.*, 1995). The volatile concentrate, which had been extracted with diethyl ether, was obtained in a *ca.* 0.1% wet weight yield and submitted to silica gel column chromatography to separate the hydrocarbon constituents and oxygenated compounds by successively eluting with pentane and diethyl ether in the usual way. The hydrocarbon fraction was approximately 26% of the volatile concentrate, its odor was weak and it did not exhibit the characteristic aroma. On the other hand, the oxygenated compounds fraction (O-fr) constituted about 74%, and its odor was characteristic of fresh rhizomes. The constituents of O-fr were analyzed by mass spectrometry combined with capillary gas chromatography (GC-MS), and identified by comparing their Kovats GC retention indices and MS data with those of the authentic compounds. An aroma extract dilution analysis (AEDA) was applied according to Ullrich and Grosch (1987), and the potent odorants were also analyzed by GC eluate sniffing of O-fr after a stepwise dilution.

Gas chromatogram of O-fr is shown in Figure 1, and the potent odor compounds with the AEDA analysis are listed in Table 1. Compounds 1,8-cineole, *trans*-2- and 3-acetoxy-1,8-cineoles (1,3,3-trimethyl-2-oxabicyclo[2,2,2]oct-6-yl and 5-yl acetate) and 1′-

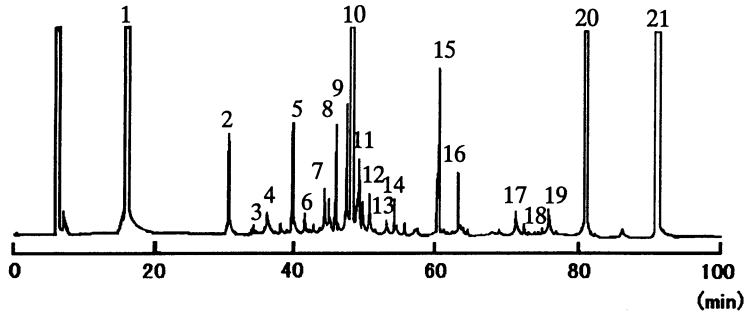


Figure 1. Gas chromatogram of the oxygenated-compound fraction of the volatile concentrate from the rhizomes of greater galangal.

acetoxychavicol acetate were quantitatively the main components and their total peak area by GC exceeded 80%. However, the spectrum of the potent odorants was a little different from the GC data. 1,8-Cineole, which has a cool and camphoraceous flavor, constituted the largest part, both quantitatively and qualitatively. Linalool, geranyl acetate, bornyl acetate and citronellyl acetate contributed greatly to the odor, although the concentration of each was low. Similarly, eugenol, methyl eugenol, eugenyl acetate, and chavicol acetate, as minor constituents, also played an important role in the flavor of the fresh rhizomes. In addition, the presence of 1,8-cineole-related compounds was noted because acetoxy-1,8-cineoles are not common volatiles in plants, although 2-hydroxy-1,8-cineoles have been found in grape (Bitteur *et al.*, 1990) and ginger lily flowers (Yamada and Ikeda, 1991). *Trans*-2-acetoxy-1,8-cineole has previously been tentatively identified in the essential oil of a weed, *Ageratum conyzoides* L. (Vera, 1993), whereas, four isomers of acetoxy-

Table 1. Potent odor compounds determined by AEDA in the oxygenated-compound fraction from rhizomes of greater galangal

Dilution factor	Peak no.	Compound	Peak area (%)	Odor impression
64	1	1,8-cineole	49.6	cool, camphoraceous
32	3	linalool	0.2	floral
	11	geranyl acetate	0.7	sourly floral
	15	chavicol acetate	1.5	cool, mint-like
	18	eugenol	0.2	clove-like
16	4	bornyl acetate	0.3	piny
	16	methyl eugenol	0.6	floral, fruity
8	2	acetic acid	1.8	sour
	6	citronellyl acetate	0.2	floral, rosy
	9	*t*-2-acetoxy-1,8-cineole	1.8	woody, musty
	10	*t*-3-acetoxy-1,8-cineole	17.3	woody, musty
	21	1′-acetoxychavicol acetate	14.0	cool, floral, ginger-like
	20	chavicol	2.7	camphoraceous
≤2	5	4-terpineol	1.2	earthy (weak)
	7	δ-terpineol	0.2	earthy (weak)
	8	α-terpineol	1.3	sweet, fruity
	13	*c*-2-acetoxy-1,8-cineole	0.3	earthy
	12	*c*-3-acetoxy-1,8-cineole	0.3	earthy
	14	thymol acetate	0.3	camphoraceous
	19	thymol	0.1	weakly spicy

1,8-cineoles, (*trans* and *cis*)-2- and 3-acetoxy-1,8-cineoles, were present in the rhizomes of galanga. Although their odor was not very strong, each showed some interesting features as described later. On the other hand, the AEDA analysis showed that the contribution of 1′-acetoxychavicol acetate to the odor of the rhizomes was not high in proportion to its concentration. Organoleptically, however its ginger-like, floral and woody odor quality seems to be indispensable for fresh rhizomes. These results indicate that the volatile constituents would be divided into two groups of monoterpenoids and C_6-C_3 compounds which seem to have originated from shikimic acid, and that 1′-acetoxychavicol acetate, 1,8-cineole and acetoxy-1,8-cineoles characterized the odor of the fresh rhizomes of greater galangal.

DETERMINATION OF THE ENANTIOMERIC PURITY AND ODOR CHARACTERISTICS OF THE MAIN COMPONENTS

It is important to investigate the enantiomeric purity of optical isomers because the flavor character can often be different between enantiomers. As just mentioned, 1′-acetoxychavicol acetate (ACA) and acetoxy-1,8-cineoles were the characteristic odor constituents of the rhizomes, each having one or three asymmetric carbons in the molecule. Although the absolute configuration of ACA in greater galangal has been determined as *S* type (Mitsui *et al.*, 1976), the enantiomeric purity in the essential oil has not been reported previously. In this contribution, we resolved the optical isomers by chiral GC or chiral HPLC, and compared the odor characteristics of the enantiomers.

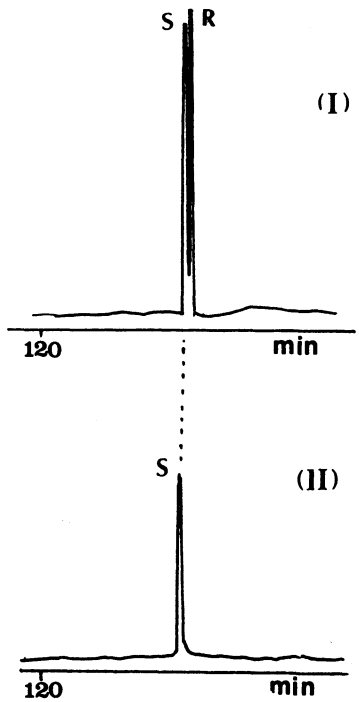


Figure 2. Gas chromatograms of synthetic (I) and natural (II) 1′-acetoxychavicol acetate with a cyclodextrin column. Kovats index: (I) *S*, 1964; *R*, 1967 (II) *S*, 1964; CP-cyclodextrin-B-236-M-19 column (50 m x 0.25 mm i.d.), 60°C→200°C (1°C/min).

1′-Acetoxychavicol Acetate

The racemate of 1′-acetoxychavicol acetate (ACA) was synthesized from 4-hydroxybenzaldehyde and vinyl magnesium bromide (Mitsui *et al.*, 1976) to compare the odor characters of (*R*)- and (*S*)-ACA. The racemate was separated by chiral column GC (CP-cyclodextrin-B-236-M-19, 50 m x 0.25 mm i.d.) into two peaks (KI 1964 and 1967) as shown in Figure 2, and co-chromatography indicated natural ACA to be coincident with the former peak. The (*R*)- and (*S*)-ACA were respectively trapped by chiral column HPLC (CHIRALCEL OB 4.6 mm ø x 250 mm, Daicel Chemical Ind. Ltd., hexane/2-propanol:80/20). (*S*)-ACA exhibited a ginger-like and floral odor, but (*R*)-ACA had almost no odor.

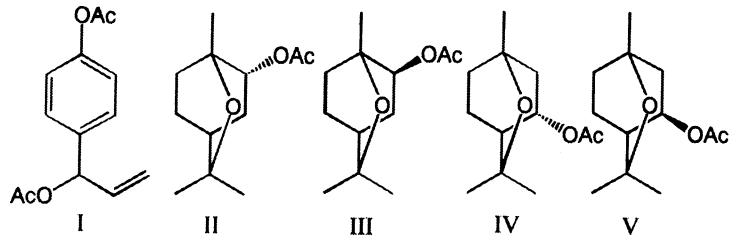
Acetoxy-1,8-Cineoles

Four isomers with the structures shown in Table 2 were identified in the rhizomes (Kubota *et al.*, 1998), the concentrations of the *trans* isomers in 2- and 3-acetoxy-1,8-cineoles being higher than those of the *cis* isomers (Table 1). Chiral GC analysis also showed that each acetoxy-1,8-cineole in the rhizomes contained optical isomers in a respective ratio, although the absolute configuration of each peak was not identified. To determine the enantiomeric purity and the absolute configuration of each peak separated by GC, each corresponding hydroxy-1,8-cineole was synthesized and tested according to the new theory of Mosher for a secondary alcohol. *Trans*-2-hydroxy-1,8-cineole ((1*SR*,4*RS*,6*SR*)-1,3,3-trimethyl-2-oxabicyclo [2,2,2]octan-6-ol) was prepared from (*R,S*)-α-terpineol by epoxidation and cyclization with *m*-chloroperbenzoic acid (mCPBA) and *p*-toluene sulfonic acid (Bitteur *et al.*, 1990). *Cis*-2-hydroxy-1,8-cineole ((1*SR*,4*RS*,6*RS*)-1,3,3-trimethyl-2-oxabicyclo [2,2,2]octan-6-ol) and *trans*-3-hydroxy-1,8-cineole ((1*SR*,4*RS*,5*RS*)-1,3,3-trimethyl-2-

Table 2. Concentration of enantiomers and their odor characteristics

Compound	Configuration	Peak area (%)	*e.e.* (%)	Odor impression
1′-acetoxychavicol acetate [I]	1′-*R*	0	100	no aroma
	1′-*S*	100		cool, floral, ginger-like
2-acetoxy-1,8-cineole (*trans*) [II]	1*R*,4*S*,6*R*	82	63.5	woody, galanga-like
	1*S*,4*R*,6*S*	18		woody (weak)
2-acetoxy-1,8-cineole (*cis*) [III]	1*S*,4*R*,6*R*	100	100	weak
	1*R*,4*S*,6*S*	0		fruity, sweet
3-acetoxy-1,8-cineole (*trans*) [IV]	1*S*,4*R*,5*R*	3	93.9	sweet floral (weak)
	1*R*,4*S*,5*S*	97		sweet floral (weak)
3-acetoxy-1,8-cineole (*cis*) [V]	1*R*,4*S*,5*R*	60	19.4	mild woody
	1*S*,4*R*,5*S*	40		camphoraceous

II and III: 1,3,3-trimethyl-2-oxabicyclo[2,2,2]oct-6-yl acetate
IV and V: 1,3,3-trimethyl-2-oxabicyclo[2,2,2]oct-5-yl acetate



oxabicyclo[2,2,2]octan-5-ol) were prepared from 1,8-cineole by oxidation with mCPBA (Asakawa *et al.*, 1988). The compound *cis*-3-hydroxy-1,8-cineole ((1*SR*,4*RS*,5SR)-1,3,3-trimethyl-2-oxabicyclo[2,2,2]ocan-5-ol) was synthesized from piperitenone by epoxidation in 35% H_2SO_4, before being reduced with $LiAlH_4$ (Kobayashi *et al.*, 1995). A diastereomeric pair of (*S*)-*O*-methylmandelate esters of each alcohol (racemate) was prepared from (S)-(+)-*O*-methylmandelate acid, oxalyl chloride, DMF and DMAP (Trost *et al.*, 1986), and the reaction mixture (including the diastereomeric pair) was submitted to HPLC to separate each diastereomer. Four pairs of *O*-methylmandelates of optically pure alcohol were resolved and submitted to ^{1}H-NMR spectrometric analysis. When the ester in each Mosher model is viewed via an extended Newman projection, the proton which eclipses the phenyl ring is always upfield because of shielding by the phenyl ring. The absolute configuration of the asymmetric carbons of the alcohol moiety in each *O*-methylmandelate ester was identified by comparing the ^{1}H-NMR chemical shift between each pair of diastereomer. Cleavage of *O*-methylmandelate with lithium aluminum hydride gave the corresponding optically pure alcohol, and the corresponding acetate was successively obtained by acetylation with acetic anhydride in pyridine. The ratio of the enantiomers in the rhizomes was determined by comparing the retention time from chiral column GC, and the odor characteristics were examined by sniffing the GC eluate (Table 2) (Kubota *et al.*, 1999).

Contribution of Acetoxy-1,8-Cineoles to the Odor of Greater Galanga

Mariani *et al.* (1995) have synthesized a series of alkyl esters of 2-hydroxy-1,8-cineoles and described the odorous note of *trans*-2-acetoxy-1,8-cineole to be woody, pine oil-like and violet-like. However, they did not mention anything about the optical isomers. In the case of *trans*-2-acetoxy-1,8-cineole, the (1*S*,4*R*,6*S*) isomer presented a woody and galanga-like odor, the odor being stronger than that of the enantiomer. In addition, the content of the stronger enantiomer was about four times higher than the other one. The compound *cis*-2-acetoxy-1,8-cineole was optically active in galanga, although its odor was weak and its content was much lower than that of the *trans* isomer. *Trans*-3-acetoxy-1,8-cineole was almost optically active in the rhizomes, but its odor was also weak. Although it was selected in the AEDA analysis as one of the potent odorants because its concentration in the essential oil was highest among the isomers, this compound seems to have had little qualitative effect on the characteristic flavor. It was noted that *cis*-3-acetoxy-1,8-cineole had a different odor than its enantiomer, although it did not affect the odor of greater galangal because of its low concentration. Thus, it was concluded that, among the acetoxy-1,8-cineoles, *trans*-2-acetoxy-1,8-cineole was the most potent odorant in the rhizomes both quantitatively and qualitatively.

THERMAL STABILITY OF THE MAIN ODOR CONSTITUENTS OF GALANGA

In a preliminary experiment it was noted that the odor of the essential oil prepared by steam distillation at atmospheric pressure was quite different from that obtained by steam distillation under reduced pressure. The former exhibited a sweeter, more floral and spicy odor than the latter which presented a refreshing, woody and floral odor as mentioned already. It has been described that 1′-acetoxychavicol acetate disappeared from both rhizomes (Janssen and Scheffer, 1988) and fruits (Dunyuan *et al.*, 1987) after boiling for a few hours. In our study, the gas chromatogram of the essential oil prepared by SDE

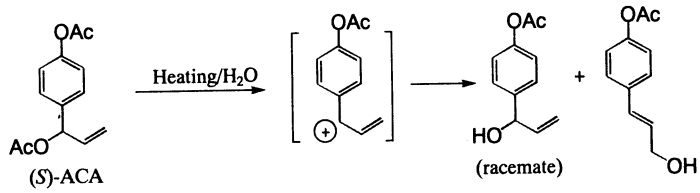


Figure 3. Degradation scheme for (*S*)-1′-acetoxychavicol acetate during cooking.

showed that the composition of the odorants were not changed to any great extent during cooking, except for the disappearance of the peak for ACA as has been shown for the fruits of greater galangal (Dunyuan *et al.*, 1987). In this study, the process for the degradation of ACA was investigated in detail by boiling synthesized ACA in water. The degradation of ACA began just after boiling and in 2 min of boiling, its concentration was reduced to only a few % of the original ACA; compounds 1′-hydroxychavicol acetate and 4-acetoxycinnamyl alcohol, at almost same portions, were found instead of ACA. Since 1′-hydroxychavicol acetate formed was racemic, the reaction mechanism shown in Figure 3 was suggested. Both degraded products presented almost no aroma. It is suggested that the disappearance of ACA brings about an increase in the role of monoterpenoids, 1,8-cineole and the acetoxy derivatives in the essential oil, and this results in enhancement of the sweet and spicy flavor of cooked greater galangal.

ACKNOWLEDGMENT

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10

VOLATILE COMPOSITION OF PANDAN LEAVES (*PANDANUS AMARYLLIFOLIUS*)

J. Jiang

Singapore Productivity and Standards Board
Food Biotechnology Centre
1 Science Park Drive, Singapore 118221

Volatile compounds were isolated from pandan leaves by liquid-liquid extraction using dichloromethane as solvent and was analysed using GC-MS. A total of 22 compounds were identified, including 9 alcohols, 4 carboxylic acids, 3 ketones, 2 esters, 3 hydrocarbons and 1 furanone. 3-Methyl-2-(5H)-furanone was the predominant component, accounting for over 70% of the total volatiles. Other major components included 3-hexanol, 4-methylpentanol, 3-hexanone and 2-hexanone.

INTRODUCTION

The leaves of Pandan (*Pandanus amaryllifolius*) have a strong aroma and are widely used in South East Asia as flavourings for various food products, such as bakery, sweets and even home cooking. In spite of its popularity as a flavouring material, pandan has not been thoroughly studied for its chemical composition, especially its volatile composition.

2-Acetyl-1-pyrroline was identified in pandan leaves by Buttery *et al.* (1982) and Lakasanalamai and Ilangantileke (1993). This heterocyclic compound was believed to be the important compound responsible for the characteristic aroma of several Asian aromatic rice varieties. Using NMR techniques, Nonato *et al.* (1993) identified three piperidine alkaloids in pandan leaves: pandamarilactone-1, pandamarilactone-32, pandamarilactone-31. Their biogenetic origin and taxonomic implication were discussed.

This chapter reports some new findings in our recent studies on volatile composition of pandan leaves.

MATERIALS AND METHODS

Fresh pandan leaves were purchased in a local market in Singapore. The washed leaves (200 g) were blended with deionised water (300 ml) and followed by centrifugation

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at 8000 rpm. The supernatant (300 ml) was extracted using 3 x 100 ml HPLC grade dichloromethane (DCM). The DCM extract was concentrated under a flow of pure N_2 to 0.2 ml for GC-MS analysis.

The concentrated extract was analysed using a Unicam Automass 150 GC-MS system (Unicam Automass, Argenteuil, France). Separation of the extracted components was achieved using a 20 m x 0.25 mm x 0.3 μm DB-Wax column (J & W Scientific, Folsom, CA, USA). Column temperature was increased from 60 to 250°C at a rate of 5°C/min. Injector temperature was set at 250°C. MS parameters were as follows: EI mode with an ionization voltage 70 *eV*, ion source temperature 130°C and scan range from 20 to 350 amu.

Identification of unknown components was mainly based on library search (NIST), but the identities of 3-methyl-2-(5H)-furanone and other major components were confirmed by both mass spectral and retention data of authentic chemicals.

RESULTS AND DISCUSSION

Figure 1 shows the gas chromatographic separation of pandan volatiles extracted using DCM.

A total of 22 components were identified, including 9 alcohols, 4 carboxylic acids, 3 ketones, 2 esters, 3 hydrocarbons, and 1 furanone (Table 1). 3-Methyl-2(5H)-furanone was the predominant component, accounting for 73% of the total volatiles, based on peak area data. Other major components included 3-hexanol, 4-methyl-2-pentanol, 3-hexanone and 2-hexanone, ranging from 2.65–7.09% of the total volatiles. In addition to a strong pandan-like aroma, the pandan extract also had fresh and green notes. The C6 compounds identified in the DCM extract would certainly make important contributions to the fresh/green attributes in pandan leaves.

3-Methyl-2(5H)-furanone was previously identified only in several processed food products, such as fermented soy hydrolysate (Liardon and Phillipossian, 1978), Finnish birch syrup (Kallio, 1989), cheese (Barbieri *et al.*, 1994) and dried bonito (Imai *et al.*, 1982). It has never been reported in any fresh food material. In our study, 3-methyl-2-(5H)-furanone was identified for the first time as a predominant volatile component in pandan leaves. Pure standard of 3-methyl-2(5H)-furanone gives an aroma characterized by caramel, sweet, honey and a bit medicinal notes. Although this compound does not resemble typical pandan-like smell, it could be an important contributor to the overall aroma of pandan leaves, especially the undertone of pandan aroma.

It is interesting to note that the tree pandamarilactones identified previously (Nonato *et al.*, 1993) all have the same moiety of 3-methyl-2(5H)-furanone. This indicates a possible biogenetical relationship between 3-methyl-2(5H)-furanone and these pandamarilactones. It is unclear at present whether 3-methyl-2(5H)-furanone is a precursor in the formation of these pandamarilactones or their breakdown product. Further studies are required to clarify this interesting question.

α-Methyl-γ-butyrolactone, i.e. 3-methyl-2(5H)-furanone is a common moiety found in a number of biologically active compounds (mostly lignans) (Chen *et al.*, 1989; Iwakami *et al.*, 1990). It is also an intermediate from synthesis of some agrochemicals, such as strigol. Therefore, there is an increasing interest in finding synthetic routes for producing this compound (Mangnus and Zwaneburg, 1992; Mangnus *et al.*, 1992). Pandan leaves may serve as a natural source for this compound.

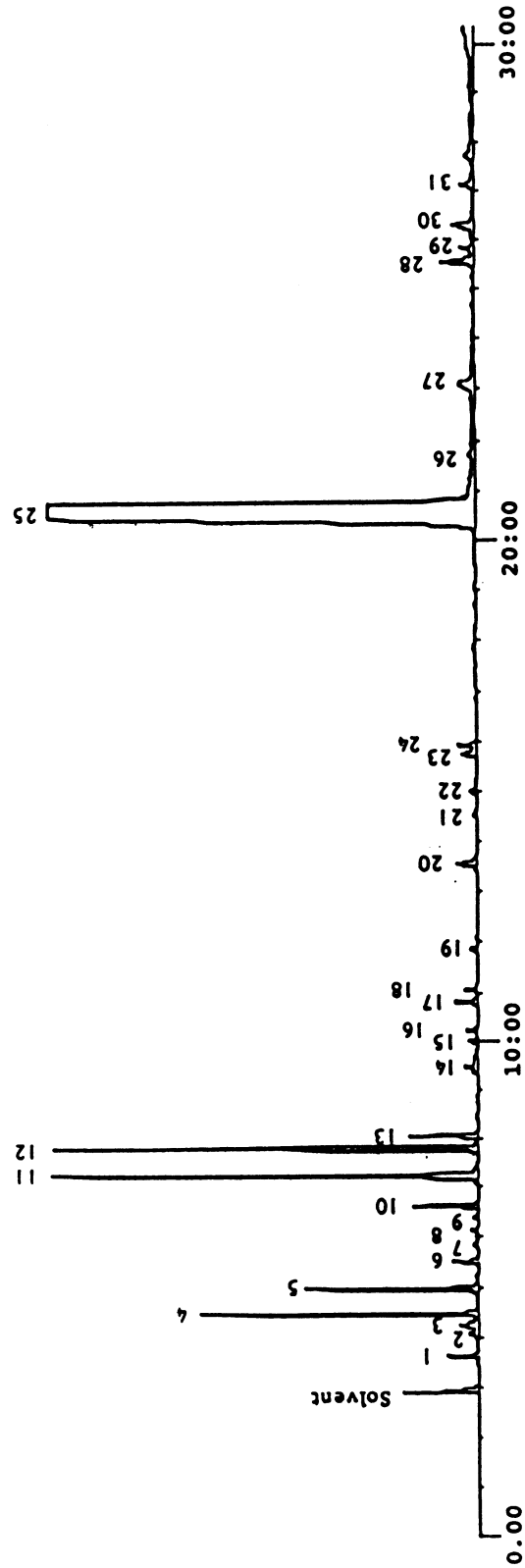

Figure 1. Gas chromatographic separation of pandan volatile compounds.

Table 1. Compounds identified in pandan leaves (*Pandanus amaryllifolius*)

No.*	Compound	CAS #	Peak area	% of total
1	decane (IS)	124-18-5	—	—
2	2-methyl-3-buten-2-one	115-18-4	5.18	0.44
3	toluene	108-88-3	1.9	0.16
4	3-hexanone	569-38-8	35.36	2.97
5	2-hexanone	591-78-6	31.55	2.65
6	3-methyl-3-pentanol	77-74-7	4.87	0.41
7	ethylbenzene	100-41-4	1.34	0.11
8	1,2-dimethylbenzene	95-47-6	1.56	0.13
9	unknown	—	0.93	0.08
10	3-penten-2-ol	1569-50-2	11.2	0.94
11	3-hexanol	623-37-0	84.39	7.09
12	4-methyl-2-pentanol	108-11-2	72.93	6.13
13	1-methylcyclopentanol	1462-03-9	11.93	1.00
14	unknown	—	3.35	0.28
15	3-methyl-2-pentanol	565-60-6	1.77	0.15
16	(E)-2-penten-1-ol	1576-96-1	2.5	0.21
17	unknown	—	4.53	0.38
18	hexyl formate	629-33-4	2.55	0.21
19	(Z)-4-hexen-1-ol	928-91-6	1.54	0.13
20	acetic acid	64-19-7	5.28	0.44
21	unknown	—	0.49	0.04
22	2,5-hexanedione	110-13-4	1.64	0.14
23	unknown	—	3.36	0.28
24	unknown	—	3.56	0.30
25	3-methyl-2(5H)-furanone	22122-36-7	869.51	73.07
26	methyl 2-hydroxybenzoate	119-36-8	2.11	0.18
27	hexanoic acid	142-62-1	8.89	0.75
28	(E)-3-hexenoic acid	1577-18-0	10.14	0.85
29	3-hexenoic acid	4219-24-3	2.22	0.19
30	eicosane (IS)	112-95-8	—	—
31	unknown	—	3.36	0.28

*Corresponding to the peak number in Figure 1.

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11

EMISSION OF BLANCHED BROCCOLI VOLATILES IN HEADSPACE DURING COOKING

Heikki Kallio, Päivi Raimoaho, and Tuomas Virtalaine

Department of Biochemistry and Food Chemistry
University of Turku, FIN-20014 Turku, Finland

Broccoli florets (*Brassica oleracea* L.) were cut, blanched at 85°C for two minutes and frozen for the experiments. The emission of 14 volatile compounds was followed by headspace gas chromatography - mass spectrometry while cooking the material in water for 13 min at 95°C. After cooking in closed or open vessels the volatiles were swept with 120 mL He into a trapping column. Five traps were tested and a liquid phase with 100 % recovery was found (OV-1/OV-25, 1/1). The percent coefficient of variance CV % of the major compounds typically varied between 3 and 30 %, including an internal variation of different broccoli samples from the same lot. The major volatiles in the headspace, measured with a flame ionization detector, were n-pentanal, 3-methyl-2-pentanone, n-hexanal, n-heptanal, cyclopentanecarboxaldehyde, ethyl acetate, 3-methylbutanal, 3-butenenitrile, 2-methylbutanal, dimethyl trisulfide and dimethyl disulfide indicating an effective inactivation of enzymes, including myrosinase. The method has been applied to optimizing the processing of broccoli.

INTRODUCTION

Glucosinolates and myrosinase are characteristic components of all wild and cultivated *Brassica* species (Mithen *et al.*, 1987). A major part of the broccoli crop (*Brassica oleracea* L. var. Italica) is stored fresh for a short time only, though industrial production of frozen florets has increased rapidly. Optimized blanching is always necessary to keep a high quality frozen product. Special use of NH_4HCO_3 in steam-blanching gave frozen broccoli better color and acceptability than that blanched in steam or water only (Odland and Eheart, 1975).

Immersion of broccoli florets in hot water (50 °C for 2 min) reduced yellowing and decay without increasing off-odor formation, even when stored at room temperature (Forney, 1995). With modified atmosphere the fresh storage time may be increased by several weeks (Bastrash *et al.*, 1993). Storage conditions are of special importance to the sensory properties of cooked broccoli (Hansen *et al.*, 1993).

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An enhanced CO_2-level is advantageous for the color retention (Lipton and Harris, 1974; Wang, 1979; Makhlouf *et al.*, 1989) but reduced aerobic conditions also enhance the formation of off-odors (Kasmire *et al.*, 1974; Makhlouf *et al.*, 1989; Izumi *et al.*, 1996), such as methanethiol which is produced by both broccoli tissues and associated microorganisms (Forney *et al.*, 1991, 1993; Obeland *et al.*, 1994). Ethanol in the storage atmosphere retains chlorophyll and retards mold growth although undesirable off-odors may occur during storage (Corcuff *et al.*, 1996).

Slight heat treatment followed by storage under anaerobic conditions has been shown to enhance the production of methanethiol, hydrogen sulfide and dimethyl disulfide in broccoli (Obeland *et al.*, 1995). The content of Dimethyl trisulfide also increases under anaerobic conditions (Hansen *et al.*, 1992) and has long been known to be one of the character impact volatiles of cooked broccoli (Maruyama, 1970).

The aim of the study was to optimize a headspace-GC-MS method for the analysis of blanched, cooked broccoli florets. The procedure was to be applied to industrial process control to support sensory evaluations.

MATERIALS AND METHODS

Samples

Broccoli (*Brassica oleracea* L. var. Italica, cultivar Arcadia) was obtained from the Agricultural Research Centre of Finland. The cut broccoli florets were blanched at 85 °C for 2 min to inactivate enzymes, packed in polyethene bags and frozen at -18 °C. Ten 200 g bags of broccoli were cut frozen with an onion cutter. The pieces (0.1–0.5 g) were blended, repacked and stored frozen at -18 °C. Two grams of broccoli were weighed into a headspace vial along with 1.85 mL of distilled water.

Headspace Gas Chromatography

The headspace analyses of broccoli volatiles were carried out with a Tekmar 3000 D/T Purge & Trap headspace concentrator (Cincinnati, OH) and a Varian 3300 gas chromatograph (Walnut Creek, CA) controlled by Teklink (Tekmar, Cincinnati, OH) and Turbochrom programmes (Perkin-Elmer, San Jose, CA). The sample vial was a 150 mm x 20 mm glass tube. Two different sampling methods were applied. 1) To investigate the formation of volatiles during cooking, the sample was heated in the vial for 7, 10, 13 or 16 min at 95 °C. Heating took place in an air atmosphere in closed vials and the emitted volatiles were swept at the water surface with He into the trapping column. 2) When volatiles of cooked broccoli were investigated, the sample was heated in an open vial not connected to the headspace concentrator. After cooking for 13 min the vial was connected to the headspace concentrator and allowed to stabilize for five minutes before collection of the sample.

During the constant 3 min sampling time, a volume of 120 mL of He was purged into the trapping column and the volatiles concentrated in the liquid phase.

Five trapping columns were tested: silica coated steel columns (length 0.3 m, i.d. 3.1 mm) packed with 3 % OV-1 (Chrom W-HP 100/120 mesh, Chromatography Research Supplies Inc., Addison, IL), 3 % OV-25 (Chrom W-HP 80/100 mesh), Vocarb 3000 (carbon absorbent, Supelco, Bellefonte, PA) and a self-packed two-phase column upper part OV-1/ lower part OV-25 (Chromatography Research Supplies, Addison, IL), as well as an

uncoated steel column with support coated OV-1 filling (Chromatography Research Supplies). The total amount of adsorbent in each column was c.a 0.2 g.

After rapid heating of the trap to 170 °C, the volatiles were swept with He for 3 min at a flow rate of 40 ml/min to an Rtx-1 fused silica capillary column (1.0 m, i.d. 0.32 mm, d_f 3.0 um, Restek Corp, Bellefonte, PA), chilled with liquid nitrogen at -60 °C. The trap was connected with a glass liner to the analytical GC-column. Injection took place by rapidly heating the cryo-trap to 170 °C.

Two analytical capillary columns were used: DB-1701 (30 m, i.d. 0.25 mm, d_f 0.25 um, J&W Scientific, Folsom, CA) and DB-1 (30 m, i.d. 0.25 mm, d_f 0.25 um, J&W Scientific). The average linear flow rate of the He carrier gas was 30 cm/s. Programme: from 30 °C (15 min) to 120 °C (5 °C/min), to 280 °C (10 °C/min). The temperature of the flame ionization detector was 280 °C.

The break through of the trapping column (OV-1 / OV-25) was tested during collection of the headspace sample. The purge gas line after the trap was connected directly to a DB-1 precolumn (-60 °C) preceeding the analytical DB-1 column.

Mass Spectrometry

The volatile compounds were identified with mass spectrometry using DB-1 and DB-1701 columns. Purge time of the sample was 10 min in order to increse the sensitivity of the analysis. VG Analytical 7070 E spectrometer and Opus V3.2X data system (VG Analytical, Wythenshawe, Manchester, UK) and Shimadzu GCMS-QP5000 (Shimadzu, Kyoto, Japan) were applied using the same programmes as in the GC-analysis.

RESULTS AND DISCUSSION

Broccoli pieces were soaked in a minimum amount of water (1.85 mL) which gave a higher total abundance of peaks in the chromatogram than with larger volumes of water. A cooking time of 13 min was selected for proper taste, aroma, and color of broccoli.

The most efficient trapping column was shown to be that with a mixed OV-1 and OV-25 filling (1:1). With this combination the total abundance of the chromatogram peaks was maximal and breakthrough was eliminated during sample collection. The trap also gave the lowest CV %-values of concentrations of the volatile compounds, being on the average 12.7 %. The metal surface OV-1 and the glass coated Vocarb 3000 gave systematically chromatograms with c.a. 20 % lower total abundances than the other traps. Both irreversible trapping and catalytic reactions of the compounds may be responsible for this phenomenon.

The column efficiency [separation numbers (SN, Tz) and number of effective plates (N_{eff})] was higher with the DB-1701 than with the DB-1 column with typical broccoli volatiles.

When the system was tested with methanol-, ethanol- and propanol-water solutions (1:200, v/v), the coefficients of variation of the peak areas were shown to be 4.4, 8.8 and 2.2%, respectively. By parallel broccoli samples the CV-values of single compounds varied between 2.3 and 65%, typical values being around 20%. Chromatograms of two broccoli samples of the same lot are shown in Figure 1. The profiles are similar but quantitative differences typical for headspace analysis of natural plant material are clear.

The results in Table 1 display the typical problems in headspace analysis of *Brassica* vegetables and at the same time the difficulties in raw material handling and stand-

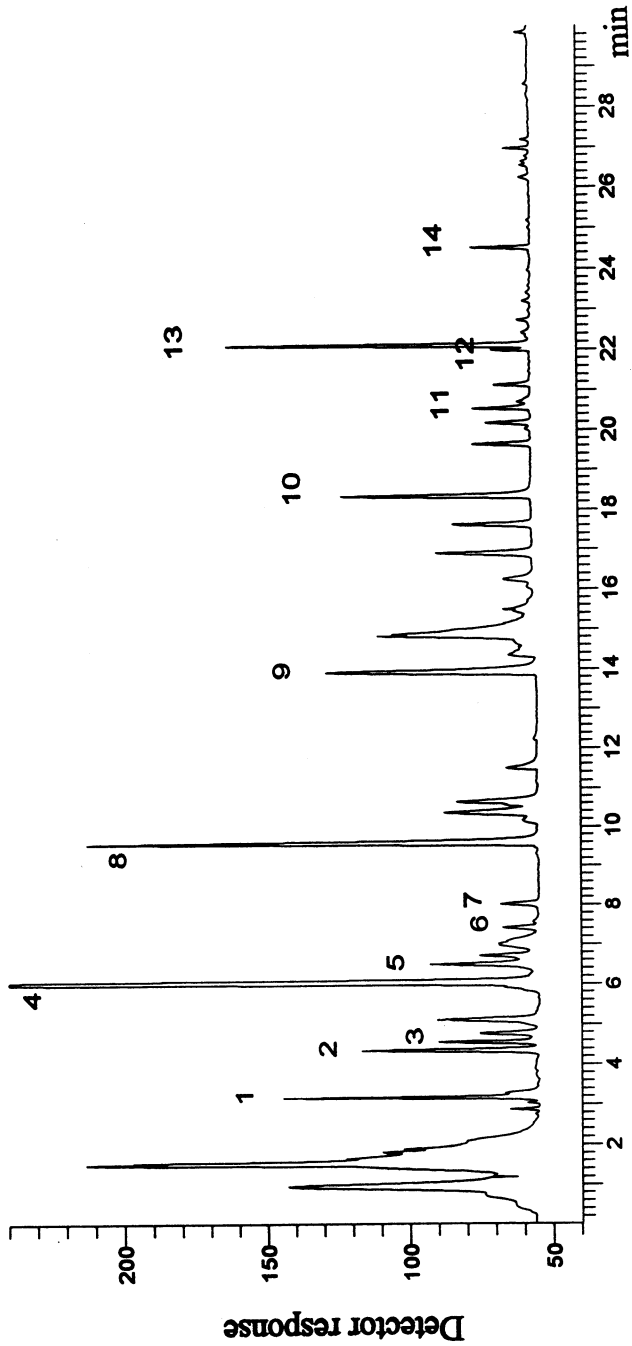

Figure 1 (Part I). Parallel headspace-GC analyses of broccoli headspace analyzed by FID. Trapping in OV-1 + OV-25, transferring into and cryofocusing at Rtx-1, analysis by DB-1701. Numbering of the peaks as in Table I.

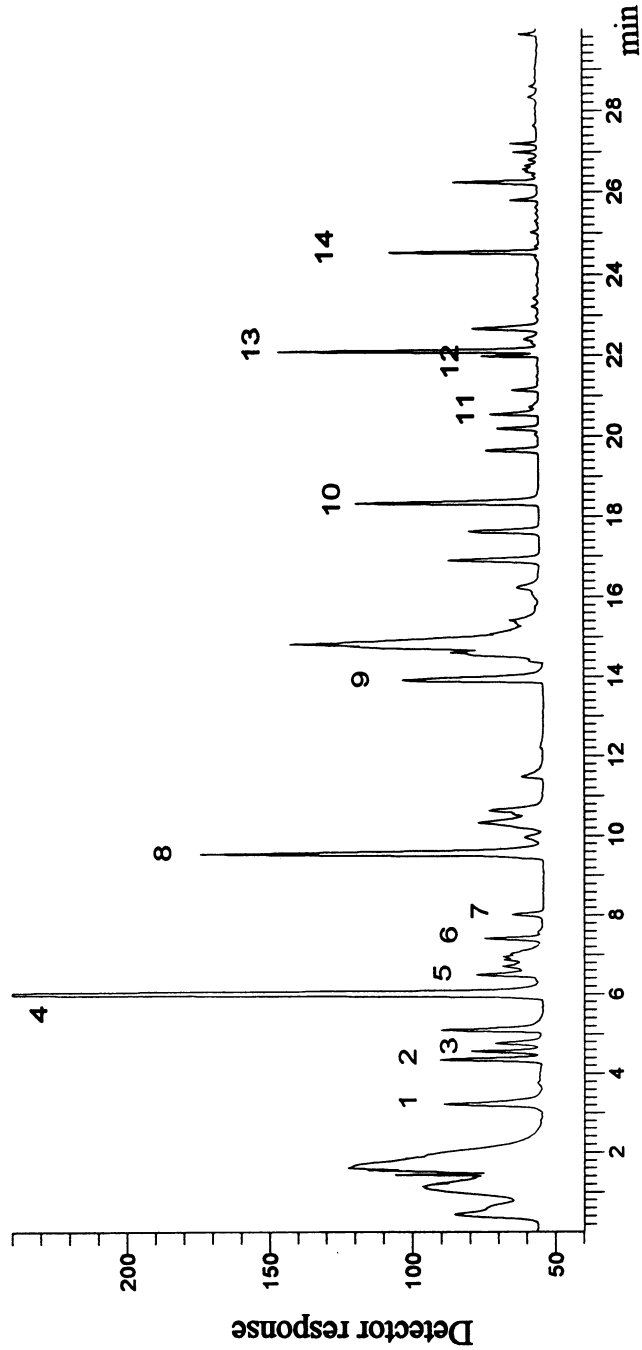

Figure 1 (Part II). Proportions of most abundant compounds, based on flame ionization detection, are shown in Table 1.

Table 1. Major volatiles in the headspace of cooked broccoli analyzed by DB-1701 column

Compound	Abundance	+/-CV%	Rel. ab.	+/-CV%
1. Ethyl acetate	283	2.3	5.2	3.6
2. 3-methylbutanal	258	27.1	4.8	25.5
3. 2-methylbutanal	170	30.8	3.1	30.4
4. pentanal	2160	5.6	39.8	4.7
5. 3-butenenitrile	207	29.6	3.8	27.7
6. dimethyl disulfide	108	64.5	2.0	65.0
7. Toluene	60	13.1	1.1	10.0
8. 3-methyl-2-pentanone	786	7.8	14.5	7.1
9. hexanal	416	24.1	7.6	21.1
10. lopentanecarboxaldehyde	322	3.9	6.0	6.1
11. 4-methylpentanenitrile	76	10.7	1.4	10.0
12. 2-heptanone	57	21.4	1.1	24.5
13. n-heptanal	375	9.4	6.9	5.2
14. Dimethyl trisulfide	146	44.8	2.7	47.1

ardization. Each unit operation, collection, transport, storage, cleaning, cutting and blanching of the product in the factory, requires special attention. The headspace method was developed for use as a practical tool to support the sensory analysis in order to optimize the industrial processing (blanching) of frozen broccoli.

Deviation based on the inhomogenity of the sample can be decreased by increasing the amount of broccoli in headspace analysis. Industrial blanching is the most critical point of quality formation: enzymes such as myrosinase, peroxydases and lipoxygenases are to be inactivated without over-cooking. The composition of headspace volatiles especially of *Brassica* and *Liliaceae* vegetables is dependent on the heat treatment. In cabbages and onions both enzymatic and non-enzymatic pathways are always significant in aroma formation.

Cooking in a "closed" vial before collection of the volatiles for headspace analysis resulted in a total area of major peaks 2.6 times higher than that in the "open" vial method. For example, the contents of of the compounds determined 3-methyl-2-pentanone and cyclopentanecarboxaldehyde were four-fold higher when compared to cooking in the open vial. The closed method is more a measure of the total chemical and enzymatic formation of aroma compounds before and during cooking whereas the open method correlates with the aroma compounds in the final cooked product. Coefficients of variation in the chromatographic analyses were typically smaller when the closed method was applied. The effect of the collection method, i.e. open *vs.* closed, was analogous when compared to the development of the onion volatiles after slicing (Kallio *et al.*, 1994).

In earlier reports most of the broccoli volatiles had been analyzed in fresh, stored florets containing active enzymes (Forney et al, 1991; Hansen at al., 1992; Di Pentima *et al.*, 1995) and less information about the cooked product was obtained. Inactivation of myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) and other enzymes by heating gives a completely different collection of volatiles in the headspace when compared to raw broccoli (Chin and Lindsay, 1994), which is known to have one of the highest lyase activities among *Brassicae* (Yen and Wei, 1993).

Our goal was to introduce a chromatographic method to supplement the sensory evaluation of cooked broccoli florets. Direct conclusions of the quality based on chemical analyses only are not possible. Wide differences in the odor threshold values of the vola-

tiles (Buttery *et al.* 1976) and their synergistic effects make the correlation of the chromatographic and sensory data the best choice. For example, methanethiol, dimethyl trisulfide, dimethyl disulfide and other sulfur compounds have very low odor thresholds compared to most of the non-sulfur compounds in broccoli (Hansen *et al.*, 1992). Sulfur compounds are typically the character impact compounds in *Brassica* species and varieties but the relative abundances and importance vary widely.

Special attention has to be paid to verify that the samples and their treatment are identical in the sensory and chromatographic headspace analyses of broccoli.

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12

FLAVOR OF KWENI (*MANGIFERA ODORATA* GRIFF), AN EXOTIC TROPICAL FRUIT

C. H. Wijaya, A. Apriyantono, T. May, H. Raharja, and T. A. Ngakan

Department of Food Tech. and Human Nutrition
Bogor Agric. University (IPB), Indonesia 16602

Kweni (Mangifera odorata Griff), an Indonesian exotic mango, has a strong fruity and spicy odor. Due to its strong and unique aroma, early studies showed the possibility to apply Kweni for flavoring purposes. Sensory evaluation showed that there was no significant difference between the aroma of flesh and peel extracts of Kweni but the aroma of the peel was of higher intensity. The extract obtained maceration for 15 min using ethanol or propylene glycol as solvent with ratio of solvent to material of 1:1 or 2:3, respectively, had an aroma most resembling that of fresh Kweni. Based on gas chromatography-olfactometry analysis, ethyl butanoate, ethyl crotonoate, α-pinene, β-myrcene and two unknowns were the character impact compounds of Kweni.

INTRODUCTION

Indonesia has been traditionally known to be a rich source of highly aromatic raw material such as tropical fruits and spices. Kweni (*Mangifera odorata* Griff) belongs to the mango family and has typical exciting strong flavor. Different from the sweet-type mango, kweni has more fruity, fresh and a little spicy note. The utilization of Kweni as fresh fruit, however, is very limited due to its fibrous flesh which causes acrid on throat. Our early studies showed that there was a high possibility to use Kweni for flavoring purposes (Muchtadi, 1994). In view of the premium value placed on sources of natural flavors, there is a great potential in developing the technology for downstream processing for the purpose of preparing value-added products from Kweni. Thus, we aimed to find the most suitable method for obtaining an extract with original flavor and to identify the volatile compounds especially those with character impact.

Flavor Chemistry of Ethnic Foods, edited by Shahidi and Ho
Kluwer Academic / Plenum Publishers, New York, 1999.

EXPERIMENTAL

Materials

Kweni fruits, which have the appearance and size of mango, were obtained from a local market in Bogor, West Java, Indonesia. Fruits were peeled and separated between the peel and flesh, then each part was kept in refrigerator until used.

Shelf-Life of Peeled Material

To study the possibility of prolonging the shelf-life of raw-material before extraction, fresh peeled-fruits and peels were stored in a tupper-ware at 4 and 25°C for 2, 4, and 6 days.

Extraction

Kweni's flesh was chopped using a blender and the peel was sliced, 3 mm thick, before extraction. Extraction was done using several methods, including water distillation, steam distillation and macerations with different solvents (ethanol, hexane and diethyl ether). The method that produced extract resembling most the original aroma of the fruit was further optimized by varying the condition of extraction. In case of potent odorants' identification, extraction was done using dynamic headspace method of analysis.

Tincture Optimization

Ethanol and propylene glycol tincture were produced by using modified method of Larsen and Poll (1990). Parameters were done in solvent concentrations, ratios of material to solvent and maceration time and duration.

Analysis

Identification of potent odorants was carried out by aroma extract dilution analysis (AEDA; Cadwallader et.al., 1994). The headspace volatile extracts of kweni's flesh and peel were injected onto the GC with sniffing port and evaluation was performed by three experienced assessors who were asked to assign odor properties to each compound detected. Identification of tincture volatile compound was held by using GC-MS. Evaluation was also done by sensory evaluation with blotter techniques including ranking test, multiple comparison test, threshold evaluation using unit dilution test method and hedonic test. Except for hedonic test, all other tests were performed by trained assessors.

RESULTS AND DISCUSSION

Shelf-Life of Peeled Raw Material

The result of this study showed that after peeling, kweni's flesh and peels could be stored at 4°C for 6 and 4 days, respectively, without significant alteration of aroma quality and intensity. The possibility of prolonging shelf-life of the raw material prior to extraction provides technical advantage for flavor production.

Optimization of Extraction Method

Based on the hedonic and multiple comparison tests, and also supported by the aroma description evaluation results, the extract obtained by maceration possessed the most resembling aroma to fresh kweni. Maceration extracts aroma had "rather better" to "better" score on comparison test and "rather like" to "like" score on hedonic test. Solvents used in the maceration method were ethanol, hexane, or diethyl ether. Each of them was chosen based on their superior specification. Ethanol and diethyl ether provided better quality than hexane, however, diethyl ether has not been chosen in the following investigation since it imported an objectionable odor even after it was evaporated.

The results of hedonic test on the flesh and peel's extracts showed that there was no significant difference in their aroma preference. In case of the aroma note, however, they have rather different characteristics as also shown by their GC profiles. The different color of extract obtained from the flesh and peel (flesh extracts have yellow color and green color for the peel one) was considered as the influence of dissolved pigment compounds in the extract.

Ethanol Tincture

Concentrating the extract, although maintained the aroma quality (until 4 fold), resulted in its decreased intensity significantly. Therefore, this phase of the study focused on tincture preparation from Kweni's flesh. According to our previous results, tinctures were prepared using two different solvents; one is the selected solvent ethanol and the other is propylene glycol which is more suitable for high temperature applications (Moyler,1991).

Concentration of ethanol has significant influence on aroma quality and intensity of the extracts. As shown in Figure 1, ethanol concentration had a positive correlation with aroma quality; higher concentrations of ethanol gave better results. In this research, the extract of 90% ethanol produced tincture with highest score on intensity and aroma quality. This phenomenon may be related to the suitability of polarity of ethanol in dissolving flavor compounds which contribute to the aroma of Kweni (Larsen and Poll, 1990).

Optimization of the ratio of material to solvent showed that the ratio of 1:1 was best. Ratios of material to solvent of less than 1 gave extracts with inferior aroma quality. On the other hand, using higher material ratio (more than 1:1) was not efficient because it did not increase the intensity of the tincture.

Maceration duration seems had no significant influence on either the aroma quality or increasing the intensity of the tinctures. For this reason, the shortest time (15 min) was chosen as optimal maceration time. Multiple usage of the extraction medium (extract was used to extract new material over and over) however, indicated that this method did not improve both the aroma quality (resemblance level) and intensity, and also did not improve the preference degree of the obtained tincture.

The best alcohol tincture obtained in this study had a yield of 123% (ie. 23% concentration), with threshold limit at 0.0004 and recognizable threshold of 0.0041. According to multiple comparison test, its intensity with the fresh one was judged as "rather weak" to "similar." The degree of preference of this tincture was scored as "like" to "rather like" and the aroma quality was evaluated as "rather similar to similar".

Propylene Glycol Tincture

Use of propylene glycol as the solvent showed no relationship between concentration of propylene glycol with either the quality of aroma or intensity of the extract (Fig-

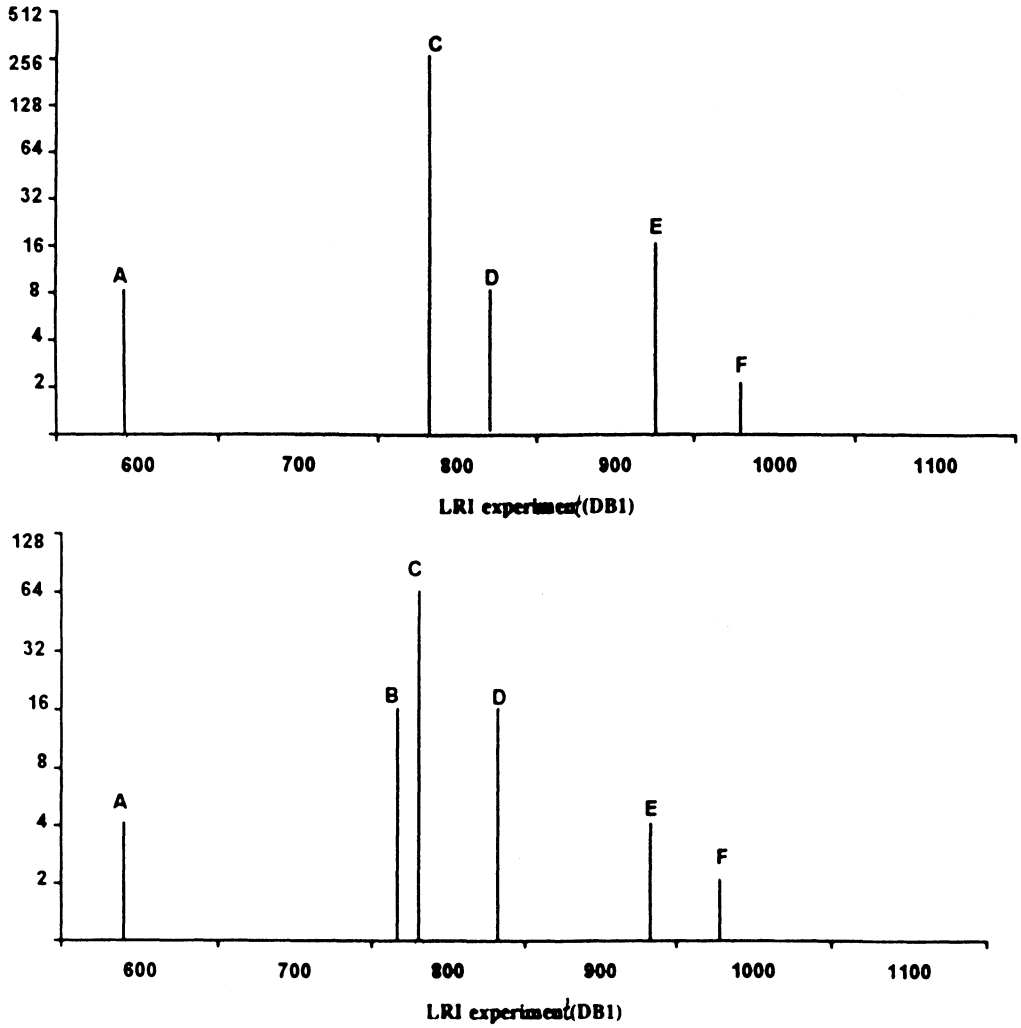

Figure 1. Flavor dilution chromatogram of kweni's flesh (top) and peel (bottom) volatiles extract.

ure 2). Due to technicallmerits, especially easiness of separation, use of propylene glycol 100% is recommended.

Similar to ethanol tincture, maceration time had significant influence on propylene glycol tincture preparation. However, the optimal ratio of material to solvent for this tincture was at a ratio of 2:3.

Multiple extraction was technically impossible because the tincture became very viscous and there was difficulty in separating the extract from the fruit pulp. The best propylene glycol tincture obtained in this study has 162.2% yield, with threshold limit at 0.00039 and recognizable threshold of 0.0036. The sensory properties were similar to ethanol tincture.

Identification of Volatile Compounds

The volatile compounds which were identified from headspace extracts of Kweni's flesh and peel are shown in Table 1. Ethyl butanoate, α-pinene, camphene, β-pinene, β-

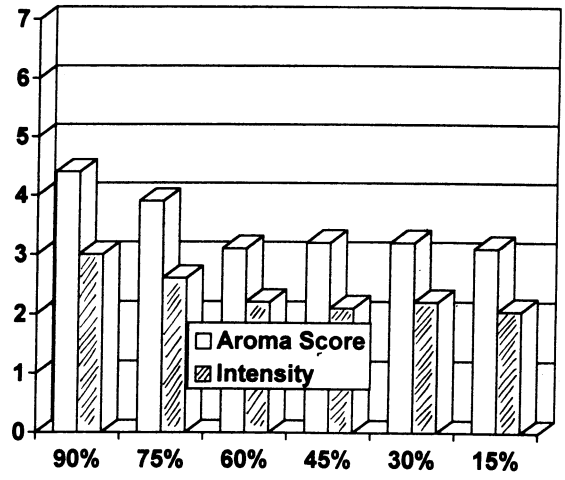

Figure 2. Quality of flavor extract of Kweni extracted by ethanol on various concentrations. Note: Intensity: 1 = weakest; 4 = neutral 7 = strongest. Aroma score: 1 = much different from the standard; 6 = greatly resembles the standard.

myrcene, butyl butanoate, ethyl hexanoate, α-terpinene, limonene, (Z)-β-ocimene, (Z)-linalol oxide-terpinolene, and linalool are the compounds that have already been reported by Wong and Ong (1993) in the Malaysian Kweni extract obtained, using a Lickens-Nickerson apparatus.

There were also several compounds that have been reported as the volatiles of different mango i.e., α-capaene, ethyl tetradecanoate, ethyl hexadecanoate, previously identified as the volatiles of mango var. Alphonso and Balady (Engel *et al.*, 1983), var. Jaffina, Willard and Parrot (MacLeod *et al.*, 1984). Allo-ocimen was also reported by MacLeod et.al. (1984) as a volatile compound of mango var. Jaffina, Willard and Parrot, and hexadecanoic acid as flavor compound of var. alphonso (Idstein *et al.*, 1985). Several compounds including α-farnesene were also identified in the Kweni's propylene glycol tincture and alcohol tincture (data not shown). There were also specific compounds like ethyl crotonoate which has not yet been reported in mango family.

Analysis of Potent Odorants

According to the sniffing judgments, sweet aroma, fruity, ripe-mango-like and mango leaf-like aromas were the dominant detectable aroma from all headspace extracts trapping by diethyl ether or dichloromethane. The flavor dilution analysis (FDA) Chroma-

Table 1. Odor-active compounds in Kweni's headspace extract

No	Code	Compounds	LRIexp DB1[b]	Lrlexp DB5[c]	LRI ref	FD-factor	Description
1	A	unidentified	600			2	Sweet, fruity, mature-mango-like
2	B	unidentified	778	809	786[d]	8	Sweet, fruity, mango's leaf-like
3	C	ethyl butanoate	792	850	826[d]	128	Sweet, fruity, mango's peel-like
4	D	ethyl crotonoate	841	935	939[e]	32	Sweet, fruity
5	E	*alpha* pinene	929	996	991[e]	16	Bitter, turpentine-like
6	F	*beta* myrcene	994			8	Bitter, metallic, young mango character

[a]Number Referring to figure 1 and 2. [b]LRI experiment column DB1. [c]LRI experiment column DB5. [d]Fischer *et al.* (1995); DB1. [e]Adams (1995); DB5.

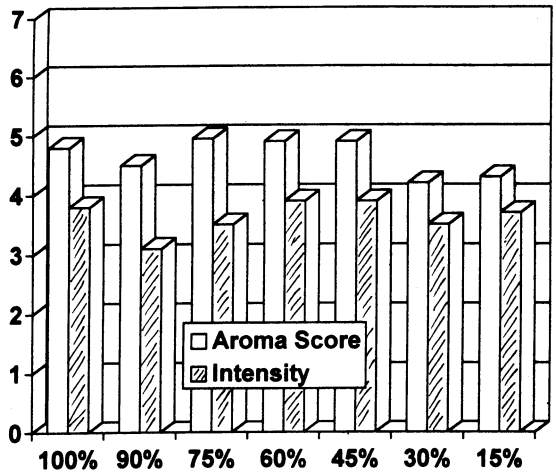

Figure 3. Quality of flavor extract of Kweni extracted by propylene glycol on various concentration. Note: Intensity: 1 =weakest; 4=neutral; 7 = strongest. Aroma score: 1 = much different from the standard; 6 = greatly resembles the standard (top, flesh; bottom, peel).

togram of the Kweni's flesh and peel are shown in Figure 3, and their odor descriptions are given in Table 2. Except for compound B, the FDA profiles of the flesh and peel were very similar.

Ethyl butanoate which described as giving a sweet, fruity and mango-peel like aroma was identified as an important compound in contributing Kweni flavor both in flesh and peel, as it showed the highest FD factor value. In view of its relative percentage area, ethyl butanoate is only the second most abundant compound after α-pinene. However, although α-pinene has the largest percentage area (almost 70%), ethyl butanoate with its high FD factor is more dominant as a major potent odorant.

Ethyl crotonoate with a specific strong sweet aroma and fruity note, along with ethyl butanoate, may also be considered as potent odorants of Kweni. This compound was detected consistently in every extract.

Furthermore, an unidentified compound with LRI below 600 that described as sweet, fruity with ripe-mango like character should be considered as a potent odorant compound since it was also consistently detected in all extracts.

Despite of having sweet, fruity aroma note, two terpenes, α-pinene and myrcene, seem to have important impact in giving fresh-spicy characteristics to kweni's flavor.

Alpha-pinene was considered as an important compound because its aroma was judged to be similar to the typical aroma of kweni's peel. Myrcene itself has been described by other researches as herbaceous, metallic, as well as fresh and green grassy (Gholap and Bhandyopadhyay, 1975; MacLeod and Pieris, 1984).

There was also one unknown compound with LRI 778 which only appeared consistently in peel extracts. Even though this compound had FD factor 4 to 8, based on its flavor description, we did not consider it as differentiating the aroma quality of peel extract from that of the flesh one. Alpha-pinene had more of a kweni-peel like aroma.

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Table 2. Positively identified volatiles of Kweni

No	Compounds	LRI exp[a]	LRI ref[b]	Percentage relatif area of flesh extract		Percentage relatif area of peel extract	
				Dichloro methane	Diethyl ether	Dichloro methane	Diethyl ether
1	Ethyl butanoate	807	800	20.54	29.62	2.53	29.66
2	Ethyl crotonoate	846	826[c]	5.82	5.17	0.68	1832
3	Propyl butanoate	903	896	0.15	nd	nd	0.10
4	*Alpha*-pinene	935	939	35.56	41.05	66.87	18.61
5	Camphene	948	953	0.66	0.67	1.07	0.53
6	*Beta*-pinene	976	980	5.68	4.91	6.92	4.96
7	*Beta*-myrcene	995	991	24.65	15.99	20.28	21.02
8	Butyl butanoate	999	993	0.52	nd	nd	nd
9	Ethyl hexanoate	1003	996	0.39	nd	nd	0.63
10	*Cis*-3-hexen-1-ol,acetate	1010	1007	0.07	nd	nd	0.06
11	*Alpha*-terpinene	1017	1018	0.28	nd	0.16	0.29
12	limonene	1030	1031	1.33	0.46	0.64	1.37
13	*Beta-cis*-ocimene	1040	1040	1.43	0.30	0.56	1.57
14	*Beta-trans*-ocimene	1050	1050	0.44	nd	0.12	0.51
15	*Gamma*-terpinene	1060	1062	0.61	nd	0.14	0.39
16	*Cis* linaloxide	1075	1074	0.07	nd	nd	0.03
17	Terpinolene	1089	1088	0.11	nd	nd	0.07
18	Linalool	1104	1098	0.92	nd	0.05	0.58
19	Allo-ocimene	1132	1129	0.09	nd	nd	0.15
20	*Alpha*-copaene	1332	1376	0.11	0.16	nd	0.06
21	*Alpha*-farnesene	1503	1508	0.09	nd	nd	0.15
22	Ethyl tetradecanoate	1797	1793	nd	nd	nd	0.12
23	Hexadecanoic acid	1972	1961	nd	1.47	nd	0.63
24	Ethyl hexadecanoate	1997	1993	0.489	0.62	0.22	0.24

[a]LRI experiment, column DB 5. [b]LRI reference (Adams *et al.*, 1995); DB 5. [c]LRI reference (Fischer et al 1995); DB 1. nd not detected.

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13

USE OF ELECTRONIC NOSE TECHNOLOGY TO EXAMINE APPLE QUALITY*

Arthur M. Spanier,[1] John C. Beaulieu,[2] Karen L. Bett,[2] and Ken Gross[3]

[1]USDA, ARS, LPSI, MSRL, BARC-East
10300 Baltimore Ave.; Beltsville, Maryland 20705
[2]USDA, ARS, SRRC
1100 Robert E. Lee Blvd.
New Orleans, Louisiana 70124
[3]USDA, ARS, BARC, BARC-West
10300 Baltimore Ave., Beltsville, Maryland 20705

Within the last 5 years there has been a major surge in the development of electronic nose technology (conducting polymers and metal oxide sensors). These multisensor electronic nose (E-nose) devices are coupled to statistical data processing packages designed to simulate the way the human brain interprets or processes the interaction of multiple sensory inputs. Fortunately, to be able to develop an electronic instrument that, in the broadest sense of the word, mimics the human olfactory system, it is not necessary to understand exactly how the human brain interprets complex vapors. E-nose technology has encouraged a wide dissemination of this instrumentation within the food and fragrance industries where it is now used primarily for quality control. Analysis time using an E-nose is often only a few minutes making it a viable alternative to longer classical gas chromatographic (GC) techniques.

The fruit component of fresh-cut produce is the fastest growing market in today's produce business, representing a value-added, ready-to-use commodity that satisfies consumer requirements for authentic flavor, texture, nutritional value, and safety. These requirements challenge the packager / producer to develop cost effective ways of producing and monitoring their products for the presence of spoilage and/or pathogenic microflora and to stabilize and maintain their product's flavor. Therefore, we developed an E-nose method to distinguish differences in and keeping-quality of whole, fresh-cut, and minimally processed Gala variety apple using a 32 sensor (AromaScan™ A32 / 50S multisampler).

* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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INTRODUCTION

Apples, traditionally an ethnic food, have become a globally desired food commodity. According to Smock and Neubert (1950), "the original home of the apple (*Malus sylvestris*) is not known but it is thought to be indigenous to the region south of the Caucasus, from the Persian province of Ghilan on the Caspian Sea to Trebizond on the Black Sea." Apples have probably existed from prehistoric times in both the wild and cultivated states in Europe from the Caspian Sea to the Atlantic Ocean (Hulme and Rhodes, 1971). Apples were available as early as 100 B.C., but "Pearmain" appears to be the first variety recorded in history appearing in 1204 in a deed relating to the lordship of Runton in Norfolk. In America, there are records as early as 1647 of apples having been grafted on seedling rootstocks in Virginia. By 1773, three years before the American War for Independence, apples from America were found in the London markets. The spread of the cultivation of apples in the United States is ascribed to Johnny Appleseed (John Chapman) who established nurseries in Pennsylvania and Ohio.

Apples became quite popular around the world with specific varieties being preferred in different geographical regions. Most of the early orchards were of seedling trees, i.e., trees growing on their own rootstocks. A multiplicity of seedling rootstocks gradually developed. As the demand for authentic, reproducible stocks has grown in recent years, so has the development of large commercial orchards. As the apple industry expanded, varieties were selected and new ones bred to produce higher average yield per tree, absence of biennial bearing, improved appearance and texture, resistance to disease, winter hardiness, etc. This process of selection has resulted in the world markets becoming dominated by less than 20 dessert and culinary varieties of apples. Perhaps the most widely distributed varieties are Bramley's Seedling (largely confined to the British Isles), Cortland, Cox's Orange Pippin, Red Delicious, Golden Delicious, Granny Smith, Jonathan, McIntosh, Newton Pippin and Winesap. From time to time there is a shift in the importance of some varieties and some of the varieties listed are unsuitable for growing in certain parts of the world. However, many countries are now growing apples on a considerable scale.

According to Robison (1997), with the start of the 1997 apple season, marketers are facing greater challenges than ever, and retailers are being offered even wider choices...which is good for the consumer. Apples are versatile, fitting into daily menus from appetizers to desserts plus between-meal snacking. The United States has 46 states with commercial apple industries, and Canada, Australia and New Zealand are a few of the countries anxious to sell their fruit to the American consumer. The first official 1997 season estimate by the U.S. Department of Agriculture is 10.6 billion pounds (253 million 42-pound bushels), an increase of 3 percent over the 1996 crop, but seven percent below the record crop of 1994. An apple a day, every day is becoming appropriate because technology now enable handlers to store and ship apples 12 months a year.

Fresh-cut fruit is among the fastest growing component in today's produce business, representing a value-added, ready-to-use commodity that satisfies consumer requirements for authentic flavor, texture, nutritional value, and safety (IFPA Convention report, 1997) and has an extremely promising future (Tilton, 1997). However, hurdles need to be jumped as this segment of the produce industry will necessarily follow in the footsteps of the now-popular, packaged salads. These requirements challenge packagers and producers to develop cost-effective methods of stabilizing and maintaining their product's flavor, particularly regarding quality changes due to oxidative changes, as well as ways to prepare and monitor their products for the potential presence of spoilage or pathogenic microflora (Kuntz, 1994). Fresh-cut apple, however, are now becoming available in regional

distributions from numerous enterprises in the United States. A new variety of apple hits the market almost every year (Robison, 1997). Among the new arrivals, and the object of the experiments to be described in the following sections, is the Gala apple developed in New Zealand as a cross between Golden Delicious and Kidd's Orange Red. Gala is heart shaped, has a distinctive yellow-orange skin with red striping, and has a crisp, sweet taste.

The Food Industry is always in search of rapid means of monitoring the flavor quality of their products. Typically monitoring food flavor is based on analysis of the hundreds of different odorous molecules that comprise the product's specific odor. However, while humans can typically detect odors in the parts per trillion range (ppt), attempts to instrumentally detect the complex odors at levels that may be below the detection limits of conventional analytical techniques are very expensive and are not always possible. Furthermore, most of the instrumental techniques can objectively discriminate odors, but the sample must be separated into its individual components.

In recent years, there has been a major surge in the development of electronic nose (E-nose) technology, i.e., instruments with arrays of conducting polymers and/or metal oxide sensors. If an array of nonspecific sensors could be compiled to rival the human olfactory system, then the sample need not be separated and could be monitored analytically as a whole (Gardner and Bartlett, 1992). Furthermore, analysis would prove rapid, nondestructive, and continuous. These multisensor devices are coupled to statistical data processing packages designed to simulate the way the human brain interprets the interaction of multiple sensory inputs. According to Barlett *et al.* (1997), "the electronic nose system parallels the human olfactory system in the following manner: Each chemical sensor represents a group of olfactory receptors and produces a time-dependent electrical signal in response to the odor." E-nose technology has encouraged a wide dissemination of this instrumentation within the food and fragrance industries where it is primarily used for quality control and rapid surveys of the products. Analysis time using an E-nose is often only a few minutes making it a valuable alternative to the longer gas chromatographic (GC) techniques. This contribution will examine the utility of using electronic nose instrumentation to survey changes in the quality of Gala apples.

MATERIALS AND METHODS

Preparation of Red Delicious and Granny Smith Apples (Figure 2)

Red Delicious and Granny Smith apples were purchased from a local Supermarket. Five apples were used in the development and optimization of methodology for electronic nose assessment of apple volatiles. To somwhat mimic the masseration of food which occurs in the oral cavity of the human, approximately half (50 grams) of each peeled apple were prepared for homogenization by mincing and mixing with 50 mL of phosphate buffered saline (PBS) at pH 7.0. The minced apple/PBS mixture (about 100 mL equivalent volume) was placed in a Sorval Omnimixer™ stainless steel holding vessel continuously maintained in an ice slurry. The mixture was homogenized (Sorvall® Omni-Mixer 17105; DuPont Instruments, Newtown, CT) 3-times for 20 seconds each at speed 10 with a 30 second pause in the ice slurry between blending periods. Five grams (± 0.1) aliquots of homogenized material from each half of 5 Red Delicious or 5 Granny Smith apples were placed via pipette into separate 22 mL multisampler vial, i.e., each variety of apple had 10 samples (2 per apple) for AromaScan analysis.

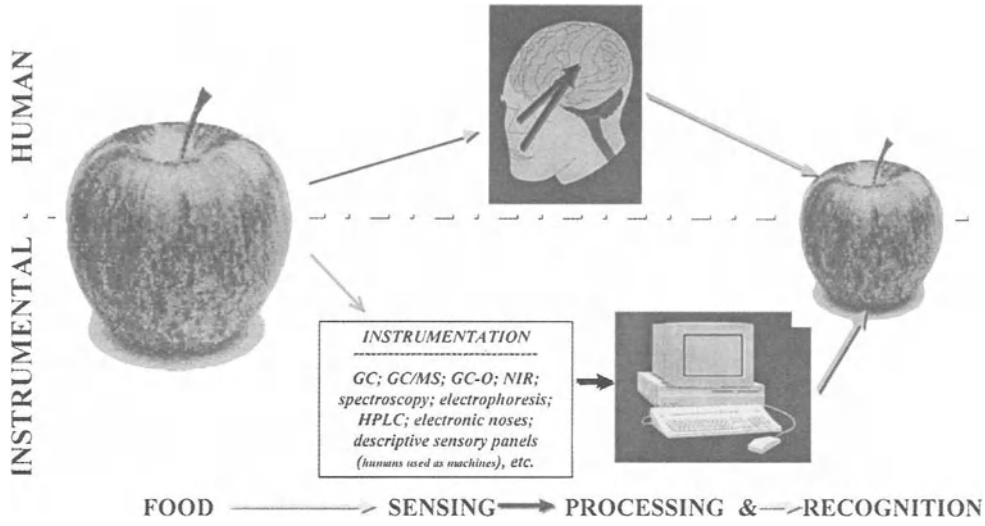

Figure 1. Simplified representation and comparison of human and instrumental means of flavor (odor and taste) sensing/detection, processing, and recognition.

Gala Apple

Gala Apple Samples. Gala apples were harvested in Pennsylvania's apple growing region in September 1997 and shipped refrigerated via overnight mail to the Southern Regional Research Center (SRRC) in New Orleans, Louisiana from Beltsville Agricultural

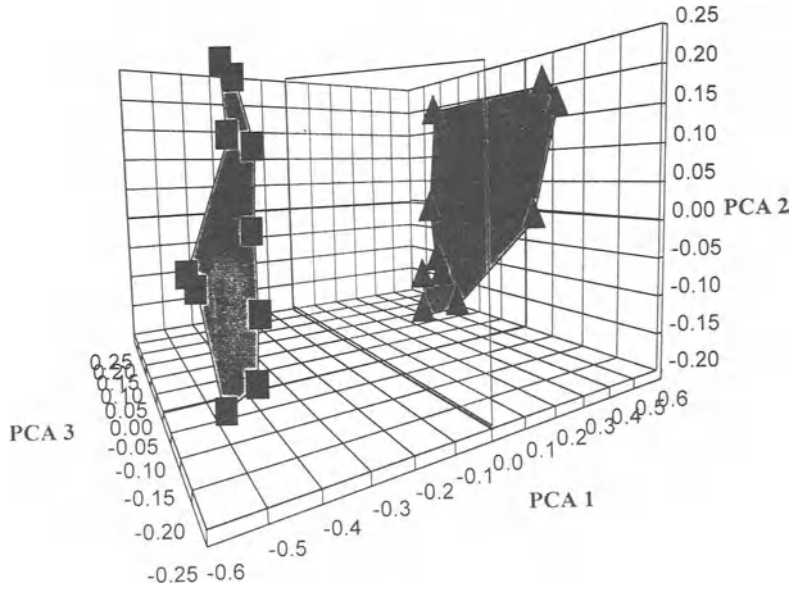

Figure 2. 3-dimentional multivariate PCA of Granny Smith ▲ vs. Red Delicious Applies ■ using the optimized loop-fill time of 0.45 with the AromaScan A32/50S electronic-nose. PCA mapping quality report (euclidian distance) = 4.451.

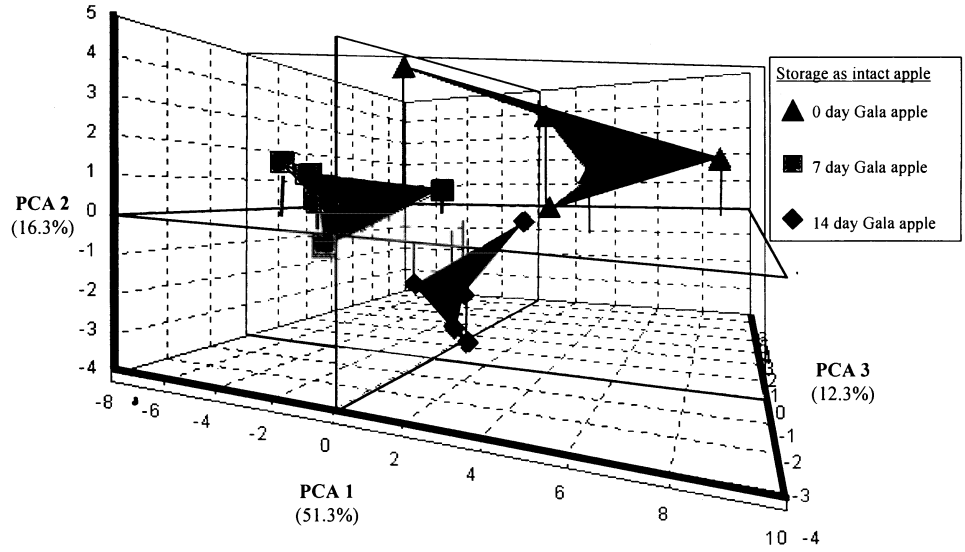

Figure 3. Multivariate principal component analysis (PCA) of Gala apples from the Fall 1997 harvest. Apples were stored whole under refrigeration (1.1 C) for 0, 7, or 14 days prior to slicing for analysis using by an array of 32 conducting polymers in an electronic nose instrument (AromaScan A32/50S). The 3 dimensional PCA map was constructed using the maximum allowable iterations of 200, using correlation matrix and the data was not normalized. The amount of variability explained by principal component 1 through 3 is listed.

Research Center (BARC) in Maryland. Upon arrival, apples were stored in their original shipping crates at the refrigerated temperature of 1.1 °C (Hobart™).

Gala apples were removed from the shipping crate, washed in a 1% chlorine/cold-water solution for about 2 minutes and then thoroughly rinsed with fresh, cold, tap-water for 2–3 minutes. Washed apples were pressure tested using a McCormick fruit pressure tester (McCormick Fruit Technology, Yakima, WA). Mean pressure of the Gala apples used in this investigation (n = 108; 27 apples/box; 4 boxes) was 16.9 lbs. (standard error = 1.9 lbs). Medium Gala apples are reported (http://www.lmg.com/twoharvest/gala.html) to have a 154 g weight. Fresh-cut apples were prepared, handled, washed, treated, bagged, and stored for analysis with slight modification to the procedure of Sapers *et al.* (1989, 1991). All apples were stored in a Hobart™ refrigerator maintained at 1.1 °C

Preparation of Gala Apples (Whole; Figure 3). Gala apples were removed from the shipping crate and treated as described in section 2.2.1 above. Storage of whole apples was in a Hobart™ refrigerator maintained at 1.1 °C. Sections of the whole Gala apple were then prepared and treated as described in the second paragraph of section 2.2.3 below.

Preparation of Gala Apples (Fresh-Cut; Figure 4). Apples were cored and sliced into 10 equal sized wedges using an aluminum apple-corer/slicer. Wedges were sliced into smaller portions and placed in a holding solution consisting of 0.25% NaCl until the next handling step. Chunks were drained in a plastic/spinning salad colander, centrifuged, placed either into LLDPE (Cryovac P640B 2.6mil) storage bags and sealed or were treated for 1–2 minutes with cold browning inhibitor (BI) solution containing 0.1% calcium chloride. BI-1 contained 4% sodium erythrobate while BI-2 contained 2% sodium erythrobate. Samples were held for 1–2 minutes in BI-1 or BI-2, drained in a colander, rinsed with

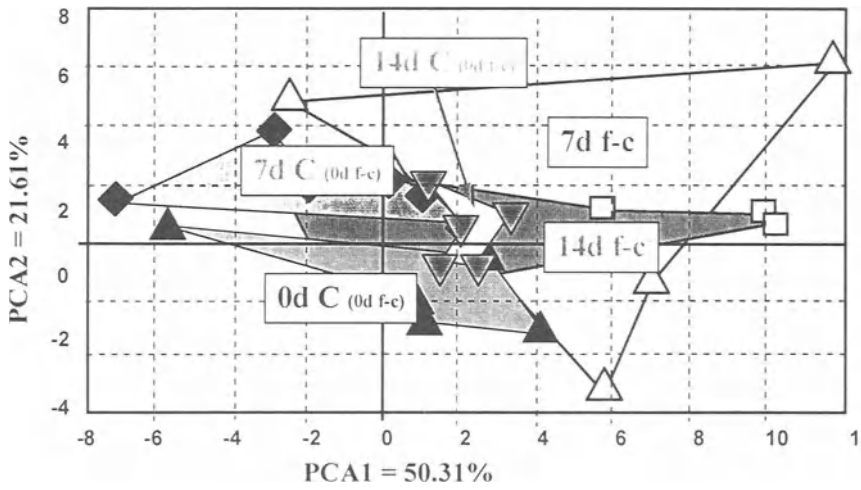

Figure 4. AromaScan™ multivariate principal components analysis (PCA). Effect of postharvest (Fall 1997) refrigerated storage (1.1 C) on Gala apples. Apples samples were prepared as homogenious mixtures taken from the identical samples that had been presented to sensory panelists. Each data point represents a separate sample taken from the homogenious mixture and reflects the variability in volatiles even in a homogenious mixture of apple. Intact/whole apples (C = control) were stored under refrigerated conditions (0d C[▲], 7d C[◆], & 14d C[▼]) and then sliced (freshly-cut) on the day of analysis with the AromaScan A32/50S instrument. Other apples were fresh-cut on day 0 and then stored in fresh-cut (f-c) form in sealed plastic bags which were placed in a refrigerator for 7 and 14 days (7d[△], 14d[□]).

deionized water, drained once more, and packaged in sealed LLDPE plastic bags (personal communication with Dr. Gerald Sapers, USDA, ARS, ERRC). All packaged, fresh-cut, apple chunks with or without BI were stored at 1.1 °C for a period of 7 or 14 days prior to experimental examination by sensory panelists (to be published elsewhere) or chemical or instrumental analysis.

On the days of experimentation, 100 g of apple/treatment was mixed with 100 mL of PBS at pH 7.0; the material was minced and then mixed for a period of 1 minute at speed 10 of the Omnimixer. The homogenization vessel was continually maintained cold by submersion in crushed ice. Five milliliters of each homogenized sample was dispensed via plastic pipette into the 22 mL multisampler vials used for analysis by the AromaScan A32/50S electronic nose (see below).

Electronic Nose

Instrumentation available for this research included the AromaScan A32/50S coupled to a Tekmar™, 50 sample, auto-sampling device which includes humidity control. The Tekmar autosampler was housed in a refrigerated cabinet (Cospolich™, New Orleans, Louisiana) held at 2–4 °C. According to the manufacturer, method optimization required that an ideal loop-fill time be established. This was done using Granny Smith and Red Delicious apples (discussed in the Results and Discussion section). Optimal loop fill time was determined to be 0.45 sec with a time-T of 24 sec.

Based on our experimentation, the optimal method for AromaScan™ analysis of apple was determined to be the following: Acquisition method: Cycles were set at Step 1 with reference set for 24 seconds (the time T), Step 2 with sample time set to 120 seconds,

Step 3 with wash time set to 30 seconds, and Step 4 with reference time set to 120 seconds. The graph scale was set to 5, the pattern average to 5, the purge humidity to 45% and the reference humidity to 50%. Multisampler method: The platen temperature was set to 35°C, the platen equilibration time to 5 seconds, the sample equilibration time to 8 seconds. The mixer was set to 'on' with a mix-time of 2 seconds and a mix power setting of 5. There was a 4 minute vial pressure-stabilization time, a vial pressure-time of 1.0 minute, and a pressure equilisation-time of 15 seconds. Loop-fill time was 27 seconds (0.45 min), loop equilibration time 27 seconds and a sample injection time of 1 minute. Sample-loop temperature was set to 35°C as was the line temperature. The A32/50S analysis time was set for 10 minutes. Data was handled by the statistical software included with the Aromascan A32/50S.

Statistical Analysis: Principal Component Analysis (PCA)

The PCA mapping algorithm supplied by AromaScan with the A32/50S unit was used to analyze and plot (chart) the data obtained from all apple samples. This method of measurement of different gasses or odors produces different patterns that are projected into multi-dimensional space (Hodgins, 1997). In order for a human to examine complex multi-dimensional data, a useful approach is to map them from the high-dimensional pattern space in which they are originally presented onto a low-dimensional pattern space as faithfully as possible.

RESULTS AND DISCUSSION

Electronic Nose Background

The human system of receptors senses a food such as an apple primarily by reception and transmission along odor and taste sensing neurons (sensors) and, to various degrees, by other visual, auditory, and tactile (mouth feel, sound, texture, etc.) cues. The data is sensed by the human neuronal endings and relayed via neurons and synapses to the brain where all of the inputs are processed ultimately to generate a word or words that describe the object (e.g. "this is an apple"; Figure 1). Unlike humans who contain multiple types of receptors, most instrumental methods of analysis such as gas chromatographs (GC), mass spectrometers (MS), GC-olfactometry (GC-O), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), near infrared (NIR), etc., contain only a single sensor/detector or are designed to examine only one chemical class such as proteins, lipids, carbohydrates, etc. (Figure 1). On the other hand, E-nose instrumentation contains multiple sensors that measure one characteristic i.e., volatile components. Thus, E-nose instruments do not measure taste, they assess the bouquet (mixture) of volatiles comprising and emitted from the food commodity under investigation.

Several companies have developed electronic sensors that detect various groups of volatile compounds. An excellent review of E-nose technology may be found in Gardner and Bartlett (1992). Operational characteristics of the three basic types of detectors may be seen in Table 1 which highlights many of their similarities and differences. Major differences to be noted are those dealing with detection temperature, which will affect sample integrity, humidity control, chemical poisons, interaction mechanisms and reported sensitivity. Basically, in all three systems the volatiles are swept across an array of sensors developed to respond to different classes of compounds. A current is passed over the sen-

Table 1. Features of electronic noses

Sensor type	Conducting polymer	'Doped' conducting polymer	Metal oxide (e.g. tin)
Sensor type	chemical, covalent	doped with metal ions, ionic	none
Poisons	none	none	sulfur compounds
Detection temperature	ambient	ambient	200-700°C
Interaction mechanism	charge, size, shape	charge and size only	charge only
Isomeric discrimination	yes	no	no
Reported sensitivity	ppb – ppm	ppm	>10 ppm
Humidity control	required	required	n/a
Sample integrity	maintained	maintained	destroyed
Neural network	yes	yes	yes

sors, the adsorption and desorption of the volatiles on the sensor cause a change in the current similar to the thermal conductivity detectors used in gas chromatography. This current change is measured and input to a computer that uses various statistical procedures and mapping techniques (algorithms) to examine this multidimensional data array and otherwise handle the data.

Statistical Handling of the Data

With an array of sensors, measurements of different gases or odors produce different patterns that are projected into multidimensional space. Within two- and three-dimensional space, human vision is very good at recognizing almost any relationship that may be present. However, in multidimensional space it is difficult to perceive structural relationships. In order for a human to examine complex multidimensional data, the information must be handled in such a manner as to map the high-dimensional pattern space onto a low-dimensional pattern space such as that seen in Figures 2–4. There are a number of ways of performing such a task where all the mapping algorithms are either linear or nonlinear.

The linear mapping algorithms are used frequently because of their simplicity and generality; these have been used in gas and odor classification as well as in chemical data, in order to visualize the multidimensional pattern space in two- or three dimensional space. For gas recognition, Gardner *et al.* (1991a, 1991b, 1992, 1994) used principal component analysis (PCA) method, one of the powerful linear mapping techniques available, to cluster and classify volatile chemicals. This linear mapping technique was recently employed by Flores *et al.* (1997) for examining the volatiles from Spanish "Serrano" dry-cured ham. Ballantine *et al.* (1986) classified vapors using the PCA method and the Karhunen-Loeve (K-L) projection, which is a linear mapping technique similar to PCA. The odor of whisky was studied by by Abe *et al.* (1988) who used the K-L projection for odor classification and by Nakamoto et al (1991), who investigated data from different whiskies using the PCA method. Kowalski and Bender (1972, 1973) used a similar linear mapping technique, using eigenvector projection, for chemical data displaying. Spanier *et al.* (1992a, 1992b, 1997) have used PCA analysis to study the interaction between sensory, instrumental, and chemical analysis of meat and meat products under various treatments. In these current studies (see below), PCA mapping was performed by direct use of the PCA mapping software supplied by the manufacturer (AromaScan™).

Methods Development

According to the manufacturer (AromaScan™), one of the principal items that must be determined when developing a method for any E-nose use, and for the AromaScan™ E-nose in particular, is to determine the "loop-fill time." Too short a loop-fill time will lead to sample variability caused by insufficient filling of the loop by the subject volatiles. Too long a loop-fill time has the potential of leading to variability caused by over-flushing or purging of the headspace causing a major dilution and difference in the equilibrium already established between the sample and the surrounding headspace of the 22 mL sample vial. According to the manufacturer, the tighter the grouping or clustering of the data points the better the loop-fill time. Thus, we examined loop-fill times of 12, 18, 24, and 30 seconds (machine times of 0.2, 0.3, 0.4 and 0.5 minutes) for loop-fill using green (Granny Smith) and red (Red Delicious) apples. While 12 seconds (0.2 min) appears to have a reasonably tight clustering, 24 and 30 seconds (0.4 and 0.5 min) appears to be somewhat better. Therefore, we examined loop-fill times of 27, 30, 33, and 36 seconds (machine set times of 0.45, 0.50, 0.55, and 0.60 min). It was determined that the tightest clustering for both red and green apples occurred at a loop fill time of 27 seconds (0.45 min).

Granny Smith versus Red Delicious Apples (Figure 2)

Using the optimal loop-fill time of 0.45 minutes we examined the pattern developed by each half of five Red delicious apples and five Granny Smith apples (Figure 2). Note the clear separation of the two cluster groups along PCA component #1 (PCA 1). According to the data handling by the AromaScan™ software, PCA component #1 has a high eigenvalue indicating that PCA component 1 explains 78.47% of the variability between Granny Smith and Red Delicious apples. Unfortunately, neither the software packet nor the documentation supplied by the manufacturer indicate what PCA 1 represents. PCA component #2 (PCA 2) explains 9.16% of the variability and PCA component #3 (PCA 3) explains 4.90% of the variability. A PCA 'mapping quality report' determined the Euclidian distance (the distance between clusters adjusted for probability) to be 4.451. According to the manufacturer, Euclidian distances in great than 2.0 indicate statistical significance in the non-complex volatile mixtures they have examined.

Gala Apples: Storage Both as Intact and as Fresh-Cut Apples

Gala apples were prepared for a major series of experiments that would involve both chemical and instrumental analysis of apple quality and quality assessment by human sensory panelists. The human panelists were to receive freshly cut apple as a control along with the apples that had been 'fresh-cut', packed and stored. The same apples that were prepared for human examination were utilized for the chemical and instrumental analysis. Since the panelists received apple portions originating from different apples (randomly sliced chunks), for the sake of consistency and because of the availability of both time and manpower it was deemed appropriate to take the same samples examined by the sensory panel and make a homogeneous suspension for chemical and instrumental analysis.

These same apples were used for E-nose evaluation of freshly-cut whole apple samples and may be seen in Figure 3. The three storage times (0d, 7d, and 14d) of whole, freshly-cut apples (C) separate into well defined groups (labeled as 0dC, 7dC, and 14dC). Freshly cut apple from day 0 (0dC) clustered above the origin of PCA component #2 (PCA 2) and behind the origin of PCA component 3 (PCA 3; Figure 3) while the control whole-

apple samples that had been stored for 7 and 14 days prior to cutting (7dC and 14dC) clustered into groups in front of the origin of PCA component #3 which explains 12.3% of the variability. However, the replicate samples from homogenized and minced apple at 7 and 14 days-stored whole apples separated into cluster groups each located on a different side of the origin of PCA component #2 (PCA 2 which explains 16.3% of the variability) and on either side of PCA component #1 (which explains 51.3% of the variability). These samples have Euclidian distances of less than 2.0 (but greater than 1.0) which is typically considered as the distance of significant difference. However, the clusters (representing the variability of 5 different samplings from the same homogeneous apple mixture) clearly segregate in different quadrants of the PCA analysis. The latter suggests that even with 5 replications of a homogeneous apple samples there is considerable scatter. The scatter in the samples taken from the same homogeneous apple (0d, 7d, and 14day) could result from instrument variation over the course of the experiment. However, in the experimental protocol, the sensors were given an extended wash time with butanol and showed the same response at the beginning and the end of the 1999 testing season and showed minimal differences. Examination of the sensors' responses indicated that there was negligible change in the sensor's response. Another possibility for the scatter of the data points comprising the group cluster may be the time in the instrument, even though the sample is homogeneous; this time would permit enzymatic browning and other enzyme reactions to occur all of which could change the volatile profile. This too was minimized by only examining one sample from each experimental group at a time (i.e. $0d_{r1}$, $7d_{r1}$, $14d_{r1}$, $0d_{r2}$, $7d_{r2}$ ...$14d_{r5}$) and maintaining all samples in a refrigerated housing (2–4 °C).

As one compares more experimental groups of data, particularly when the data are from the same commodity that share many common volatile components, one still can see a separation into different cluster groups, but the separation is not as sharp (another Spanier *et al.* chapter in this monograph). Thus, when whole apples that had been stored for 7 and 14 days were compared to their fresh-cut/then stored counterparts (Figure 4), a separation along PCA component #1 (explaining 50.31% of the variability) and PCA component #2 (explaining 21.61% of the variability) was observed. There was so much similarity in the samples that it is difficult to examine this number of sample on a 2 dimensional plot and even worse on a 3-D plot. The three whole-apple groups (i.e., apples stored as whole

Table 2. Effect of postharvest (Fall 1997) refrigerated storage (1.1 °C) of whole and fresh-cut GALA apples[‡]

	0d C[a]	7d C	14d C	7d f-c[b]	14d f-c
0d C		1.276	1.061	0.930	0.801
7d C			1.914	1.221	1.218
14d C				0.800	0.600
7 f-c					0.138
14 f-c					

[‡]. The numbers in the table are AromaScan's[TM] "PCA mapping quality report," which are actually the Euclidian distance (Srivastava and Carter, 1983).

[a] C represents intact whole apples that were cut on the day of examination, i.e., 0, 7, or 14.

[b] f-c represents apples fresh-cut on day 0 and stored as fresh-cut for the listed time, i.e. 7 or 14 days.

apples and then freshly-cut on day of examination (0dC, 7dC, and 14dC, respectively) still separated primarily along PCA component #2 as seen in Figure 4. However, the two fresh-cut apple groups that were stored as fresh-cut apples (groups 7d f-c and 14d f-c) separated from the whole-stored apples along PCA component #1 and showed major overlap with one another. Table 2 shows the Euclidian distance (PCA-mapping quality report; ED) generated by the AromaScan™ software used in this study. The greater the Euclidian distance the greater the significance of the difference between the clustered groups. Thus, the 0dC group was different from each of its stored whole apple comparisons, i.e. 7dC and 14dC (1.276, and 1.061, respectively Table 2) and somewhat different from the fresh-cut and stored groups, 7d f-c and 14d f-c (0.930 and 0.801, respectively). The 7dC group of apples is different from that of the 14dC and was different from the 7d f-c and 14d f-c groups. However, the fresh-cut stored groups were not shown to be different from each other in this comparison.

The results presented in Figures 2, 3, and 4 show that as the complexity of the sample increases (i.e., going from two different types of apples to the same type of apple that are stored, towhole and fresh-cut stored apple), the AromaScan instrumentation (an perhaps other electronic noses as well) has potential to see differences among the samples. However, much larger sample sizes would be needed to generate libraries to confidently use the AromaScan™ to identify an unknown. Unfortunately, limitations in manpower, in experimental design constraints, etc. have limited the number of samples we were able to examine without contribution of other variables such as enzymatic browning for one. Thus, as sample complexity increases (with sameness of commodity) and as more treat-

Table 3. Effect of postharvest (Fall 1997) refrigerated storage (1.1 °C) of whole and fresh-cut GALA apples with or without browning inhibitor (BI) at two concentrations‡

	0d C[a]	7d C	14d C	7d f-c[b]	14d f-c	0d f-c BI-1	7d f-c BI-1	14d f-c BI-1	0d f-c BI-2	7d f-c BI-2	14d f-c BI-2
0d C		1.098	1.119	0.932	0.822	0.298	0.598	0.510	0.395	0.892	0.607
7d C			2.111	1.271	1.266	0.983	0.999	1.481	1.267	0.584	1.162
14d C				0.859	1.698	0.986	0.387	1.061	2.051	0.800	0.071
7d f-c					0.125	0.948	0.183	0.718	1.493	0.313	0.440
14d f-c						0.852	0.099	0.583	1.368	0.161	0.347
0d f-c BI-1							0.605	0.729	0.573	0.967	0.553
7d f-c BI-1								0.433	1.033	0.131	0.184
14d f-c BI-1									1.031	0.542	0.543
0d f-c BI-2										1.550	1.117
7d f-c BI-2											0.389
14d f-c BI-2											

‡. The numbers in the table are AromaScan's[TM] "PCA mapping quality report," which are actually the Euclidian distance (Srivastava and Carter, 1983).

[a] C represents intact whole apples that were cut on the day of examination, i.e., 0, 7, or 14.

[b] f-c represents apples fresh-cut on day 0 and stored as fresh-cut for the listed time, i.e. 7 or 14 days.

ment groups are added to the data analysis (2, 3, and 5 sets, respectively), the likelihood for cluster overlap increases making interpretation of the graph more difficult. Visual data analysis becomes particularly difficult (Figure 4). Therefore, visual examination of the 11 different treatment groups in this experiment are impossible to perform on a two dimensional graph and even more on a 3-d graph. Table 3 was prepared to present the data as a 'quality mapping report' giving Euclidian distances (Srivastava and Carter, 1983) as an indicator of difference between treatments. The greater the MD between clusters, the greater their dissimilarity.

The data in Table 2 show the quality mapping report (Euclidian distances) of whole stored apples (0dC, 7dC, 14dC) compared to untreated fresh-cut/stored apples (7d f-c, 14d f-c) or those treated with BI-1 or BI-2. The PCA mapping quality report indicates that 0, 7, and 14 day stored whole gala apples are not similar (0dC Gala ≠ 7dC Gala ≠ 14dC Gala; also see Figure 2). The data in Table 3 indicated that the untreated fresh-cut Gala apples stored without browning inhibitor solution (7d f-c; 14d f-c) were found to be somewhat similar at 7 and 14 days to the BI-1 treated 0d fresh-cut group (0d f-c BI-1; 0.948 and 0.852, respectively). However, they were different (1.493 and 1.368, respectively) from the BI-2 treated fresh cut (0d f-c BI-2). According to the quality mapping factors shown in Table 3, the fresh-cut apples treated with BI-2 (2% erythrobate + 0.1% calcium chloride) were similar to their control apple [0.395 = MD for 0dC vs. 0d f-c BI-2; 0.584 = MD for 7dC vs. 7d f-c BI-2; 0.071 = MD for 14dC vs. 14d f-c BI-2]. On the other hand, the fresh-cut apples treated with BI-1 (4% erythrobate, 0.1% calcium chloride) were different than the control at 14 and 7 day, but the same as the control on day 0 [1.061 = MD for 14dC vs. 14d f-c BI-1; 0.999 = MD for 7dC vs. 7d f-c BI-1; 0.298 = MD for 0dC vs. 0d f-c BI-1].

Ponting *et al.* (1972) demonstrated that apples dipped at neutral and basic pH with calcium in the form of calcium chloride at 0.1% were more firm than untreated apples, an important quality factor to maintain during food storage. These same authors found that higher calcium concentrations (0.2%) made "apples too firm and woody." When used in conjunction with sulfite (SO_2) in a dip "the qualities of firmness, color, and flavor can be balanced to give the most desirable product after storage. The storage life can be extended by this means from a maximum of about 3 weeks to as long as 8 weeks at 34°F."

Other investigators have reported that sodium and calcium are the compounds most commonly used in the food industry for the inhibition of browning (McEvily and Iyengar, 1992; Steiner and Rieth, 1989). However, the effect of sodium and calcium may have been due to their anion which in both cases was the chloride anion. McEvily and Ivengar (1992) also showed that both the anion and cation affect quality; they suggest that the halide anion inhibits browning while the cations may have an affect on texture by maintaining "the firmness of the pulp tissue by interacting with pectin in the cell walls." Thus, it is possible that the calcium dip we used in this study could have maintained apple quality in a manner similar to that of uncut control apples even without the presence of erythrobate. However, further experimentation to determine the effect of calcium and halide anions should be performed before any firm conclusion may be reached in this regard.

Browning of fruits and vegetable during postharvest handling and processing is typically attributed to release and activation of browning-inducing enzymes and components by the mechanical injury such as slicing associated with preparation of fruits as fresh-cut and minimally processed products; browning (enzymatic or oxidative) is an important cause of loss in quality and value of the commodity. Sapers *et al.* (1987a, 1987b, 1989, 1991) have shown that erythrobate (an isomer of ascorbic acid) is an effective anti-browning compound. In our studies of stored whole Gala apple versus stored fresh-cut Gala apples using the electronic nose, no significant difference in response was observed for the

two erythrobate concentrations used. Howerver, there appears to be a very slight differential response to erythrobate such that 2% erythrobate appears to be more effective in maintaining the fresh-cut Gala apple quality more similar to a whole apple than is 4% erythrobate (based on Euclidian distances between clusters). However, many more experiements must be performed before this conclusion regarding erythrobate becomes a reality.

CONCLUSION

Different varieties of apple have many measurable differences in their rate of maturation, in flavor quality, lipids, peptides, amino acids, malic enzymes, sugars, and other components (Hulme and Rhodes, 1972; Knee, 1993). It would, therefore, seem reasonable to predict that E-nose technology might prove itself useful to examine the differences in apple types and, perhaps, among different treatments of the same apple variety. Our data suggest that the electronic nose technology has a usefulness in examining the differences in different varieties of apple such as the Granny Smith and Red Delicious (Figure 2), and may be quite useful in examining Gala apples during storage and fresh-cut storage (Figures 3 and 4, Tables 2 and 3). At this time we are evaluating data obtained from trained human sensory panelists and from other instrumental methods of assessing Gala apple flavor. These data in conjunction with the further experimentation and development of E-nose sensors may produce sensors that have the potential to be used for the rapid assessment of apple quality.

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14

FLAVOR AND CHEMISTRY OF UNCURED AND CURED MEAT OF HARP SEAL (*PHOCA GROENLENDICA*)

Fereidoon Shahidi and Ming-Xia Liu

Department of Biochemistry
Memorial University of Newfoundland
St. John's, New Foundland, A1B 3X9, Canada

Seal meat is an excellent source of high quality protein for human consumption. Its intramuscular lipids are also composed of high proportions of omega-3 polyunsaturated fatty acids. In this study, the flavor volatiles of uncured and nitrite-cured seal meat were isolated by a continuous steam distillation extraction (SDE) technique. Aroma concentrates were prepared using a Likens-Nickerson extraction procedure and subjected to GC-MS analysis. Samples were cured with 50, 150 and 500 ppm sodium nitrite in the presence of 550 ppm sodium ascorbate and 1.5% sodium chloride. The uncured sample gave rise to 86 components while samples cured with 50, 150 and 500 ppm sodium nitrite contained 72, 45 and 27 components, respectively. The volatile components were composed of aldehydes, ketones, alcohols, hydrocarbons and miscellaneous compounds. The major contributors to seal meat flavor were (E,Z)-2,6-nonadienal and its breakdown product (Z)-4-heptenal, both of which are degradation products of omega-3 polyunsaturated fatty acids; 2-octenal, a breakdown product of omega-6 fatty acids was also present. Benzaldehyde was another important volatile present in both cured and uncured seal meat.

INTRODUCTION

Flavor is an important sensory attribute of foods in general and of muscle foods in particular. While the nutritional aspects of food are important upon consumption, flavor and color are among the primary factors influencing the customers even prior to consumption. Flavor of foods is influenced by both taste and aroma-active components (Shahidi, 1989).

The flavor of meat is affected by the chemical nature and proportion of its water-soluble components which interact with one another or with lipids and lipid oxidation products to produce an array of volatile compounds. The chemical nature of these volatiles is of course influenced by the proportion of low-molecular-weight non-volatile components as well as by the compositional characteristics of the lipids which are responsible for species

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identification of meat from different animals. The main flavor precursors were suggested to be free sugars, sugar phosphates, nucleotide-bound sugars, free amino acids, peptides, nucleotides and other nitrogenous components such as thiamine (Mottram, 1998).

Cured meats are treated with nitrite, salt, ascorbic or erythorbic acid or their salts, possibly together with polyphosphates and spices. Nitrite is a unique ingredient in the curing of meat and meat products due to its role in the development of color, flavor as well as oxidative and microbial stability. Each of these effects could be duplicated, however, no single compound has yet been found with such multifunctional properties. Although nitrite is closely associated with cured-meat aroma, the chemical changes that are responsible for the unique flavor of such products are not clearly understood. However, Cross and Zeigler (1965) reported that the volatile constituents of both cured and uncured ham were qualitatively the same, but quantitatively different. They also passed the volatiles of uncured beef and chicken through a solution of 2,4-dinitrophylhydrazine, where volatile carbonyl compounds were stripped, and found that the remaining volatiles had flavor effects which were similar to one another as well as their cured counterparts. Later on, it was postulated that the flavor of cured meat from different species might be the same as the natural flavor of meats without being influenced by their lipid components that are responsible for species identification (e.g. Shahidi, 1992). However, it was later found that there were exceptions to this simple postulate as the flavor of cured mutton was different from those of other species.

In this chapter, chemical and nutritional characteristics of seal meat is discussed. The role of nitrite in the curing of seal meat and its influence on the aroma volatiles of products is also reported.

SEALS AND THEIR CHEMICAL AND NUTRITIONAL CHARACTERISTICS

Seals or pinnipeds are found in every sea in the world and along every coast of every continent. Harp seal (*Phoca groenlendica*) is the third most abundant species of seal in the world after ringed and crabeater seals with a population of over 4.8 million animals. The average size of an adult harp seal is approximately 135 kg during the breeding season and smaller at other times of the year (Shahidi, 1998).

The body of slaughtered harp seal is composed of approximately 29.3 ± 5.7% blubber, 7.8 ± 1.6% pelt, 43.6 ± 3.8% carcass and 18.2 ± 3.8% viscera. The yield of meat from carcass depends on the method of deboning; mechanical deboning provides a higher yield

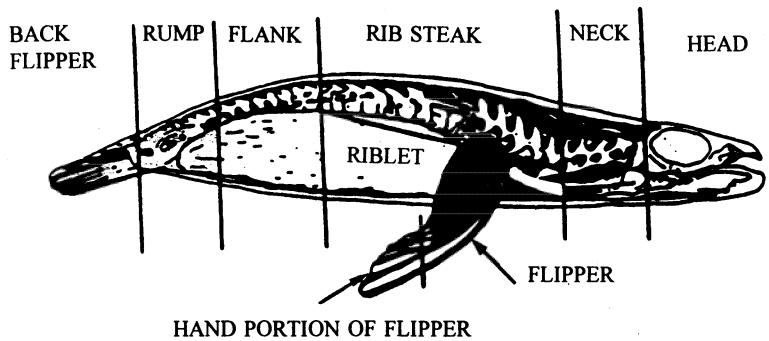

Figure 1. Different meat cuts of seal carcass.

of approximately 80–85%. Figure 1 shows the carcass of seal with different cuts identified. An examination of the freshness quality of seal meat, as determined from changes in the content of its nucleotides, revealed that keeping quality of seal meat resembles other red meats, particularly beef, and is different from that of cod (Shahidi *et al.*, 1994a).

Table 1 summarizes the nutritional quality of mechanically-separated seal meat (MSSM) as compared to other sources of muscle food (Shahidi and Synowiecki, 1993). Based on the data provided, seal meat may be considered as an excellent source of nutritionally valuable proteins, lipids, minerals and vitamins. The crude protein content of MSSM varies between 21.6 and 24.3% which is somewhat higher than other species of red meat, perhaps due to its high content of hemoproteins (5.7–5.9%) (Shahidi *et al.*, 1992). Seal proteins contain 2.1 and 1.8% more histidine and 0.7 and 1.3% more lysine than beef and pork, respectively. Meanwhile, the calculated protein efficiency ratio (PER) of seal meat was 2.99 as compared with that of beef (2.85), pork (2.52) and cod (2.90). Furthermore, PER value of MSSM proteins exceeds the value of 2.50 set as the American Standard requirements for mechanically deboned meats.

The lipids in seal meat, similar to those of other marine oils/lipids, are composed of approximately 74.3–79.9% unsaturated fatty acids. Table 2 summarizes the fatty acid composition of MSSM as compared to other species of red meat and cod (Shahidi, 1998). While 98.9% of blubber lipids were composed of triacylglycerols (TAG), intramuscular lipids contained only 78.9% TAG. The content of polar lipids in the intramuscular lipids was 21.1% and these were mainly derived from structural components of the cells. Approximately 30% of the unsaturated fatty acids of seal meat were composed of polyunsatu-

Table 1. Nutritional quality of mechanically-separated seal meat (MSSM) as compared with other meats

Constituents in 100 g meat[a]	Dimension	MSSM[b]	Beef	Pork	MSCM[c]	Cod
Total protein	g	22.3	22.0	22.0	14.6	17.8
Collagen	g	0.9	0.5	0.7	1/1	0.5
EAA value	—	116	117	119	117	119
PER value	—	3.0	2.8	2.5	2.8	2.9
Lipids	g	3.7	1.9	1.8	9.8	0.6
Caloric value	kJ	528	481	479	612	344
Calcium	mg	591	3.5	3.2	78/4	24.0
Phosphorus	mg	504	194	204	195	184
Potassium	mg	288	370	418	229	356
Sodium	mg	159	57	56	87	72
Iron	mg	64	1.9	1.0	1.8	0.4
Magnesium	mg	34	21	27	17	25
Zinc	mg	2.8	4.2	1.9	2.2	0.5
Copper	mg	0.1	0.1	0.0	0.1	0.2
Manganese	mg	0.1	0.0	0.1	0.1	0.0
Thiamine (Vitamin B1)	mg	0.3	0.2	0.2	0.2	0.1
Riboflavin (Vitamin B2)	mg	0.1	0.2	0.9	0.1	0.1
Niacin (Vitamin B3)	mg	6.1	7.5	5.0	4.5	2.3
Pantothenic acid	mg	0.9	0.6	0.7	1.6	0.1
Vitamin B6	mg	0.2	0.4	0.5	0.3	0.2
Vitamin B12	μg	7.7	5.0	5.0	0.8	0.5
Folic acid	μg	3.3	15.3	6.0	11.5	12.0

[a]EAA, essential amino acid; PER, protein efficiency ratio.
[b]MSSM - mechanically separated seal meat.
[c]MSCM - mechanically separated chicken meat.

Table 2. Fatty acid composition (%) of mechanically-separated seal meat as compared with beef, pork, lamb, and cod

Fatty acid	MSSM	Beef	Pork	Lamb	Cod
14:0	1.5	3.1	1.2	2.2	1.9
16:0	8.1	23.9	22.1	20.8	18.7
18:0	4.7	13.2	11.1	13.4	6.1
20:0	0.9	—	—	0.8	—
22:0	1.2	—	—	—	—
24:0	1.2	—	—	—	—
16:1	3.3	3.4	3.1	2.0	3.3
18:1	29.7	38.7	41.0	35.4	12.6
20:1	15.9	—	0.7	—	3.1
22:1	6.3	—	—	—	0.6
24:1	1.3	—	—	—	—
18:2	4.1	2.4	8.6	3.7	1.0
18:3	0.8	0.2	2.9	1.0	0.2
18:4	2.0	—	—	—	0.2
20:3	1.3	—	—	—	—
20:4	1.8	0.3	0.5	1.3	4.6
20:5	5.7	—	—	—	13.2
22:5	3.4	—	—	—	2.0
22:6	5.5	—	—	—	24.8
Total	98.7	85.3	91.2	80.6	92.3

rates. In MSSM, the contents of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docasahexaenoic acid (DHA) were 6.9, 5.6 and 10.1%, respectively (Shahidi *et al.*, 1994b). Meanwhile, the content of cholesterol in manually-separated and MSSM ranged from 66 to 116.3 mg/100g sample (Shahidi and Synowiecki, 1991). Thus, seal meat provides an excellent source of muscle food for human nutrition.

TASTE-ACTIVE NITROGENOUS COMPOUNDS OF SEAL MEAT

Consumption of seal meat may require an acquired taste. Products based on seal meat are intensely flavored and also possess a dark color because of their high hemoprotein content. Thus, sensory characteristics of seal meat might benefit from processing, including curing. The taste active components of seal meat are low-molecular-weight water-soluble non-protein nitrogenous compounds. However, low-molecular weight volatile components are responsible for the aroma of the products arising from interaction of different precursors.

The non-protein nitrogen (NPN) compounds of seal meat also influence quality, taste component of flavor and nutritional value of products. Non-protein nitrogen compounds of MSSM were present at 3.41 mg/g and composed of nucleic acids, nucleotides, free amino acids, peptides as well as minute quantities of trimethylamine oxide and other amines (see Table 3) (Shahidi *et al.*, 1993). Except for imidazole dipeptides, free amino acids are the major fraction of NPN compounds in seal meat and their content was 0.35% in MSSM. Approximately 55% of free amino acids consisted of taurine, alanine, glutamine, glutamic acid, leucine and lysine. Free amino acids of seal meat also contained 32.8% sweet- and 22.1% bitter-taste compounds (Table 3). Free amino acids, amines and peptides contribute to the flavor and taste of seal meat.

Table 3. Non-protein nitrogen (NPN) compounds of seal meat (mg N/100g sample)

Component	Manually separated	Mechanically separated
Total NPN	172	342
Free amino acids	30.0	51.9
Immidazole dipeptides	79.4	94.9
Nucleic acids	23.8	40.7
Amines	1.36	1.74
Ammonia	2.33	5.10
Nucleotides	17.7	24.3

AROMA-ACTIVE COMPONENTS OF UNCURED AND CURED SEAL MEAT

Ground seal meat (80g) was cooked with 20 ml of water at 85°C for 45 min, with intermittent stirring in order to facilitate uniform cooking. Similarly, cured meats were prepared simultaneously by adding different amounts of sodium nitrite (50ppm, 150ppm, and 500ppm as well as 550 ppm of sodium ascorbate and 1.5% sodium chloride, in addition to 20 ml of water and processed. The cooked meat (uncured and cured) samples were cooled to room temperature and stored in a refrigerator at 4°C for 24 to 48h. Distilled water was added to the cooked-meat samples (2:1, w/w) before the extraction of volatiles.

Aroma concentrates were prepared from 15g ground seal meat using a modified Likens-Nickerson steam distillation-extraction (SDE) apparatus. The flavor components were extracted into n-pentane (2ml). The pentane extract was dried over anhydrous sodium sulfate. Then 1 μl of pentane extract was injected into gas chromatograph-mass spectrometer (GC-MS). Mass spectra obtained were compared with those of known compounds in the NIST liberary by using a Gateway 2000 (486/33c) computer. Tentative identification of the individual components was based on the MS data.

Hexanal and decanal were used as internal standards in uncured and cured seal meat. From the peak areas of different known concentrations of hexenal and decanal, the amount of individual constituents present in uncured and cured seal meat was calculated and expressed as milligrams per kilogram of meat.

The total ion chromatograms of aroma concentrates of uncured and cured seal meats, analyzed by gas chromatography-mass spectrometry, are shown in Figure 2. A close scrutiny of the chromatograms indicates that as the amount of nitrite in the cure was increased there was a descimation of the number and concentration of volatile components. Shahidi (1992) has reported that the flavor of meat from different species, without being influenced from its lipid components, might be the basic flavor of meat and similar to that of cured meat. However, it has been recognized that other factors affect the fine tuning of this simplified approach as mutton flavor, even after curing, was found to be different from that of the desirable flavor of other cured products. Nonetheless, the simplification of the chromatograms upon curing of seal meat indicates the important impact of lipids on flavor of such products (Shahidi, 1992; Ramarathnam, 1998).

The volatile flavor constituents of uncured seal meat were composed of 29 aldehydes, 10 ketones, 11 alcohols, 16 hydrocarbons and 9 other compounds. The list and contents of different volatiles in uncured and cured seal meat are provided in Tables 4–6. Curing of the meat with 50, 150 and 500 ppm sodium nitrite resulted in a decrease in the

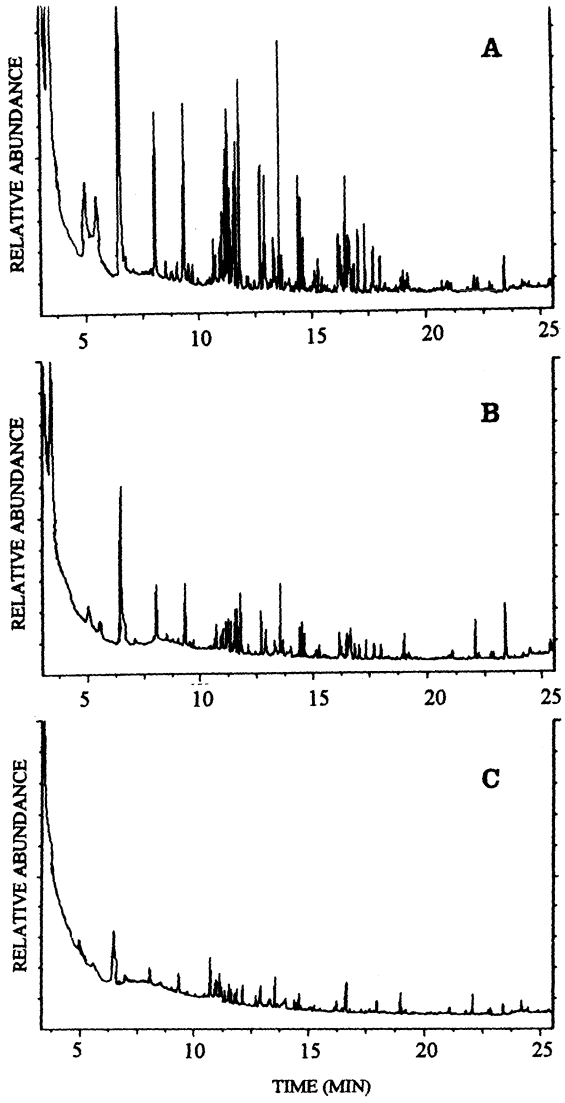

Figure 2. Total ion chromatograms of seal meat. A, uncured; B, cured with 50 ppm sodium nitrite; and C, cured with 150 ppm sodium nitrite.

number of volatiles from 86 to 72, 45 and 27, respectively. Of the 72 compounds presented in the volatiles of seal meat cured with 50 ppm sodium nitrite, there were 25 aldehydes, 7 ketones, 9 alcohols, 10 hydrocarbons and 7 miscellaneous compounds. Corresponding numbers for seal meat cured with 150 and 500 ppm sodium nitrite were 17 and 15 aldehydes, 5 and 1 ketones, 8 and 5 alcohols, 2 and 1 hydrocarbons and 6 and 2 others, respectively.

Aldehydes (Table 4) were the main flavor volatile constituents of uncured and even cured seal meat. In the uncured seal meat, (E,E)-2,4-hexadienal, hexanal, 2-hexenal and heptanal were detected in high amounts; propanal, a major breakdown product of omega-3 fatty acids was not separated/detected under the experimental conditions employed.

The compound (E,Z)-2,6-nonadienal, a breakdown product of omega-3 polyunsaturated fatty acids (PUFA) was present in the volatiles of seal meat. This compound might

Table 4. Carbonyl compounds in aroma concentrates of uncured and nitrite-cured seal meat[a]

Component	Uncured	Cured with nitrite, ppm		
		50	150	500
Aldehydes				
2,4-hexadienal, (E,E)-	16.85	9.82	9.25	—
2-butenal, 3-methyl-	0.19	—	—	—
2-pentenal, (E)-	0.56	—	—	—
Hexanal	6.11	1.53	0.27	0.36
4-pentenal, 2-methyl-	0.03	0.01	—	—
2-hexenal	1.23	0.57	0.11	0.55
4-heptenal, (Z)-	0.20	0.12	0.03	—
Heptanal	1.14	0.64	0.13	0.16
2-heptenal, (Z)-	0.22	0.09	—	—
Benzaldehyde	0.18	0.18	0.22	0.20
Octanal	0.63	0.29	0.08	—
2,4-heptadienal, (E,E)-	0.89	0.42	0.06	—
4-pentenal, 2-ethyl-	0.08	0.08	0.10	0.08
2-octenal, (E)-	0.77	0.33	0.06	0.04
2,4-octadienal	0.22	0.16	0.04	0.22
2,6-nonadienal, (E,Z)-	0.54	0.22	0.04	0.11
2-nonenal, (Z)-	0.56	0.28	0.06	0.08
Benzaldehyde, ethyl-	0.24	0.17	0.09	0.08
Decanal	0.19	0.14	0.05	0.03
Ketones				
3-pentanone	3.77	4.94	—	—
2,3-pentanedione	2.24	3.14	—	—
2-heptanone	0.17	0.05	0.02	—
4-hepten-3-one, 5-methyl, (E)-	0.43	0.14	0.13	—
2,4-octanedione	0.07	—	—	—
2-nonanone	0.27	0.11	0.06	—
3,5-octadien-2-one, (E,E)-	0.24	—	—	—
2-octanone	0.05	0.01	0.01	—
3-hexene-2-one, 3,4-dimethyl, (Z)-	0.24	0.01	—	—
5,9-undecadiene-2-one, 6,10-dimethyl, (Z)-	0.04	—	—	—
2-acetonylcyclo-hexanone	0.02	—	—	—
3-octene-2-one	—	—	0.03	0.01

[a]All samples were cured with specified levels of sodium nitrite along with 550 ppm sodium ascorbate and 1.5% sodium chloride. Concentrations of volatiles are in ppm.

have derived via enzymatic breakdown of omega-3 PUFA and is reported to possess a cucumber-like aroma (Josephson and Lindsay, 1987). Further degradation of this compound through retro-aldol condensation in the aqueous environment would lead to the formation of (Z)-4-heptenal (Josephson and Lindsay, 1987). This latter compared was also present in the volatiles of samples examined. The compound 2,4-decadienal, derived from autoxidation of omega-6 fatty acids was also present and may have a profound effect on the flavor of foods through involvement in the formation of Maillard reaction products or via degradation to a variety of secondary products, including 2-octenal and hexanal (Chang *et al.*, 1994; Yu *et al.*, 1994). Another ubiquotous compound present in the volatiles of both uncured and cured seal meats was benzaldehyde which has also been reported to be present in the crayfish tail meat and other seafoods.

Further work, using aroma dilution technique (gas chromatography-olfactometry), is necessary in order to unravel the character-impact flavor volatiles of the raw, cooked and

Table 5. Alcohols and hydrocarbons in aroma concentrates of uncured and nitrite-cured seal meat[a]

Component	Uncured	Cured with nitrite at, ppm		
		50	150	500
Alcohols				
1-penten-3-ol	6.32	8.30	—	—
1-hexanol	0.11	0.07	—	—
Phenol, 3-ethyl-	0.12	0.12	0.02	—
1-heptanol	0.30	0.13	0.03	0.36
7-octen-4-ol	0.78	0.24	0.15	—
1-octanol	—	—	0.03	—
2-nonen-1-ol	1.09	0.77	0.16	0.33
3,6-nonadien-1-ol, (E,Z)-	0.17	0.13	0.03	—
2-decyn-1-ol	0.42	0.22	0.05	0.02
2-octylcyclopropene-1-heptanol	0.05	—	—	—
1-octanol, 2-butyl-	0.13	—	0.15	0.50
4-morpholineethanol	0.03	0.04	—	0.24
Hydrocarbons				
1-hexyne, 5-methyl	0.05	3.58	—	—
1,2-hexadiene, 5-methyl-	0.05	2.85	—	—
2-hexyne, 4-methyl-	0.01	0.10	—	—
1,4-hexadiene, 3-ethyl-	0.20	0.07	—	—
Cyclohexene, butenyl-	0.79	0.23	0.12	0.14
1,3-hexadiene, 3-ethyl-2-methyl-	0.10	0.01	—	—
3-tetradecen-5-yne, (Z)-	0.63	0.27	—	—
3-undecen-5-yne, (Z)-	0.11	—	—	—
3-hexadecyne	0.02	—	—	—
4-undecyne	0.02	—	—	—
1-nonene, 4, 6, 8-trimethyl-	0.02	—	—	—
1-undecyne	0.02	—	—	—
Bicyclo [2.2.1] heptane, 2,2-dimethyl-3-methylene	0.01	—	—	—
9-eicosyne	0.25	0.11	—	—

[a]All samples were cured with specified levels of sodium nitrite along with 550 ppm sodium ascorbate and 1.5% sodium chloride. Concentrations of volatiles are in ppm.

Table 6. Miscellaneous compounds in aroma concentrates of uncured and nitrite-cured seal meat[a]

Component	Uncured	Cured with nitrite at, ppm		
		50	150	500
Hydroperoxide, heptyl-	1.12	0.26	—	—
Furan, 2-pentyl-	0.62	0.26	0.07	—
Benzene, 1,3-dichloro-	0.15	0.04	0.09	0.16
7-oxabicyclo [4.1.0] heptane, 3-oxiranyl-	1.02	0.12	0.08	—
Hexanenitrile	—	—	0.01	—
Fomic acid, heptyl ester	0.06	0.04	0.03	0.06
Ethanone, 1-(3,3-dimethyl bicyclo [2.2.1] hept-2-yl)-endo-	0.02	—	—	—
Benzene, (1-cyclopropyl-1-methylethyl)-	0.10	0.11	0.03	—
1,2-dipentylcyclopropene-3-carboxylic acid	0.09	0.07	—	—
1H-3a, 7-methaneazulene, octahydro-1,4,9,9-tetramethyl-	0.06	—	—	—
1,2-benzenedicarboxylic acid, butyl, 2-methylpropyl ester	0.06	—	—	—

[a]All samples were cured with specified levels of sodium nitrite along with 550 ppm sodium ascorbate and 1.5% sodium chloride. Concentrations of volatiles are in ppm.

cured seal meats (Shahidi and Cadwallader, 1998). Results of these studies would hopefully have a positive effect on better understanding of factors involved in process-induced aroma generation in seal meat and other marine foods.

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CHARACTERIZATION OF VOLATILE ALDEHYDES AND PYRAZINES IN PAN-FRIED ZOUSOON

Tzou-Chi Huang,[1] Yea-Chyi Chiou,[1] and Chi-Tang Ho[2]

[1]Department of Food Science and Technology
National Pingtung University of Science and Technology
912, Pingtung, Taiwan
[2]Department of Food Science
Rutgers University
65 Dudley Road New Brunswick, New Jersey 08901-8520

Volatile aldehydes and pyrazines were characterized from freshly prepared and aged, pan-fried zousoon. The effect of fluorescent light exposure on the oxidative stability of lipids in packaged zousoon was investigated. The aroma quality of zousoon was evaluated by both sensory and electronic aroma sensing techniques, while lipid oxidation products in stored zousoon were assessed using peroxide value and volatile aldehyde analysis. Results of this study indicate that oxidation of lipids occurred in zousoon after prolonged exposure to fluorescent light. In addition to the lipid derived aldehydes, substantial amounts of Strecker aldehydes and pyrazines were formed during the pan-frying processing.

INTRODUCTION

Short chain fried pork bundle (zousoon), a popular pork product in Taiwan and China, is produced by roasting threaded pork ham muscle using pan-frying. Zousoon is produced in a variety of textures and flavors. Various packaging materials such as tin can, glass and plastic bottles have been used for zousoon and commercially packed products are sold in grocery aisles of retail food stores and supermarkets.

Despite its importance to consumer acceptability, very little information is currently available regarding the flavor of zousoon. Huang *et al.* (1989) utilized a headspace GC-MS technique to analyze the volatile compounds in zousoon; alkylpyrazines were found to contribute to its unique and pleasant aroma. Lin *et al.* (1981) and Chang *et al.* (1996) have reviewed the processing and palatability traits of fried pork bundles.

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Table 1. Crude fat, peroxide value (POV) and aroma intensity of the illuminated and unilluminated zousoon

Samples no.	Illumination (%)	Crude fat (mg/Kg)	POV	Aroma intensity	
				Rancid	Roasty
1	without	18.1	1.1	41.2	68.4
2	with	18.8	4.6	69.5	61.8
3	with	18.6	4.8	62.6	58.8
4	without	18.9	0.7	39.6	71.4
5	with	18.2	4.3	72.7	63.8
6	without	19.2	0.8	36.8	69.5
7	with	18.5	4.3	76.9	65.3
8	without	18.6	0.6	32.8	73.5
9	control	18.5	0.2	23.8	81.4

Sample No. 9 (control) is the canned freshly pan-fried zousoon stored in a refrigerator under – 4° C for 1 year.

It is well established that light exposure accelerates the deterioration of oil or fat-containing foods (Chahine and deMan, 1971). Rancid flavor is so common that many Oriental consumers tolerate and accept it in food products. However, increased awareness of the adverse biological effects of some oxidation products has caused concern over the possible formation and presence of these biologically active compounds in fat-based foods. Approximate composition of fried zousoon showed that significant amounts of lard (ranging from 18.1% to 19.2% by weight) is used in manufacturing pan-fried zousoon as shown in Table 1. The objective of this study was to investigate the effects of light on lipid stability in the illuminated zousoon in an attempt to simulate supermarket storage conditions.

EXPERIMENTAL PROCEDURES

Model systems composed of previgor (<3 h post mortem) ham (1000 g), sucrose (100 g), salt (15 g), lard (120 g) and bean powder (200 g) were prepared to study the effect of fluorescence light illumination on the flavor stability of Chinese fried short pork bundle. The mixes were pan-fried following the method described by Huang *et al.* (1989) and a product with a final moisture content of about 5% was obtained. Finished fried zousoon was placed in either tin cans (250 g/can x 2 cans) or glass bottles (250 g/bottle x 2 bottles). The experiment was duplicated. All of the packed zousoon samples were stored in an incubator at 25°C for 1 year with a fluorescent light suspended approximately 30 cm above the samples. The light intensity was approximately 1500 lux (Luna Pro, Gossen, Germany).

SENSORY ANALYSIS OF ZOUSOON

An eight-member panel consisting of students and staff was trained to become familiar with the products to evaluate the rancid and roasty aroma of zousoon. Panelists scored the attributes using a 150-mm line scale, which was anchored at 10, 42.5, 75, 107.5 and 140 mm corresponding to intensities of none, slight, moderate, strong and extremely strong, respectively. Panelists evaluated rancid aroma and roasty aroma, respectively, by three short sniffing from a slightly opened sample cup containing 20 g zousoon. Only

slight differences were detected in sensory scores for the roasty aroma attribute between the illuminated and unilluminated zousoon samples (Table 1). The rancid notes for the illuminated and unilluminated zousoon were scored less than slight (42.5 mm) and moderate (75 mm), respectively, as shown in Table 1.

A possible explanation is that increases in lard content may lead to an increase in the formation of volatile aldehydes. Substantial amounts of these short-chain volatile aldehydes may mask the sensational, roasty aroma of pan-fried zousoon. Guadagni *et al.* (1963) noticed that the C6-C10 aldehydes having low odor thresholds (0.1–5 μg kg^{-1} in water) could be expected to play some role in the overall aroma of the cooked meats. Autooxidation occurred in the pan-fried samples causing a significant increase in peroxide values and the rate of oxidation was accelerated in the illuminated samples (Table 1). These data were consistent with the findings of Armstrong (1967) who reported that a peroxide value of 1.0 is a critical point at which oxidized flavor is easily recognized. Hydroperoxides are the major initiation products of autocatalytic oxidation of unsaturated lipids (Shahidi *et al.*, 1986). Further decomposition of hydroperoxides may lead to the formation of volatile aldehydes, which are the major class of compounds responsible for flavor reversion of lipids.

ELECTRONIC AROMA SENSING ANALYSIS OF ZOUSOON

An electronic aroma measuring system with sensors responding specifically to individual flavor notes was recently developed and applied to cork stopper quality control (Rocha *et al.*, 1998). An AromaScan A32S/8S Labstation System (AromaScan plc, Crewe, UK) with system software including data mapping tools and an artificial neutral network (ANN) was used to investigate the aroma qualities of the illuminated and unilluminated zousoon. Five grams of zousoon representing different storage conditions were sealed into a polyester/polyet+hylene bilayer pouch, which was filled with charcoal and molecular sieve filtered air to approximately 35% relative humidity (RH). The samples were then analyzed on the A32S unit, using by the 32 sensors provided by the supplier. Typical histograms representing the odor fingerprint for the illuminated zousoon are shown in Figure 1A. Significant differences in response between the illuminated and unilluminated zousoon could be observed Figure 1B. The results obtained were based on the response of the complete sensor array. Among the 10 categories (amines, short chain alcohols, long-chain alcohols, carboxylic acids, aromatics, chlorinated hydrocarbons, short-chain esters, long-chain esters, ketones and water) could be detected by the 32 sensors. The sensors of 17, 18, 22, 23, 24, 25 and 28 having relative stronger response to the carbonyl compounds were chosen to improve the differentiation between slight rancid and moderate rancid odors. The selected sensors effectively increased the discrimination power between the two different odors as shown in Figure 2. The map demonstrated that the samples are clearly discriminated between the illuminated and unilluminated zousoon by this Electronic Aroma Sensing system. This map (Figure 2B) can be split into two distinctive sections corresponding to slight rancid and moderate rancid odors.

ANALYSIS OF VOLATILE ALDEHYDES IN ZOUSOON

A considerable amount of Strecker and lipid-derived aldehydes were characterized by the modified cystamine derivatization method (Huang *et al.*, 1998). Quantitative analy-

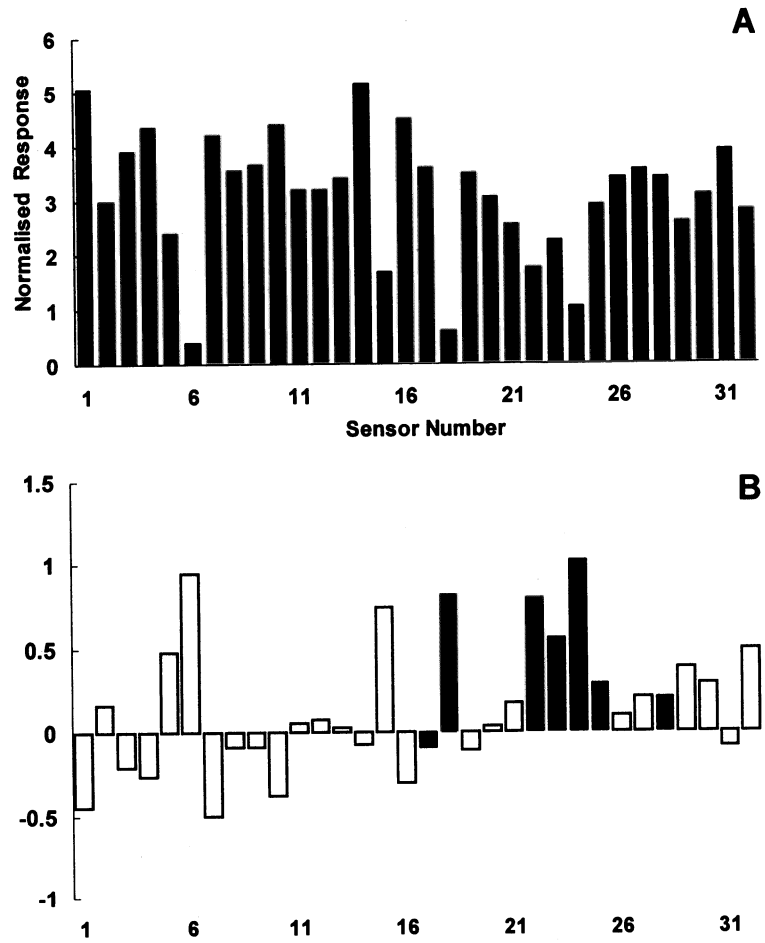

Figure 1. Histogram representing the fingerprint for (A): Zousoon stored at 25°C with fluorescent light illumination (B): variation in response between the illuminated and unilluminated zousoon.

ses of volatile aldehydes from the freshly roasted (canned and stored under – 4°C for 1 year) and the illuminated zousoons are listed in Table 2. Substantial amount of formaldehyde (7.28 ppm), acetaldehyde (5.72 ppm), 2-methylpropanal (7.74 ppm), 3-methylbutanal (20.66 ppm) and 2-methylbutanal (17.62 ppm) formed in zousoon during the pan-frying processing. Strecker aldehydes, formaldehyde (mouse-urine, ester-like), acetaldehyde (sharp, penetrating, fruity), 2-methylpropanal (penetrating, green), 3-methylbutanal (malty, green) and 2-methylbutanal (ethereal, bitter, almond, green) may derive from glycine, alanine, valine, leucine and isoleucine, respectively (Ho, 1996).

In addition to the Strecker aldehydes, volatile aldehyde analyses indicated that lipid in zousoon underwent slight oxidative changes during exposure to fluorescent light. The volatiles from the illuminated zousoons were dominated by acetone followed by the short chain aldehydes, formaldehyde, acetaldehyde, butanal, pentanal and hexanal. Noticeably, 1.6-, 3.5-, 4.7-, 3.5- and 3.4-fold increases in formaldehyde, acetaldehyde, butanal, pentanal and hexanal, respectively, were observed in the illuminated samples as compared to those without illumination. Significant amounts of butanal, hexanal, heptanal and nonanal

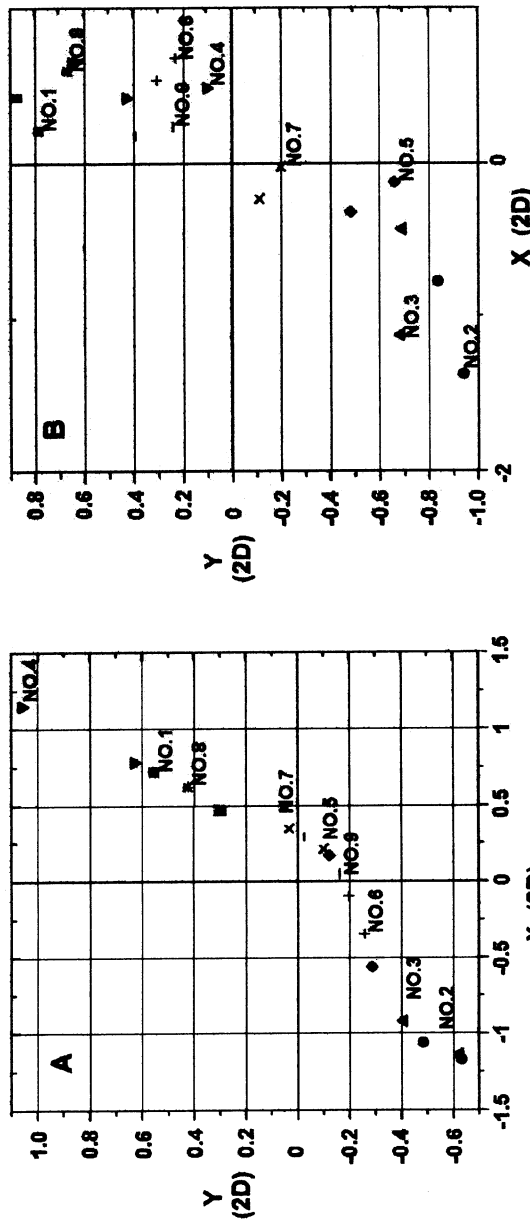

Figure 2. Map of control (9), illuminated and unilluminated zousoons according to (A): 32 sensors (B): 7 sensors.

Table 2. Carbonyls in pan-fried zousoon stored for one year

Carbonyls	Concentration (ppm)		
	Control[a]	Unilluminated	Illuminated
Formaldehyde	7.28	13.81	22.16
Acetaldehyde	5.72	17.71	62.14
Acetone	36.20	51.38	121.24
Propanal	0.64	2.67	2.07
2-methylpropanal	7.74	9.16	8.26
Butanal	0.80	4.97	23.52
2-methylbutanal	20.66	21.48	22.83
3-methylbutanal	17.62	19.24	23.69
Pentanal	trace	0.50	1.74
Hexanal	1.48	7.61	25.88

[a]Control is the canned freshly pan-fried zousoon stored in a refrigerator under -4° C for 1 year.

have been characterized from heated pork fat (Yasuhara and Shibamoto, 1989). They proposed that these aldehydes were thermally derived from the corresponding fatty acids, e.g. palmitoleic, linolenic, linoleic, and oleic acids found in lard. A series of straight-chain aldehydes (n-C_1 to n-C_9) has also been found in the headspace of cooked pork (Mottram *et al.*, 1982). The authors attributed the formation of hexanal to the oxidation of esterified or free linoleic acid. Forss (1972) reported that aldehydes and alcohols were the major contributors to the volatiles from cooked pork and postulated that most of the headspace volatiles in the meat system were of lipid origin. Fatty acid composition analyses revealed that the lard in freshly pan-fried zousoon contained oleic acid (43.5%) followed by palmitic acid (23.2%), myristic acid (7.3%), palmitoleic acid (4.8%), and linoleic acid (13.5%), respectively. The amount of various aldehydes recovered from the pan-fried zousoon may correspond to the amount of possible fatty precursors in pork fat.

PYRAZINE FORMATION IN ZOUSOON

Volatile compounds are frequently of varied origin or may arise from secondary reaction between substances stemming from different catabolic pathways. In addition to the aldehydes mentioned above, other compounds including pyrazines, pyridines and thiazoles may appear as well. Among them, pyrazines deserve special attention. Pyrazines result from Maillard reaction involving components stemming from the catabolism of lipids, carbohydrates and proteins. Carbohydrate degradation produces compounds such as glycoeal dehyde, pyruvaldehyde, 3-hydroxy-2-butanone, 2,3-butanedione and other four-carbon carbonyl compounds (Weenen, et al, 1994). Dicarbonyl compounds can initiate Strecker degradation of amino acids to yield α-amino ketones, which in turn can undergo condensation and subsequent oxidation to form substituted pyrazines (Buckholz, 1989). A selective purge-and-trap method (Kuo *et al.*, 1989) was used to collect the basic fraction in Zousoon. A powered sample (100 g) was soaked in water in a two-arm round bottom flask and volatile compounds were purged with nitrogen and trapped in an Erlenmeyer flask containing a 1 N HCl solution. After titration to pH 13, the basic fraction was extracted with dichloromethane. The basic fraction of the volatiles characterized in the freshly fried pork bundle contained at least 16 alkylpyrazines as listed in Table 3. Among these, methylpyrazine (nutty, roasted), 2,5-dimethylpyrazine (grilled chicken, roasted pea-

Table 3. Alkylpyrazines in seasoned and pan-fried zousoon

No.	Compound	Concentration (ppm)	
		Unilluminated	Illuminated
1	pyrazine	0.3	0.4
2	methylpyrazine	7.3	7.2
3	2,5-dimethylpyrazine	10.4	10.1
4	2,6-dimethylpyrazine	0.7	6.2
5	2,3-dimethylpyrazine	4.1	4.3
6	vinylpyrazine	1.2	1.1
7	5-methyl-2-ethylpyrazine	0.8	0.6
8	6-methyl-2-ethylpyrazine	1.3	4.9
9	3-methyl-2-ethylpyrazine	8.1	7.6
10	propylpyrazine	2.5	2.1
11	2-methyl-vinylpyrazine	0.5	0.4
12	3,6-dimethyl-2-ethylpyrazine	12.5	11.9
13	3,5-dimethyl-2-ethylpyrazine	2.9	2.6
14	tetramethylpyrazine	1.6	1.4
15	5-methyl-2,6-diethylpyrazine	1.2	0.9
16	3-methyl-2,6-diethylpyrazine	0.5	0.6

nut), 2,6-dimethylpyrazine (ether-like), trimethylpyrazine (nutty, roasted) and 2-ethyl-6-methylpyrazine (grassy) were predominant. Propylpyrazine which is also present may be formed from the interaction product of lipids, proteins and carbohydrates following a similar mechanism proposed by Chiu *et al.*, (1990). The combination of these alkylpyrazines may lead to the formation of the characteristic fried meaty aroma of Chinese fried pork bundle. Pyrazine concentration of both illuminated and unilluminated zousoon did not differ significantly even after one year of storage. Pyrazines were found to be stable in the sealed containers. No significant variation of pyrazine composition was observed in the comparison between samples with and without fluorescent light irradiation.

CONCLUSION

Our results reveals that only minor lipid oxidation occurred in the pan-fried zousoons stored under the experimental conditions in this study. It is well known that the moisture which forms an effective barrier between atmospheric oxygen and lipid constituents of the food may retard lipid oxidation, and has a beneficial effect on the flavor of stored food products. The water activities of the pan-fried and stored zousoons were found to be in the range of 0.3 to 0.5. Despite the slight differences in rancidity sensory scores, illuminated and unilluminated zousoon can be differentiated by electronic aroma sensing techniques. Only minor differences were observed in the zousoon's roasty sensory scores and the pyrazines contributing to the aroma. This study indicates that the typically used packaging materials provide satisfactory protection for zousoon.

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CHANGES IN FLAVOR-RELATED COMPOUNDS IN MEAT TREATED WITH ORGANIC ACIDS

Héctor Escalona,[1] Sharon K. Ogden,[2] Isabel Guerrero,[1] and Andrew J. Taylor[2]

[1]Departamento de Biotecnología
Universidad Autónoma Metropolitana-Iztapalapa
Apartado Postal 55-535, C.P. 09340 Mexico D.F., Mexico
[2]Department of Applied Biochemistry and Food Science
University of Nottingham, Sutton Bonington Campus
Leics. LE12 5RD, United Kingdom

Meat preservation in areas with no refrigeration facilities or in conditions of temperature abuse is affected by the surface microbial load. The use of food-grade organic acids to reduce the number of microorganisms on meat surface is well documented. However, addition of these acids may modify meat flavor. In order to study the effects on volatiles present in the headspace and alteration in sensory characteristics, minced pork was treated with lactic and propionic acids and stored at 4 and 19°C for 48 h. Volatiles were collected on Tenax traps and analyzed using a bench top mass spectrometer connected to a gas chromatograph. Of the fourteen most abundant headspace volatiles identified, most had originated from lipid oxidation. Sensory characteristics were analyzed in pork treated with lactic acid and subjected to a controlled surface fermentation with *Staphyloccocus carnosus* and *Lactobacillus pentosus*. Rancidity was not detected in acid-treated and fermented meat, however, aextension of shelf life at 19°C by fermentation with *L. pentosus* caused alteration in sensory characteristics of fabricated meat products, but this did not happen in meat stored at 4°C. Lactic acid caused production of acid flavor and odor.

INTRODUCTION

A healthy animal has natural defenses against microbial invasion, therefore it is considered that meat of freshly slaughtered animals is sterile. Microbial contamination occurs firstly on the meat surface, due to contact of the carcass with walls, floors, slaughtering equipment or handling. Although this contamination is mainly superficial, a number of microorganisms find their way to the inner part of the muscle during evisceration and cleaning operations. Meat is frequently exposed to conditions that increase growth of pathogens and spoilage microorganisms. Among these are lack of suitable temperature, ambient con-

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ditions of high humidity and temperatures above 15°C, lack of suitable transportation and failure in the refrigeration system. In order to reduce surface microbial population, some inexpensive methods have been designed, most of them based on spraying chemicals on the carcass surface (Anderson *et al.*, 1977; Leistner, 1983; Snijders *et al.*, 1985; Ogden *et al.*, 1995). Controlled lactic fermentation on the meat surface has also been tested (Guerrero *et al.*, 1995). In order to apply these methods in developing areas, they should be inexpensive, simple, long lasting and with minimal effects on physicochemical, chemical and sensory characteristics of meat.

AROMA PRODUCED BY CONTAMINANT MICROFLORA

Due to the variety of chemical components present in the meat, microorganisms of a wide phylogenetic variety can be present (Nychas *et al.*, 1988). In extending meat shelf-life it is necessary to consider that the meat is a suitable substrate for the growth of pathogens and spoilage microorganisms. Their nutritional requirements are well known according to their ability to deplete the chemical components of the meat. Therefore it is possible to predict the compounds produced as a results of their metabolic activity, some of them are related to odor and flavor. (Gill, 1986; MacMeekin, 1982; Greer, 1989; Dainty and Hibbard, 1983; Dainty *et al.*, 1989; Pitchard and Coolbear, 1993).

There is a reproducible sequence of chemical events in the meat which results in different aromas. It starts with the presence of acetoin, diacetyl and 3-methyl-1-butanol, due to the metabolism of *B. thermosphacta* (Dainty and Hibbard, 1980). Later some sulfur odors are produced due to ethyl esters and short-chain fatty acids generated by *Pseudomonas* spp. Finally, when *Pseudomonas* population reaches 10^9 cfu/g sulfur odors are prevalent (Greer, 1989).

AROMA PRODUCED BY CHEMICAL REACTIONS OF MEAT COMPONENTS

Chemical reactions involved in formation of meat flavor and aroma are due to degradation of individual components such as amino acids, carbohydrates, lipid oxidation and interactions between these compounds. Degradation of saccharides and amino acids involves heat treatment. In this case, saccharides produce furanones and furfurals, but amino acids remain stable unless direct heat is applied. In such a case, pyrolysis caused in localized areas (Baines and Mlotkiewics, 1984).

Degradation of thiamine, however, can occur at a lower temperature, producing compounds related to meat aroma, such as thiophenes, thiazols, furanes, and other heterocyclic compounds. Reactions between amino acids and peptides cause changes due to Maillard reaction and produce up to 100 volatiles. The resulting compounds, related to aroma, were aldehydes, ketones, furans, pyrrols, pyrazines, pyrimidins, sulfur-containing amino acids, thiophenes, thiazoles and thiols. Of special interest is the reaction of cysteine and reducing sugars, which produce a typical meaty aroma. Around 68 meat-related compounds have been detected in this reaction (Farmer, 1992).

Lipids contribute to meat aroma in two way: as solvents to other aroma-related compounds originated in animal feeds, metabolism or external contamination or as a source of varied oxidation reactions. Lipid oxidation gives a wide range of aliphatic compounds such as saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, acids, es-

ters, as well as cyclic compounds such as furans, lactones and cyclic ketones, many of these compounds with an intense aroma (Gray and Crackel, 1992).

AROMA PRODUCED BY THE APPLICATION OF PRESERVATION METHODS

Methods to extend of shelf-life meat include from heating and freezing, application of chemicals to reduce or inhibit enzymatic and microbial spoilage. Treatments with organic acids from chemical or microbial origin besides extending shelf-life of raw meat, but they also promote changes in aroma of meat and meat products fabricated from treated carcasses or cuts (Guerrero and Taylor, 1994).

Treatments to reduce surface contamination include acids, water spray at high pressure, chlorine solutions and even antibiotics. However, the most efficient treatments are those involving organic acids. The inhibitory effect is related to the amount of undissocited acid. It diffuses into the cell and dissociated once inside. This promotes acidification of the cytoplasm and disruption of metabolic pathways (Baird-Parker, 1980).

An alternative method to apply organic acids to meat surface is producing it *in situ*, especially lactic acid by a controlled fermentation using lactic acid bacteria (LAB) which are the dominant microflora in meat stored under reduced oxygen supply or anaerobiosis (Guerrero *et al.*, 1995). The use of *Lactobacillus* and *Pediococcus* as a means to extend meat shelf-life is a very old method, and is achieved by inoculating selected strains in minced meat. In the case of a controlled surface fermentation, the same mechanisms of inhibiting the growth of undesirable microorganisms are applied: production of bacteriostatic compounds such as lactic acid, peroxides and bacteriocines, and successful substrate competition of LAB against pathogens and spoilage microorganisms.

Since volatile compounds are mainly responsible for meat aroma, any factor affecting their production also affects flavor and odor. Therefore, meat subjected to lactic acid fermentation shows production of aroma-related compounds. Triacylglycerol degradation has been studied with respect to degradation of free fatty acids, di- or monoacylglycerols during meat ripening, as well as the increase the content of carbonyl oxidation products such as aldehydes and ketones. Carbonyls have an important effect on meat aroma due to their low detection thresholds. As fatty acids are carbonyl precursors, lipolytic activity of microorganisms present in meat, either as contaminants or intentionally added is very important in fermented meats. However, carbonyls may also originate from free fatty acids or nondepleated triacylglycerols (Ogden *et al.*, 1996).

Since LAB cannot synthesize most of the amino acids necessary for their metabolism, the presence of proteolytic systems in protein-rich substrates, such as meat, is fundamental. These enzymatic systems also produce the typical meaty aroma. Montel and coworkers (Montel *et al.*, 1993) reported products of lipolysis in sausages inoculated with *Staphylococcus carnosus, S. saprophytocus* and *S. warnerii*. Lipolysis is related to release of free fatty acids but also with presence of breakdown products such as alkanes, aldehydes, alcohols and furfurals. Stahnke (Stahnke, 1996), inoculating *S. xylosus*, a highly lipolytic LAB in a model meat system, detected 11 aldehydes, 8 alcohols, 4 sulfides, 9 ketones, 9 acids and 5 nitriles in the headspace of minced meat. Berdague and coworkers (Berdague *et al.*, 1993) reported 86 volatiles in fermented sausages of which 78 where identified as 15 alkanes or alkenes, 11 aldehydes, 11 ketones, 10 alcohols, 7 aromatic hydrocarbons, 5 carboxylic acids, 4 chlorine-containing compounds, 3 furans, 3 S-compounds, 1 pyrazine, 1 amine and 1 terpene.

EFFECT OF THE USE OF ACETIC AND PROPIONIC ACIDS, AND LACTIC FERMENTATION IN PORK

Treatments with lactic acid in concentrations up to 2% have been reported to increase meat shelf-life without considerably affecting its physicochemical characteristics. Although sensory evaluation has been carried out in acid-preserved meat, no studies have been done with respect to the production of flavor-related compounds during its storage.

The objective of the present work was to study some of the major volatile compounds related to the flavor of the meat treated with lactic and propionic acids, and the possible changes in sensory characteristics of acid-treated and fermented meat.

Acid-Treated Pork

Batches of 300 g of minced pork (*Longissimus dorsi* muscle) were placed in impermeable plastic bags where 1, 2 and 3% of propionic and lactic acid was added. Completely randomized samples were allocated to a 2×3×5 complete factorial arrangement with three replicates, including the following factors and levels: acid (propionic or lactic), acid concentration (1, 2 and 3%) and storage time (1, 3, 5, 7, 11 days). Volatile compounds analyzed were: 2-methylfuran, pentanone, 2-ethylfuran, dimethyl disulfide, toluene, pentanal, hexanal, ethylbenzene, 3-heptanone, 1-octen-3-ol, 2-pentylfuran, nonanal, 3-pentanone, octanal and 2-heptenal. They were removed from the headspace with a stream of dry nitrogen and collected on a Tenax trap, thermally desorbed and identified using a VG MD800 bench top mass spectrometer (Fisons Scientific, Manchester, U.K.) connected to a Hewlett Packard 5890 Series II gas chromatographer fitted with a headspace injector (Unijector, SGE). The meat volatiles were desorbed from the traps at 240°C for 2 min. During desorption, a 400 mm region of the column was cooled in liquid nitrogen to cryotrap the volatiles. Following desorption, the sample was chromatographed from 30 to 240°C. The key volatiles from the meat were identified and quantified on the basis of their retention times and characteristic ions, using a reference library. The data were analyzed using a SAS package adapted to a personal computer.

Table 1. Volatiles in headspace (analysis of variance)

	Source of variation (P>)		
Response variable	Acid	%	Time
2-methylfuran	0.059	0.006	0.031
Pentanone	0.721	0.410	0.192
2-ethylfuran	0.0001	0.0001	0.001
Dimethyl disulfide	0.519	0.0001	0.002
Toluene	0.787	0.142	0.034
Pentanal	0.165	0.0001	0.0001
Hexanal	0.0001	0.0001	0.0001
Ethyl benzene	0.169	0.0001	0.0001
3-heptanone	0.0001	0.0001	0.0001
2-pentylfuran	0.471	0.0001	0.053
Nonanal	0.041	0.709	0.153
3-pentanone	0.111	0.011	0.004
Octanal	0.884	0.149	0.285
2-heptenal	0.946	0.615	0.021

Production of 2-ethylfuran, hexanal and 3-heptanone was significantly higher in propionic than lactic acid-treated meat (Table 1). Acid percentage promoted significantly higher amounts of 2-ethylfuran, dimethyl disulfide, pentanal, hexanal, ethylbenzene, 3-hepatnone and 2-pentylfuran ($P>0.001$). With the exception of 2-pentylfuran, concentration of these volatiles also increased with storage time, as well as 3-pentanone. 2-Metylfuran is one of the main compounds responsible for meaty aroma, whereas pentanal and hexanal were promoted by lipid oxidation.

Aldehydes were also responsible for loss of desirable flavors due to their fast formation during lipid oxidation and low detection threshold. Formation of saturated aldehydes (hexanal and nonanal) was fast and abundant. Acid treatment, particularly lactic acid, apparently induced lipid oxidation throughout the storage period. However, hexanal concentration was lower in the control. Contribution of alcohols to off flavors was less marked, probably due to their high detection thresholds. Contribution of ketones to off-favors was apparently less than that from alcohols, since 2-heptanone was not present in high concentrations until after 9 days. Carbonyls had an important effect on flavor due to their low odor threshold, as compared to hydrocarbons, furans and alcohols.

Fermented Pork

After screening several lactic acid starters, two strains were kindly supplied by Ms. Lone Andersen, Chr. Hansen's, Denmark: *Staphylococcus carnosus* MC-1 and *Lactobacillus pentosus* LP-1. The criterion for selecting a strain was mainly the reduction of undesirable microflora by acidifying the medium. Lack of refrigeration conditions, where meat is normally stored in subrural areas in Central Mexico, were simulated by storing the meat at 19°C, whereas samples stored at 4°C were used as a means of comparison. From preliminary trials, it was shown that lactic acid was the most efficient organic acid in reducing microbial populations, therefore treatments with this acid were included in the experimental design.

The treatments were:1) Fermentation with *Staphylococcus carnosus* at 4°C; 2) Fermentation with *Lactobacillus pentosus*, stored at 4°C; 3) Treatment with lactic acid, stored at 4°C; 4) Fermentation with *Staphylococcus carnosus*, stored at 19°C; 5) Fermentation with *Lactobacillus pentosus* (SC), stored at 19°C; 6) Treatment with lactic acid (LA), stored at 19°C. Strains of *S. carnosus* and *L. pentosus* were inoculated in MRS broth and incubated at 35 and 30°C, respectively, until reaching a minimum cell suspension O.D.=1, at 590 nm. Pork loins were sectioned and and excess fat removed. Samples, 1 cm thick, were prepared aseptically and randomly allocated to treatments. The cell suspension was adjusted for each inoculum diluting with sterile water, and 5% glucose was added to each inoculum. Pork samples were immersed in this cell suspension during 2 min. A third batch of samples was immersed in 1.7% lactic acid for the same period. All samples were vacuum packaged and stored at 4 or 19°C for a total of 48 h.

Sensory Evaluation

It was carried out in fermented and lactic acid-treated meat. Control samples included meat purchased from local retail shops in Mexico City few hours before the sensory test, therefore considered in optimal conditions for the consumers' acceptance. Sensory characteristics were evaluated by R-index, i.e. defining the difference between treatments and a non-treated meat sample (Brown, 1994). This was performed by rating and ranking (O'Mahony, 1983). In addition, a descriptive flavor profile was obtained (Stone *et al.*, 1974).

Table 2. R-index for raw pork stored at 4°C

		Raw meat			Cooked meat		
Attribute	Statistics	Lactic acid	*S. carnosus*	*L. pentosus*	Lactic acid	*S. carnosus*	*L. pentosus*
Odor	mean	0.642	0.710	0.558	0.607	0.665	0.661
	P>|t|	0.028	0.0057	0.369	0.061	0.004	0.008
Juiciness	mean	0.718	0.886	0.639	0.330	0.520	0.569
	P>|t|	0.028	0.091	8.36×10^{-6}	0.007	0.827	0.357
Color	mean	0.973	0.962	0.878	0.666	0.638	0.783
	P>|t|	1.5×10^{-12}	3.1×10^{-11}	1.5×10^{-7}	0.002	0.010	1.6×10^{-5}
Flavor	mean				0.604	0.658	0.682
	P>|t|				0.281	0.079	0.028
Toughness	mean				0.492	0.432	0.508
	P>|t|				0.962	0.444	0.942

Besides differences in storage temperature, the samples were also evaluated as raw and cooked. Raw meat was cut into approximately 1 cm^3 pieces. The samples were placed in plastic containers in order to visually evaluate their attributes: odor, juiciness, and color. Samples of the same size were cooked in a microwave oven over a 4-min period at high power. They were presented in plastic containers and compared against a control for their attributes: odor, color, flavor and texture. Results for raw and cooked samples are shown in Table 2. R-index for odor of raw samples stored at 4°C was significantly different in lactic acid-treated samples as well as samples fermented with *S. carnosus* (P>0.028 and 0.0057, respectively). Conversely, treatments with *L. pentosus* did not alter odor in fermented meat as compared to a control. All three treatments had a pronounced effect for juiciness and color.

The panel detected significance differences in the odor of cooked samples treated with *S. carnosus* and *L. pentosus* (P>0.0042 and 0.0082, respectively). The only significance difference in flavor was detected in samples treated with *L. pentosus* (P>0.028). Toughness was not affected by any of the treatments, and juiciness only for the application of lactic acid (P>0.0068). Color was significantly different for samples subjected to the three treatments studied (P>0.002, 0.009 and 1.63×10^{-5}). When the three treatments were compared, only juiciness and odor had significant differences (P>0.035 and 0.032, respectively). *L. pentosus* produced the most juicy meat, and *S. carnosus* the best odor, as compared to a control.

Similarly, as with samples stored at 4°C, acid treatment in raw samples stored at 19°C as well as fermentation with *S. carnosus* had a significant difference in odor (P>0.028 and 0.005, respectively) and color (Table 3). Juciness was not different from the control in any of the treatments. Cooked samples were evaluated only for their odor and flavor. Differences in odor with respect to the control were detected in acid-treated samples and fermented with *L. pentosus* (P> 0.0002 and 0.0018, respectively), but not in samples fermented with *S. carnosus* (P>0.0593). However, flavor in all samples was significantly different from that of the control (P>0.009, 0.003 and 0.021).

Treatments with lactic acid bacteria and lactic acid, together with a control were presented to the panelists in 1 cm^3 pieces. The evaluation was carried out in cooked meat prepared in a similar manner as for R-index tests. A qualitative descriptive analysis (QDA) was carried out. In order to identify the generated attributes, the panel agreed in the attributes and characteristics shown in Table 4.

As *L. pentosus* had the least effect on sensory characteristics in raw and cooked meats, stored at two temperatures, treatments included in this analysis were: lactic acid-

Table 3. R-index for raw pork stored at 19°C: qualitative descriptive analysis

		Raw meat			Cooked meat		
Attribute	Statistics	Lactic acid	*S. carnosus*	*L. pentosus*	Lactic acid	*S. carnosus*	*L. pentosus*
Odor	mean	0.810	0.790	0.675	0.737	0.680	0.805
	P>|t|	0.025	0.005	0.162	0.0002	0.0593	0.0018
Juiciness	mean	0.471	0.810	0.699			
	P>|t|	0.623	0.061	0.111			
Color	mean	0.725	0.623	0.502			
	P>|t|	0.002	0.024	0.969			
Flavor	mean				0.646	0.727	0.756
	P>|t|				0.009	0.003	0.021

treated meat, fermentation with *L. pentosus* and untreated meat as control. Samples were stored at 4 and 19°C. Only cooked samples were evaluated.

In samples stored at 4°C before cooking, acid odor was noticeable in acid-treated samples (P>0.0085). Acid flavor and after taste were also detected in these samples. A chewy texture was also described by the panelists in acid-treated samples. Fermented samples were significant in rancid and refrigerated odor, but in both cases these attributes were less intense in fermented meat than in the control.

Fermented samples stored at 19°C prior cooking showed a significant difference only in acid flavor, they had also a significant difference in egg-like and acid odor, acid flavor, juicy and chewy texture and an acid after taste (Figure 2). Therefore, extension of shelf-life at 19°C by fermentation with *L. pentosus* cause alteration in sensory charac-

Table 4. Qualitative descriptive analysis: acid-treated and fermented samples stored at 4 and 19°C (P-values)

Attribute	Characteristics	Lactic acid	*L.pentosus*	Lactic acid	*L.pentosus*
Odor	fatty	0.112	0.936	0.941	0.161
	egg	0.170	0.006	0.169	0.729
	smoke	0.896	0.388	0.143	0.916
	roast meat	0.509	0.540	0.615	0.744
	acid	0.096	0.004	0.008	0.078
	rancid	0.489	0.022	0.047	0.022
	sweet	0.056	0.677	0.548	0.259
	refrigerated storage	0.154	0.334	0.055	0.011
Flavor	acid	0.024	0.005	0.001	0.496
	smoke	0.676	0.567	0.854	0.501
	refrigerated storage	0.938	0.661	0.131	0.819
	bready	0.233	0.146	0.137	0.354
Texture	juicy	0.396	0.0007	0.067	0.062
	hard	0.317	0.030	0.118	0.089
	fibrous	0.850	0.158	0.307	0.922
	dry	0.842	0.052	0.646	0.369
	cohesive	0.068	0.043	0.135	0.819
	chewy	0.121	0.007	0.021	0.676
After taste	durable	0.381	0.069	0.086	0.328
	acid	0.421	0.002	0.006	0.219
	roast meat	0.269	0.237	0.544	0.969

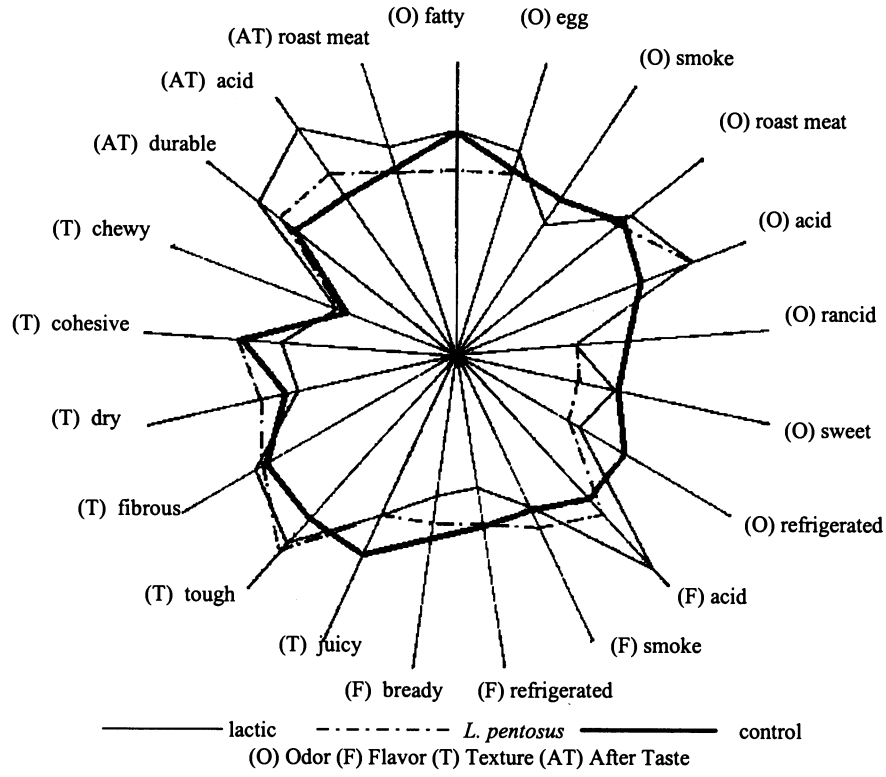

Figure 1. Qualitive descriptive analysis of cooked pork stored at 4°C for 48 h.

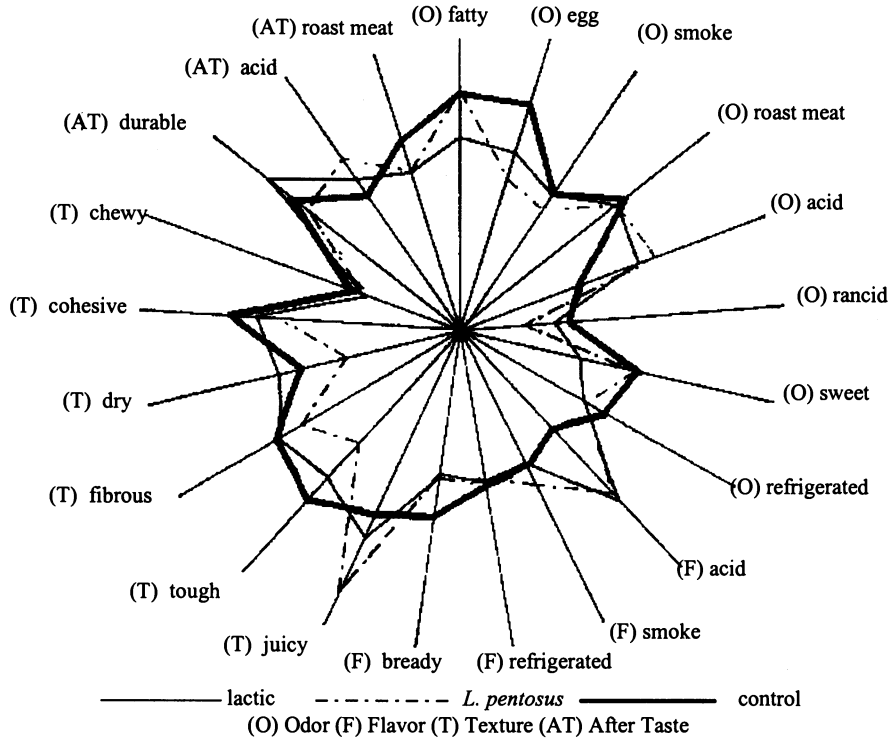

Figure 2. Qualitive descriptive analysis of cooked pork stored at 19°C for 48 h.

teristics of fabricated meat products, but this does not happen in meat stored at 4°C. *L. pentosus* has an optimum growth and metabolite production temperature of 30°C which explains the significant alteration of aroma in samples stored at 19°C as compared to samples 4°C. Lactic acid caused production of acid flavor and odor.

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FLAVOR DIFFERENCES DUE TO PROCESSING IN DRY-CURED AND OTHER HAM PRODUCTS USING CONDUCTING POLYMERS (ELECTRONIC NOSE)*

Arthur M. Spanier,[1] Monica Flores,[1,2] and Fidel Toldrá[2]

[1]USDA, ARS, BARC, LPSI, MSRL
10300 Baltimore Ave., Beltsville, Maryland 20705
[2]IATA (CSIC)
Box 73, 46100 Burjassot (Valencia), Spain

Dry-cured ham is popular in Spain and other European countries not only because of its flavor, but also because it is convenient, ready-to-use, and has a long shelf-life. The salt and nitrate used during the curing process play an important role in development and fixation of the characteristic cured-meat color, in prevention of growth of spoilage and/or pathogenic microflora, and in developing and stabilizing product flavor. The ripening/drying process involves numerous time-temperature interactions that serve as a major source of flavor variability. Some studies have been performed to demonstrate the relationship of dry-cured ham flavor to chemical data (direct and indirect instrumental methods) and sensory attributes. However, studies are often too expensive or too time consuming. Therefore, developing a rapid, reliable, and effective method to distinguish differences in dry-cured ham products is desirable. Using an electronic nose with 32 sensors (AromaScan™ A32/50S multisampler) we were able to differentiate between Spanish "Serrano" dry-cured hams processed for 7 month (short) and 12 month (long) periods. Since the conducting polymers of the electronic nose distinguished between Serrano ham processing times we examined its ability to distinguish among other processed hams including Prosciutto ham, Country ham, Virginia ham and Deli ham; the instrument effectively distinguished these products.

* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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INTRODUCTION

The origin of salting, originally used to preserve meat products in general, is lost in ancient time. However, Catón, in "De Re Agricola," described several salted-meat recipes that today are still being used in several Mediterranean areas (Pineda, 1989). With today's widespread availability and use of refrigeration, salting of meat for preservation has become of less importance. Today, salting has been modified and improved to dry-curing wherein additives and adjuncts such as nitrates and ascorbic acid are added to the salt; furthermore, processing time is for a long periods to permit optimal maturation and flavor development (Flores and Toldrá, 1993).

An example of a dry-cured meat product is the Spanish dry-cured ham, such as the "Serrano" and "Iberian" hams, that have been considered as tasty morsels that smelled and tasted so fine that they were eulogized not only by Spanish kings, from Emperor Carlos I of Spain to Juan Carlos I, but also by European Royal Houses and by illustrious and famous personalities. Spanish dry-cured ham in particular, and some of the other dry-cured hams such as Italian Parma, San Daniele hams and French Bayonne ham in general, have become so well known that they are now served in Europe's most prestigious restaurants, at banquets and at ceremonies throughout the Mediterranean area (González, 1994). Indeed, the impact of the desirable Spanish dry-cured ham is seen from data that indicates that in 1993 production was as high as 181,500 metric tons, representing about 30 million hams (Anonymous, 1994).

Many factors affect the final flavor quality (Toldrá *et al.*, 1997a), but of primary importance are the initial quality of the raw or starting material and the processing conditions to which the material is exposed. A few studies have been reported on the influence of different breeds of swine on quality of dry-cured ham (Berdague *et al.*, 1993; Gou *et al.*, 1995; Flores *et al.*, 1994). Other studies have shown the influence of processing on the volatile components of dry-cured ham (Buscailhon *et al.*, 1993; Careri *et al.*, 1993; Hinrichsen and Pedersen, 1995).

In general, dry-cured hams reach their optimum flavor after a long ripening or drying stage which may well be more than 12 months (Toldrá *et al.*, 1997b); this long period increases production costs. The objective of the producer, therefore, is to lessen production costs; this may be accomplished by modifying the curing process by such mechanisms as modifying the length of curing time among other factors (Toldrá *et al.*, 1997b). However, most producers currently desire the availability of rapid means for determining their products readiness for sale. Many studies have been performed to demonstrate the relationship of dry-cured ham flavor to chemical data (liquid and instrumental) and sensory attributes. However, these are often too costly in their requirement for necessary personnel and hardware or are too time consuming for a typical production facility. We, therefore, set out to develop a rapid, reliable, and effective method to distinguish differences in dry-cured ham products.

Within the last 7 years there has been a major surge in the development of electronic nose (E-nose) technology, i.e., instruments with conducting polymers and/or metal oxide sensors. These multi-sensor devices are coupled to statistical data processing packages designed to simulate the way the human brain interprets the interaction of multiple sensory inputs. Fortunately, it is not necessary to understand exactly how the human brain interprets complex vapors in order to develop an electronic instrument that, in the broadest sense of the word, mimics the human olfactory system. Electronic nose (E-nose) technology has encouraged a wide dissemination of this instrumentation within the food and fragrance industries where it is used now primarily for quality control and rapid product survey. Analysis time using an E-nose is often only a few minutes making it a valuable alternative to the longer gas chromatographic (GC) techniques. This contribution will report

upon the utility of using electronic nose instrumentation to survey the differences among some dry-cured hams and between Spanish "Serrano" dry-cured ham processed for two different lengths of time.

MATERIALS AND METHODS

Ham Samples

Twenty Spanish "Serrano" dry-cured hams were purchased from a factory in Castellon, Spain (Jamones Segorbe). Prosciutto, Country, Virginia (smoked), and Deli (pressed) ham was purchased from local food stores in the greater New Orleans, Louisiana area.

Short (7 Month) and Long (12 Month) Dry-Curing Method for "Serrano" Hams

Ten hams were submitted to a 7 month process (short), consisting of the traditional stages of salting (12 days at 3°C), post-salting (50 days at 4°C) and ripening-drying (1^{st} phase: 60 days at 12°C, 2^{nd} phase: 60 days at 18°C, and 3^{rd} phase: 30 days at 25°C). A second group of 10 hams was ripened for a 7 months process (long) where hams were held, after ripening for 7 months, for an additional 150 days at 15°C. Following these processes, 750 grams of tissue were excised from the center of each ham, perpendicular to the femur that included the *biceps femoris*, *semitendinosus* and *semimembranosus* muscles (Figures 1 and 2). Samples for analysis were sent within 3 days at 4°C to the Southern Re-

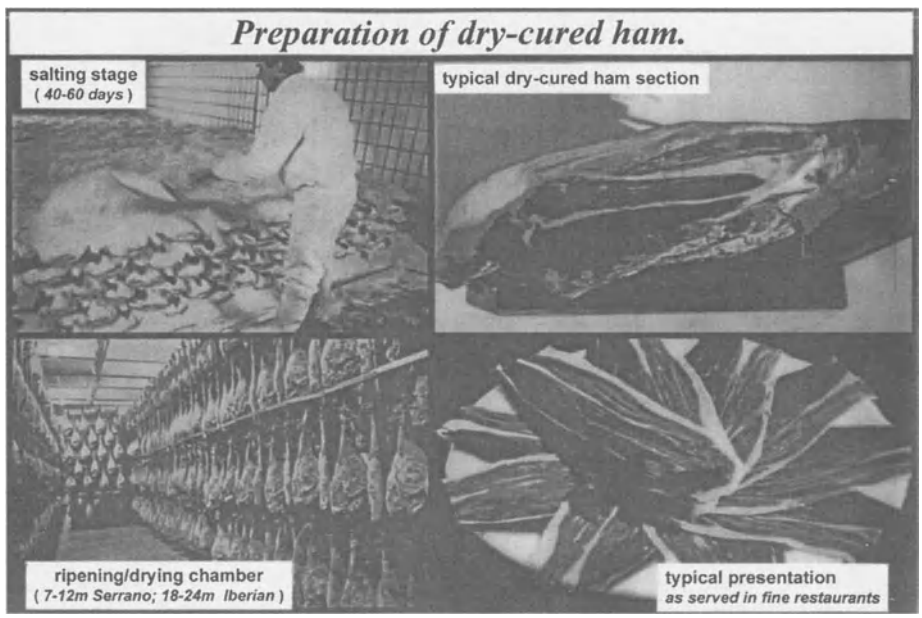

Figure 1. Photos of different steps in the processing of dry-cured ham. *Upper left* shows the salting stage in processing of dry-cured ham. *Lower left* shows the storage facility for the ripening/drying of dry-cured ham. *Upper right* shows a section of a typical dry-cured ham. *Lower right* shows the typical presentation of dry-cured ham as served in fine restaurants. All photos courtesy of Navidul S.A., Toledo, Spain.

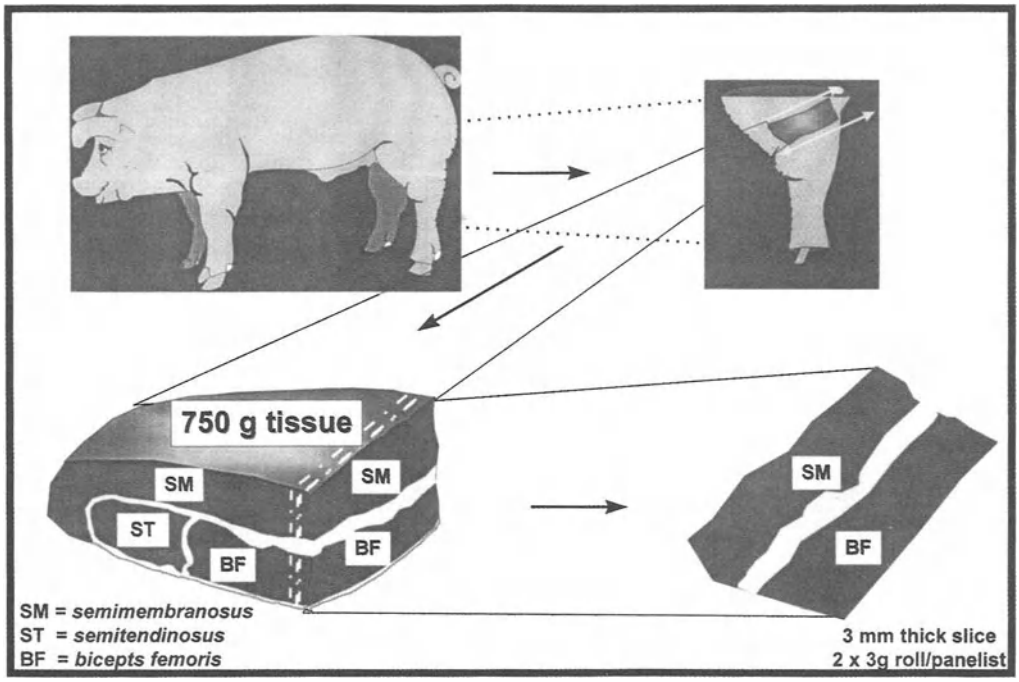

Figure 2. Schematic diagram indicating sections of the cured ham used in this investigation. SM = *semimembranosus* muscle. ST = *semitendinosus* muscle. BF = *biceps femoris* muscle.

gional Research Center (USDA, New Orleans, LA), following appropriate documentation with the Food Safety Inspection Service (FSIS) and the U.S. Customs Bureau.

Preparation of Ham for Electronic Nose

Ham from each source were finely minced with a new single edged razor blade. Precisely 5 grams of the minced ham was placed into 22 milliliters capacity multisampler vials (AromaScan, Hollis, New Hampshire). One milliliter of deionized water was added to each vial and the vials crimped and sealed with the Teflon side of a septum facing inward. Each ham had 3 samples analyzed via the e-nose thus the final data point presented on the Sammon's map represents the average of the 3 measurements per ham.

Electronic Nose

Instrumentation available for this research included the AromaScan 32S coupled to a Tekmar 50-slot autosampling device. The AromaScan instrumentation included humidity control. The Tekmar autosampler and AromaScan instrument were held at room temperature (22 °C) in an airconditioned room to control major humidity fluctuations.

The instrumental method was optimized to the following hardware control conditions: *Acquisition method*: Cycles were a Step 1, reference for 27 seconds (the time T), Step 2, sample time of 120 seconds, Step 3, wash time of 30 seconds, and Step 4, reference time of 120 seconds. The graph scale was set to 5, the pattern average to 5, the purge humidity to 45 and the reference humidity to 50. *Multisampler method*: The platen tem-

perature was set to 40°C, the platen equilibration time to 10 seconds, the sample equilibration time to 10 seconds, the vial size was 22 mL. The mixer was set to on with a mix time of 2 seconds and a mix power of 7. There was a 5 minute vial pressure stabilization time, a vial pressure time of 0.50 minutes, and a pressure equilization time of 0.15 minutes. Loop fill time was 0.40 minutes, loop equilibration time 0.20 minutes and an inject time of 1 minute. Sample loop temperature was set to 40°C as was the line temperature, with a sample injection time of 1 minute. The A32S analysis time was set for 6 minutes. Data was handled by the statistical software included with the A32S E-nose.

Statistical Analysis: Sammons Mapping Technique

The nonlinear mapping algorithms of Sammon (1969, 1970) were used to analyze and to plot/map the data obtained from all ham samples. This method was chosen since with an array of multiple sensors, measurements of different gasses or odors produce different patterns that are projected into multi-dimensional space (Hodgins, 1997). Within two- and three-dimensional space, human vision is very good at recognizing almost any relationships present; however, in multidimensional space it is difficult to perceive structural relationship. In order for a human to examine complex multi-dimensional data, a useful approach is to map them from the high-dimensional pattern space in which they are originally presented onto a low-dimensional pattern space as faithfully as possible.

RESULTS AND DISCUSSION

Chemical, Instrumental, and Human Sensory Analysis of Dry-Cured Ham: Background

Data obtained from sensory (Flores *et al.*, 1997a) and from instrumental and chemical analysis of volatile (Flores *et al.*, 1997b) and non-volatile (Flores *et al.*, 1997c) components of dry-cured Spanish "Serrano" ham have indicated that there were significant differences in short (7 month) and long (12 month) dry-cured ham. It was felt that the differences observed made it feasible to explore the possibility of utilizing the conducting polymers of the electronic nose (e-nose) for surveying the hams and that any differences observed utilizing the e-nose would most likely be related to the flavor differences observed by the other instrumental and sensorial methods of analysis.

Sensory differences are observed (Flores *et al.*, 1997a) between the short (7 month) processed and the long (12 month) processed hams. Production techniques have been studied that report to accelerate the dry-curing process (Marriott *et al.*, 1992). Although the techniques can accelerate the drying stage, it is necessary to determine the optimal conditions to ensure the development of the typical and desirable dry-cured ham flavor. The long drying process method is necessary for the development of the typical dry-cured ham flavor (Flores *et al.*, 1997a). Three factors, cured, port, and off flavors are present in hams cured for long period (12 months). While the flavor of the short (7 month) dry-cured hams is acceptable, the data clearly indicates that the dry-cured flavor has not fully developed. Thus, the time period between 7 and 12 months at which dry-cured flavor is optimized must be determined if we are to select the most advantageous processing time.

The volatile components of the headspace of Serrano dry-cured ham contributed, both individually and in combination, to the distinctive aroma properties of the product (Table 1; Flores *et al.*, 1997b). Ketones, esters, aromatic hydrocarbons, and pyrazines

Table 1. Volatile compounds detected by gas chromatography flame ionization detection. Only those compounds showing significant difference in peak area are shown. Samples are from Spanish short-term cured (7 months) and long-term cured (12 months) "Serrano" dry-cured ham

Group	Compound	KI[a]	Short[b] (7month)	Long[b] (12 month)	*P*
Alcohol					
	2-propanol	555	1.55	3.66	<0.001
	2-methylpropanol	677	1.03	0.48	<0.001
	1-butanol	712	0.39	0.28	<0.001
	1-penten-3-ol	728	1.54	0.62	<0.001
	2-pentanol	740	0.84	0.46	<0.001
	1-hexanol	915	0.77	0.45	<0.001
Aldehydes					
	3-methylbutanal	694	3.38	2.32	<0.05
	hexanal	838	0.91	0.66	<0.001
	heptenaL	942	0.78	0.66	<0.05
	octanal	1049	3.23	2.65	<0.05
	2,4-decadienal (E,Z)		0.51	0.44	<0.01
	2-undecenal		0.60	0.50	<0.01
Ketones					
	2-propanone	542	1.03	1.34	<0.05
	2,3-butanedione	633	0.44	0.37	<0.01
	2-heptanone	934	0.66	0.53	<0.01
Aliphatic hydrocarbons					
	heptane	700	0.59	1.53	<0.001
	1-octene	814	1.21	0.53	<0.001
	decane	1000	0.76	0.48	<0.05
	dodecane	1200	0.54	0.49	<0.05
Esters					
	methyl butanoate	745	0.38	0.28	<0.001
	ethyl butanoate	823	0.52	0.44	<0.01
	ethyl 2-methylbutanoate	875	0.28	0.32	<0.05
	ethyl 3-methylbutanoate	892	0.15	0.12	<0.01
Aromatic hydrocarbons					
	p- or m-xylene	910	0.20	0.17	<0.01
Halide compounds					
	chloroform	657	0.57	0.37	<0.001
	2,2-dichloroethanol	861	0.10	0.08	<0.01
Nitrogen compounds					
	methylpyrazine	847	0.09	0.08	<0.05
Sulfur containing compounds					
	dimethyl disulfide	782	2.92	1.49	<0.001

[a]Kovats index (KI) calculated dfor a DB-624 capillary column (J&W Scientific: 60m, 0.32mm i.d.; film thickness 1.8 μm) installed on a gas chromatograph (GC) equipped with flame ionization detector (FID). [b] Results expressed as the mean of ten samples ± sem of the area of GC-FID peak normalized to the area of the internal standard.

were essentially the volatile compounds that correlated with the pleasant aroma of the hams, while hexanal, 3-methylbutanal, 1-penten-3-ol, and dimethyl disulfide were related with the short ripening/drying stage. On the other hand, in the long process an increase in the pork flavor shows a low correlation with 2-butoxyethanol and 2-pentylfuran. These volatile compounds appeared to be mainly formed by lipid oxidation except for the sulfide compounds, methyl branched aldehydes, and pyrazines which were generated by Strecker degradation of amino acids. It would be important to continue these studies to identify

those unknown compounds responsible for specific aromas in Serrano dry-cured ham and relate their contribution to the development of the characteristic dry-cured ham flavor.

Few studies have been reported to establish any relationship between non-volatile components of dry-cured ham and its flavor. However, such a study would be important since the contributions of proteinaceous components, peptides and amino acids to meat taste, have been shown to occur during postmortem aging (Nishimura *et al.*, 1988; Kato *et al.*, 1989; Spanier *et al.*, 1997) and at different cooking temperatures (Spanier *et al.*, 1988; Spanier and Miller, 1993; 1996). The increase in amino acids has been attributed to the action of muscle aminopeptidases active at neutral pH (Nishimura *et al.*, 1988; 1990). Spanier *et al.* (1990) reported that thiol proteinases, cathepsins B and L, were most likely involved in the production of peptide flavor precursors during beef postmortem aging and they retained considerable activity even after cooking. Many changes during processing of dry-cured ham are a result of endogenous hydrolytic activity, since low counts of microorganisms have been reported inside the hams (Toldrá and Etherington, 1988; Molina and Toldrá, 1992). As suggested by Spanier *et al.* (1990), cathepsins were found to be active through the entire process (Toldrá *et al.*, 1993) while the activity of calpains was restricted to the initial curing (Rosell and Toldrá, 1996). The last step in the proteolytic process would be the generation of free amino acids by the action of aminopeptidases on peptide fragments from the activity of the cathepsins (Flores *et al.*, 1993, 1996). These aminopeptidases have also been found to be active during the processing of dry-cured ham (Toldrá *et al.*, 1992, 1995).

The effect of the two different curing processes (short and long) on the concentration of free amino acid and peptide content of Spanish "Serrano" dry-cured ham has been shown (Flores *et al.*, 1998). All amino acids exhibited increased concentration during the long curing process compared to the shorter curing process; the additional 5 months curing time permits the proteolytic system to continue its action (Toldrá *et al.*, 1993). Capillary zone electrophoresis of the dry-cured ham extracts indicated significant changes in peptide mapping between short and long processed hams (Flores *et al.*, 1998). All peptide peaks increased in the long process except for one that almost disappeared and another that showed a slight decrease. The generation of free amino acids from proteins and peptides is attributed to the action of exopeptidases especially alanyl aminopeptidase and aminopeptidase B which have been characterized from porcine skeletal muscle (Flores *et al.*, 1993, 1996). Alanyl aminopeptidase may be mainly responsible for the increase in amino acids, due to its broad substrate specificity and because it accounts for more than 80% of the total aminopeptidase activity found in porcine skeletal muscle (Flores *et al.*, 1996; Toldrá *et al.*, 1995).

The flavor of dry-cured ham is due to the interactions of combinations of flavor (odor and taste) compounds originating from ham proteins, lipids and carbohydrates. The non-volatile components, peptides and amino acids, constitute taste-active compounds (Nishimura and Kato, 1988) that have a large impact on the final flavor of the dry-cured ham product as seen by the increase in the contents of glutamic acid, aspartic acid, methionine, isoleucine, leucine, and lysine (Flores *et al.*, 1998); these amino acids have been shown to contribute to the dry-cured ham flavor by their combinated interaction and not by individual interaction (Flores *et al.* 1997c). The amino acids of dry-cured ham also contribute to flavor volatile formation as a result of Strecker degradations and the formation of Maillard reaction products and include sulfide compounds, methy-branched aldehydes and alcohols, and pyrazines. The remainder of the volatile compounds are formed by lipid oxidation that takes place during ham ripening. The volatile components of the headspace of Serrano dry-cured ham contribute, both individually and in combination, to

the distinctive aroma properties of the product (Flores *et al.*, 1997b). Ketones, esters, aromatic hydrocarbons, and pyrazines are essentially the volatile compounds that correlate with the pleasant aroma of dry-cured ham, while hexanal, 3-methylbutanal, and dimethyl disulfide are related with the short ripening-drying process.

Evaluation of Differences in Hams and Ham Processing Time by Electronic Nose Technology

Almost all of the components described above have an impact on the final flavor of dry-cured ham. Many of these are volatile in nature and should be capable of interacting with the conducting polymers to give an idea for a flavor profile of the various hams. However, before examining short and long processed "Serrano" hams for differences, it was determined that we should explore other varieties of dry-cured and processed hams for differences that could become visible after analysis by the electronic nose. For this purpose we utilized Prosciutto ham and Country ham which were processed (salted and cured) similar to the Serrano ham, Virginia ham which had been slightly smoked and which represented the flavor of the hams typically sold in the east-central coastal region of the U.S., and Deli ham which was cooked-and-pressed and which represented the typical type of ham sold throughout the U.S.

Several companies have developed electronic sensors to detect groups of volatile compounds. An excellent review of current technology may be found in Gardner and Bartlett's (1992) text. Characteristic of the three basic types of detectors may be seen in Table 2 which highlights many of their similarities and differences. Major differences to be noted are those dealing with detection temperature which will affect sample integrity, humidity control, certain metabolic poisons, interaction mechanisms and reported sensitivity. Basically in all systems the volatiles are swept across an array of sensors, each developed to respond to a different class of compounds. A current is passed over the sensors, the adsorption and desorption of the volatiles cause a change in the current similar to the thermal conductivity detectors used in gas chromatography. The data is handled by various statistical procedures and mapping techniques.

Figure 3 shows the Sammon (1969, 1970) map generated from evaluation of multiple data sets each from five different sample lots (hams purchased at different times) of four types of hams (Prosciutto, Country, Virginia, and Deli). All 4 ham types were readily distinguishable as individual cluster groups. Before proceeding further, some discussion must be given to the method of analysis. With an array of sensors (32 in the case of the AromaScan) it can be said that measurements of different gases or odors produce different

Table 2. Features of electronic noses

Sensor Type	Conducting polymer	'Doped' conducting polymer	Metal oxide (e.g. tin)
Sensor type	chemical, covalent	doped with metal ions, ionic	none
Poisons	none	none	sulfur compounds
Detection temperature	ambient	ambient	200-700°C
Interaction mechanism	charge, size, shape	charge and size only	charge only
Isomeric discrimination	yes	no	no
Reported sensitivity	ppb - ppm	ppm	>10 ppm
Humidity control	required	required	n/a
Sample integrity	maintained	maintained	destroyed
Neural network	yes	yes	yes

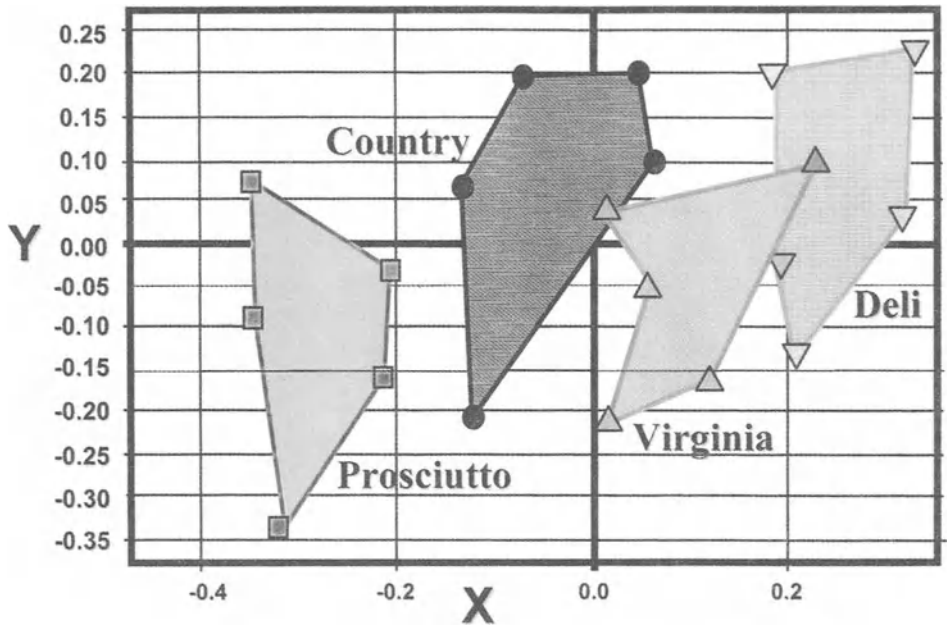

Figure 3. Sammon plot by AromaScan A32S50 software of data from five different samples of four different processed hams, i.e., Prosciutto, Country, Virginia, and Deli.

patterns that are projected into multidimensional space. Within two- and three-dimensional space, human vision is very good at recognizing any relationship(s) that may be present. However, in multidimensional space it is difficult to perceive structural relationships. In order for a human to examine complex multidimensional data, the information must be handled in such a manner as to map the high-dimensional pattern space onto a low-dimensional pattern space (see Figure 3). There are a number of ways of performing such a task where all the mapping algorithms are either linear or nonlinear. The linear mapping algorithms are used frequently because of their simplicity and generality; these have been used in gas and odor classification as well as in chemical data, in order to visualize the multidimensional pattern space in two- or three dimensional space. For gas recognition, Gardner *et al.* (1991a, 1991b, 1992, 1994) used principal component analysis (PCA) method, which is one of the powerful linear mapping techniques, to cluster and classify volatile chemicals as recently shown by Flores *et al.* (1997b) for the volatiles from Spanish "Serrano" dry-cured ham.

Ballantine *et al.* (1986) classified vapors using the PCA method and the Karhunen-Loeve (K-L) projection, which is also a linear mapping technique similar to PCA. The K-L projection was used in odor classification by Abe *et al.* (1988) and Nakamoto et al (1991), who investigated the odor of whisky data sets, using the PCA method. Kowalski and Bender (1973) used a similar linear mapping technique, using eigenvector projection, for chemical data displaying. Spanier *et al.* (1992a, 1992b) have used PCA analysis to study the interaction between sensory, instrumental, and chemical analysis of meat and meat products under various treatments. By contrast, nonlinear mapping algorithms can be used when linear mapping are unable to preserve complex data structures as is the case with real life data from complex foods. These techniques have complicated mathematical formulations compared with linear mapping, and have, thus far, been rarely used for gas and odor classification. However, the responses of the multisensor arrays, such as the instrument utilized in this study, present a nonlinear multidimensional pattern structure,

which contains the concentration-independent pattern data set describing gasses and odors. Therefore, the nonlinear mapping technique is more applicable in this case than linear mapping techniques for volatile classification. Sammon (1969) proposed a nonlinear mapping algorithm, which showed a highly effective method of multivariate data analysis, and clearly visualizes the multidimensional patterns on to two- or three-dimensional patterns. Sammon's nonlinear mapping technique was compared with the linear mapping technique, such as eigenvector projection, using twenty Gaussian-generated clusters located in a nineteen-dimensional pattern space. This comparison indicated that the nonlinear mapping technique gave superior results to the linear mapping technique (Sammon, 1970); Sammon's nonlinear mapping algorithm was also applied to "handprinted" character recognition.

Kowalski and Bender (1972) used nonlinear mapping techniques, including Sammon's algorithm, as visual display of chemical structures. They also investigated Sammon's algorithm to interpret chemical data (Kowalski and Bender, 1973) where they concluded that the true value of pattern recognition was realized when several methods, including linear and nonlinear mapping techniques, were used in combination as a system. When the nonlinear mapping technique was used for the applications, which mainly needed the mapping of the multidimensional pattern structure onto two- or three- dimensional patterns, the most popular technique was, probably, Sammon's nonlinear mapping algorithm. Since Sammon's paper, several modifications of his algorithm have been attempted, but mostly to save both memory space and computational requirements. However, current computer technology supplants this necessity and several investigators have demonstrated that the Sammon's nonlinear multidimensional algorithm may be used without any modifications for volatile (gas and odor) classification. For example, Hatfield *et al.* (1994) used this technique for gas and odor application in order to observe possible correlations between data sets of different alcohols.

While the Sammon's mapping of the data shown in Figure 3 shows a good separation of the four different ham varieties (Prosciutto, Country, Virginia, and Deli), Figures 4

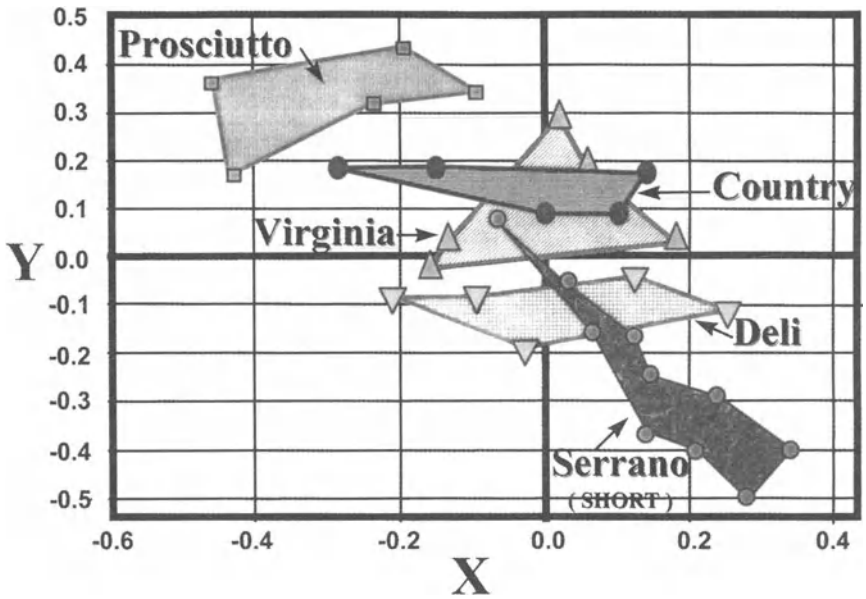

Figure 4. Sammon plot by AromaScan A32S50 software. Same as Figure 3, but with data from the ten Serrano short term (7 month) dry-cured ham.

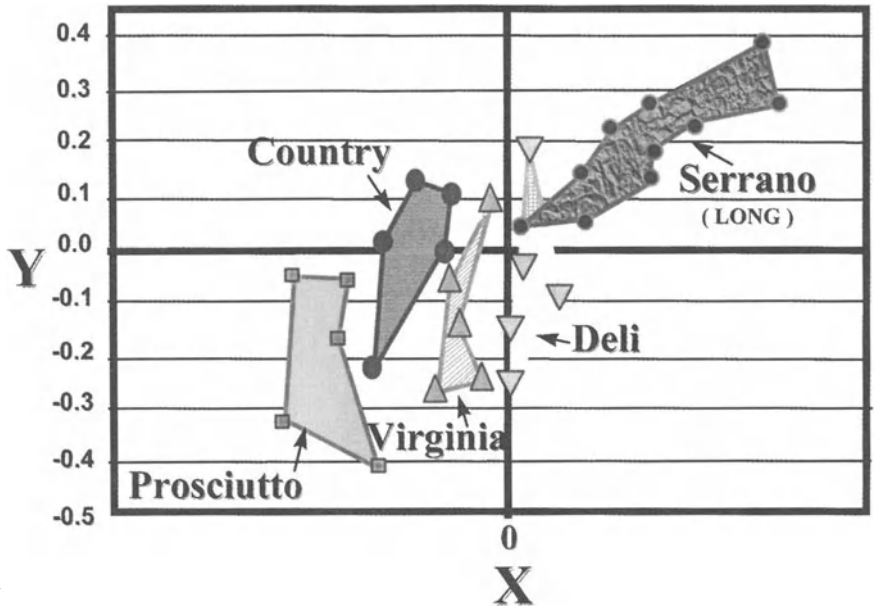

Figure 5. Sammon plot by AromaScan A32S50 software. Same as Figure 3, but with data from the ten Serrano long term (12 month) dry-cured ham.

and 5 show an overlap of some cluster groups. In these figures there is an overlap of the Deli and Virginia hams by four out of ten Serrano short cured hams (Figure 4) while the Serrano long cured ham overlaps only the Deli ham and only for one of the 10 hams examined (Figure 5). This overlap is seen to a greater degree when data from a larger number of experimental groups is examined (Figure 6). Part of this overlap can be explained by the manufacturer-published cross-sensitivity of several of the sensors in the ar-

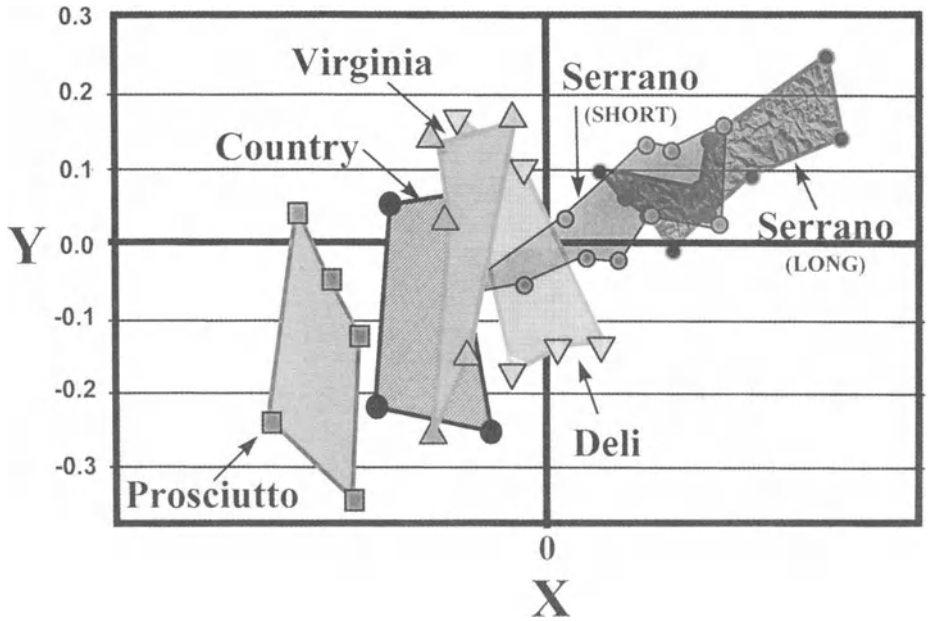

Figure 6. Sammon plot by AromaScan A32S50 software. Same as Figure 3 plus the data from the ten Serrano short term (7 month) dry-cured ham and the ten Serrano long term (12 month) dry-cured ham.

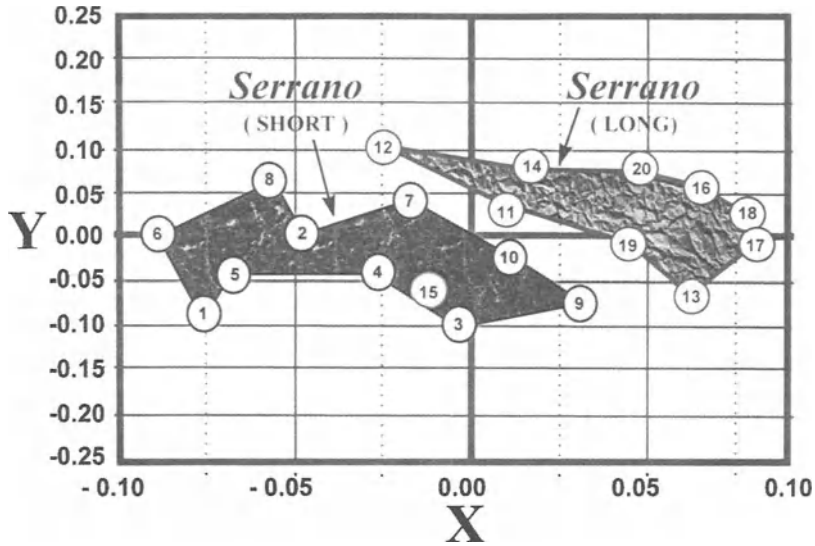

Figure 7. Sammon plot by AromaScan A32S50 software of the data obtained from the ten Serrano short term (7 month) dry-cured ham and the ten Serrano long term (12 month) dry-cured ham. Numbers indicate the number of the animal from which the ham was produced.

ray of 32. These comments are in agreement with Horner and Muller (1992) who indicated that "cross-sensitivity data is inaccurate and shows spread between samples of the same type." Another possible explanation is that all of the samples are hams and thus share similar volatile components. Multivariate analysis must, therefore, distinguish between these similar volatiles and those volatiles that may be fewer in number and concentration in the different hams. Our experience with electronic nose instrumentation suggests that an increase in the number of samples evaluated leads to a tightening of the clustering.

The Sammon's plot of Figure 6 contains data from 4 hams shown in Figure 3 plus both the short and the long dry-cured Serrano hams; the Figure 6 plot is quite different from the plot of the data for the 4 hams alone (Figure 3) and quite different from the Serrano hams alone (Figure 7). Figure 6 shows a fair amount of overlap in the cluster group for Country, Virginia and Deli hams and an overlap of about half of the Serrano long and short cured samples with each other. The Serrano short as seen in Figure 6 showed some samples clustering with the Virginia and Deli hams just as in Figure 4, while the Serrano long did not overlap with any of the other hams except the Serrano short. When the data set for only the Serrano short and Long hams are examined and plotted with Sammon's algorithm (Figure 7), only one of the Serrano long-cured ham samples (#15) showed any overlap with the 10 hams of the Serrano-short processed samples.

CONCLUSION

Short and long dry-cured Serrano hams have many measurable differences in their flavor, lipids, peptides, amino acids and other components that can be evaluated by sensory, instrumental and chemical means (Flores *et al.*, 1994, 1996, 1997a, b, c, 1998). It would seem reasonable to predict that electronic nose technology might prove itself useful to examine the differences in Serrano ham processing. Our data show that the technology has a usefulness in examining differences in different varieties of ham. It is hoped that

with further experimentation and development of electronic nose sensors ham processors will have a rapid means of assessing the flavor quality of their product with the final end-point of production of high quality dry-cured ham at a cost effective level for both themselves and the consumer.

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THERMALLY GENERATED VOLATILES IN ROSELLE TEA

Pi-Jen Tsai,[1] Tzou-Chi Huang,[1] Shyh-Hung Chen,[1] and Chi-Tang Ho[2]

[1]Department of Food Science and Technology, National Pingtung Polytechnic Institute, 912, Pingtung, Taiwan
[2]Department of Food Science, Rutgers University New Brunswick, New Jersey 08903

Likens-Nickerson steam distillation (L-N) and thermal desorption (TD-3) were used to mimic the preparation of roselle tea. Thermally generated volatiles from roselle were collected and analyzed by GC and GC-MS. Samples were frozen, cold-air dried at 25°C or hot-air dried at 50, 75 or 85°C and used to elucidate the effect of heat treatment on thermal generation of volatiles in roselle tea. Volatile of roselle tea were classified into four groups: fatty acid derivatives, sugar derivatives, phenolic derivatives and terpenes. As compared with L-N extraction, volatiles extracted by TD-3 apparently had a higher content of aliphatic C6 components and terpenes, but thermally generated volatiles, such as furfural, linalool oxide, and eugenol were present in much smaller amounts. Drying process reduced the content of aliphatic C-6 lipid derivatives and terpenes dramatically, but markedly increased the amount of furfural. A combination of the terpene derivative and sugar derivative were found responsible for the roselle aroma as evidenced by Principle component analysis. Discriminant analysis of the volatiles extracted by TD-3 revealed that principle component 2 is most important in contribution of the model and thermally generated furfural is responsible for its correct classification.

INTRODUCTION

Roselle *(Hibiscus sabdariffa L.)* tea with its brilliant red color (Esselen *et al.*, 1975), is one of the most popular drinks in Taiwan (Tsai, 1995). However, its unique flavor is very delicate and barely discernable to detect. There are few reports on the aroma of roselle, except one referring to the volatiles in seed oil of roselle (Jirovetz *et al.*, 1992). In this study, two methods of extraction (Likens-Nikersen, L-N and thermal desorption, TD-3) were used to mimic the extraction of thermally generated volatiles of roselle prior to their identification by GC and GC-MS, in order to understand the effect of drying temperature on flavor. Further analysis of these volatiles by principle component analysis (PCA) intended to find the responsible aroma for roselle. Discriminant analysis was used to classify the samples into groups of different drying temperatures according to these volatiles.

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SAMPLE PREPARATION

Roselles harvested from Taitung, Taiwan, were frozen (FZ) at -20°C, cold-air dried at 25(C25)or hot-air dried at 50(D50), 75 (D75)or 85(D85) for 36 hours respectively. Extraction of roselle volatiles was carried out by Likens-Nickerson apparatus(L-N) (Nickerson and Likens, 1966) and Short Path Thermal Desorption System (Model TD-3)(Alonso *et al.*,1996; Overton and Manura,1995). In the L-N system, roselle samples were mixed with 700 ml distilled water, refluxed for 2 hours with the solvent flask filled with 50 ml diethyl ether. Then the diethyl ether layer was concentrated using a condenser to about 100 μl and injected onto a GC.

In the TD-3 apparatus, 60 g sample was immersed in 300 ml boiled water for 10 min, volatiles were then purged for 2 hours at 40°C by N_2 at 60 ml/min onto a stainless steel desorption tube packed with 200 mg of Tenax TA. The desorption tube with sample was then attached to the thermal desorption system. After desorption, the content of the tube was injected onto the GC. After GC analysis (Majlát *et al.*, 1974), roselle volatiles exhibited over 50 peaks, which were identified by the Mass spectrometer (Pfannhauser *et al.*,1989).

Statistical analysis used were principle component analysis(PCA, Factor), analysis of variance and discriminant analysis and all of them were conducted using the SAS statistical software (SAS,1988). In the discriminant analysis, linear discriminant analysis (LDA) was performed on either principle components or the log transformation of standardized peak areas. The classification ability of the discrimination functions was verified using a cross-validation methodology.

RESULTS AND DISCUSSION

In order to understand the effect of extraction method and drying temperature on the thermal generation of volatiles in roselle tea, volatiles extracted by different methods from samples were compared, as summarized below.

Effect of Extraction Method and Drying Temperature

Volatiles extracted from all samples were found to contain numerous monterpenoid and sesquiterpenoid compounds and flavor-active components such as furfural, hexanal and eugenol. According to their sources, they could be divided into four groups: (I) fatty acid derivatives, (II) sugar derivatives, (III) phenolic derivatives and (IV) terpenes. Most of the group (I) volatiles extracted by L-N method (Table 1) or TD-3 method (Table 2), which were classified as fatty acid derivatives, belong to aliphatic C6 aldehydes and alcohols, and contribute to the green aroma notes. Sugar derivatives, mainly furfural, were found to be formed only in the dried roselles. As to the terpenes, -terpineol, limonene, linalool and linalool oxide were four of the dominant compounds in roselle calyces.

Comparison of the Volatiles Extracted by L-N and TD-3. In comparison, volatiles extracted by L-N and TD-3 were considerably different. Figure 1 shows the comparison of the peak area percentage of volatiles extracted by L-N and TD-3 in (a) frozen, (b) 50 or (c) 75 oven dried roselles, respectively. As compared with L-N extraction, volatiles extracted by TD-3 apparently had a higher content of aliphatic C6 components (hexanal, (E)-2-hexenal, etc.) and terpenes (especially in linalool and limonene). However, thermally

Table 1. Classification of volatile constituents in roselle by L-N

Volatile Compound	Fresh**	Frozen	Oven dried,°C	
			50	75
Fatty acid derivatives*				
2-ethylfuran	0.776	0.179	—	—
Hexanal	4.287	1.291	1.337	0.353
(E)-2-hexenal	6.465	3.265	0.534	—
(Z)-3-hexenol	32.870	1.899	1.192	0.898
2-hexenol	27.721	6.650	—	—
1-hexanol	21.936	—	—	
Heptanal	0.295	0.278	0.791	—
(E)-2-heptenal	0.246	0.359	—	—
2-pentylfuran	1.308	1.391	1.347	—
Octanal	—	—	—	0.349
Nonanal	3.887	5.548	3.335	0.617
Sugar derivatives				
Furfural	0.292	0.641	23.006	43.606
5-methyl-2-furaldehyde	—	—	0.931	1.915
Phenolic derivatives				
Eugenol	9.128	9.089	9.052	9.014
Terpene components				
2H-pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	2.577	2.728	1.258	—
1,4-cineole	0.626	0.552	—	—
1,8-cineole	1.546	2.584	—	—
Limonene	2.288	2.229	—	—
Linalool oxide (a)***	6.380	7.110	4.797	1.436
Tetrahydro-2,2-dimethyl-5-(1-methylpropyl)-furan	0.522	0.596	0.653	0.213
Linalool oxide (b)	3.039	3.581	2.086	0.759
Linalool	1.007	1.498	0.384	0.349
2-methyl-6-methylene-7-octen-2-ol	1.750	1.612	0.525	0.390
1-methyl-4-(1-methylethyl)-3-cyclohexenol	3.423	4.101	0.833	0.383
2,6-dimethyl-5,7-octadien-2-ol	3.125	4.009	0.600	0.131
α4-dimethyl-3-cyclohexene-1-acetaldehyde	3.661	2.800	1.271	0.495
α-Terpineol	12.322	18.014	2.002	1.450
α-Terpinylacetate	3.369	3.560	1.074	0.684
3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-, acetate	3.267	3.122	0.633	0.311
Caryophyllene	4.510	2.329	0.213	0.517
α-Farnesene	4.075	3.313	1.315	1.906
Exo-2-hydroxycineole	2.758	2.427	1.366	1.596
Miscellaneous				
2,3-dimethylbutane	0.246	1.003	2.633	0.499
Acetic acid	1.095	2.511	12.670	6.512
2,2-dimethylhexanal	1.198	0.516	—	—
Methyl salicylate	0.744	—	0.447	0.968
6,10,14-trimethyl-2-pentadecanone	2.073	1.052	0.955	0.867

* The class of volatile by source of reaction.

** concentration by GC-FID (mg/kg dry wt.).

***linalool oxide(a) and (b) are cis- and trans-linalool oxide of furanoid type

generated volatiles such as furfural, terpene derivatives (linalool oxide, -terpineol) and eugenol were present in much smaller quantities. Due to the long heating period during the distillation in L-N, it is believed that the volatiles extracted by TD-3 method resemble more the true aroma of roselle tea and the thermal generation of volatiles in roselle can be more clear by defined using this extraction method.

Table 2. Classification of volatile constituents in roselle calyces

Volatile constituents	M.W.	** Frozen	Cool dried, °C		Oven dried, °C	
			25	50	75	85
Fatty acid derivatives*						
Hexane	86	0.900	0.937	0.980	0.486	0.463
Hexanal	100	4.570	2.400	3.075	1.475	0.841
Octane	114	0.091	0.172	0.045	—	—
2-Hexenal,(E)-	98	1.445	0.928	1.038	0.805	0445
3-Hexen-1-ol,(Z)-	100	0.310	—	—	—	0.579
1-Hexanol	102	1.639	—	—	—	—
Heptanal	114	0.456	0.718	0.728	0.374	0.185
2-Heptenal,(E)-	112	0.395	0.663	0.741	0.405	0.247
Furan,2-pentyl-	138	0.792	0.290	0.417	0.196	0.128
2-Octenal,(E)-	126	0.333	0.152	—	—	—
2-Nonenal,(E)-	140	0.203	0.239	0.259	0.123	0.066
Decanal	156	0.288	1.729	0.515	0.237	0.345
Sugar derivative						
Furfural	96	—	—	1.629	4.972	28.20
Phenolic derivative						
Eugenol	164	0.238	0.268	0.328	0.218	0.144
Terpene components						
5-Hepten-2-one,6-methyl-	126	0.186	0.367	0.444	0.304	0.663
β-Pinene	136	1.531	1.114	0.474	0.361	0.237
α-Phellandrene	136	0.342	2.089	1.653	0.837	0.444
α-terpinene	136	0.327	0.758	0.841	0.448	0.215
Limonene	136	1.655	0.582	0.645	0.509	0.793
(E)-Ocimene	136	0.288	1.667	0.654	0.353	0.261
3-Carene	136	0.557	0.493	0.081	—	—
γ-terpinene	136	0.164	1.324	1.718	0.912	0.484
Linalool oxide	170	0.178	0.408	0.194	0.101	0.102
Terpinolene	136	0.143	0.505	0.682	0.381	0.264
Linalool	154	6.408	3.847	2.579	1.236	0.537
2,4,6-Octatriene,2,6-dimethyl,(E,Z)-	136	0.134	0.340	0.505	0.350	0.250
4-terpineol	154	0.308	0.485	0.594	0.262	0.040
α-terpineil	154	1.005	0.483	0.458	0.446	0.351
p-Menth-1-en-9-al	152	0.197	0.728	0.535	0.462	0.137
Nerol	154	0.065	—	0.069	0.045	0.065
2-Bornene	136	0.234	0.160	0.146	0.106	0.116
Geraniol	154	0.258	0.530	0.524	0.376	0.289
(+)-Longifolene	204	0.427	0.371	0.438	0.288	0.231
Caryophyllene	204	0.538	0.326	0.220	0.144	0.101
α-Caryophyllene	204	0.294	0.215	0.299	0.166	0.132
Miscellaneous						
Toluene	92	0.305	0.384	0.524	0.235	0.238
Colriue	136	0.114	1.009	1.115	0.650	0.363
Undecane	156	0.086	0.516	0.041	0.100	0.094
Naphthalene	128	0.202	0.252	0.553	0.111	0.189
Dodecane	170	0.122	0.210	0.179	0.217	0.246
Tridecane	184	0.299	0.214	0.146	0.129	0.267
Tetradecane	198	0.436	0.655	0.701	0.488	0.336
Pentadecane	212	0.498	0.504	0.905	0.594	0.354

*The classification of volatiles by source of reaction.

**Concentration detected by TD-GC-FID (mg/kg dry wt.)

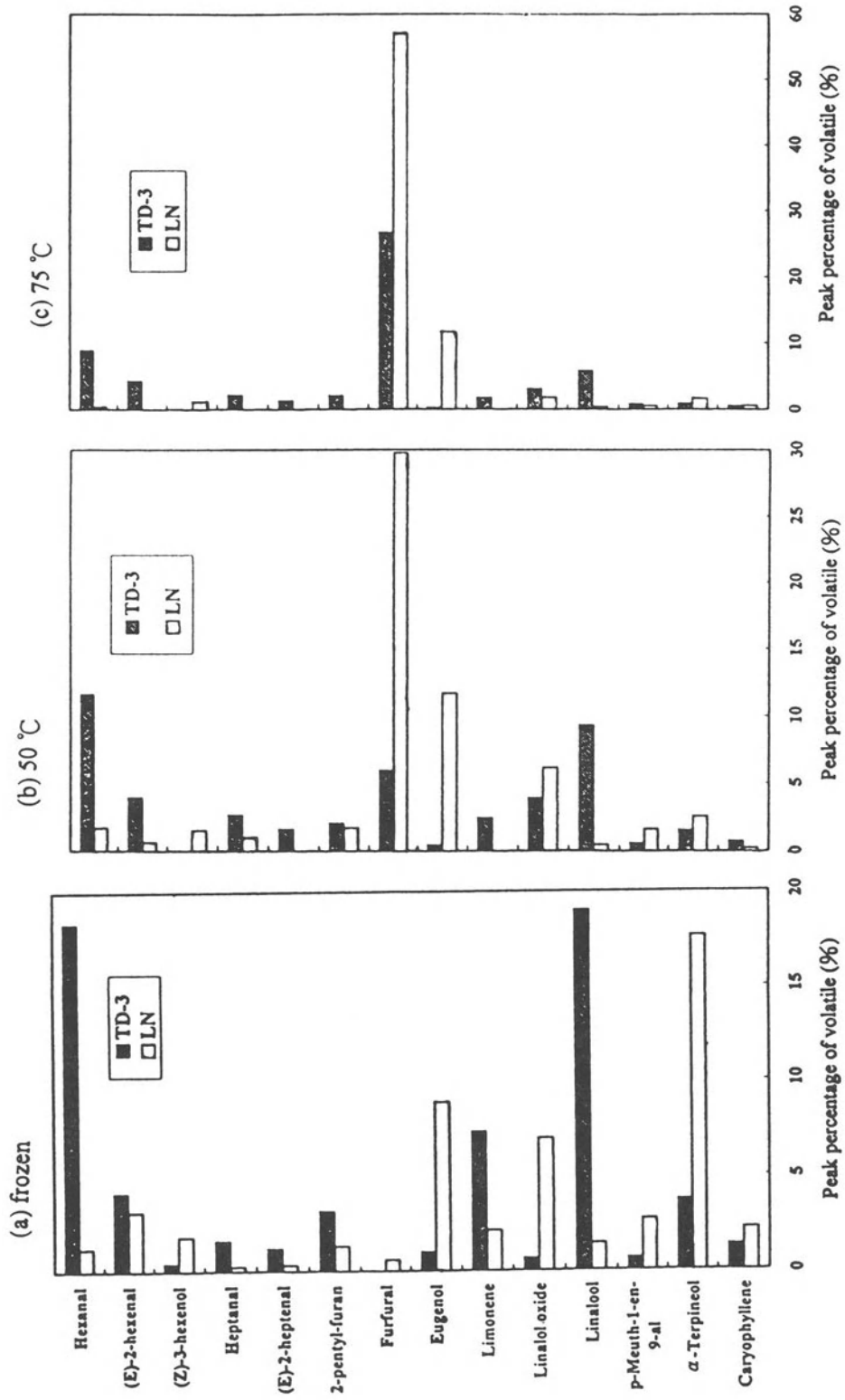

Figure 1. Comparison of peak area percentage of the volatile constituents in (a) frozen roselle (b) 50°C roselle (c) 75°C roselle extracted by TD-3 and L-N system.

Effect of drying Temperature. As shown in Table 1, green note volatiles were found to mask other aromas in the fresh sample. However, in the frozen and oven dried samples, their concentration was reduced considerably. Further fatty acid analysis showed the possibility that these green notes might have been generated from the action of lipoxygenase on unsaturated fatty acids (Luning *et al.*, 1995; Olias *et al.*, 1993). Freezing and oven drying seem to inactive the lipoxygenase (Belitz *et al.*, 1986). This explains why frozen and oven dried samples had much less intense green notes aroma. Although only trace amounts of 2-pentylfuran was detected, there is a possibility for thermal decomposition of fatty acids via other routes to form the Group I volatiles (Min *et al.*, 1989; Belitz *et al.*, 1986).

In the sugar derivatives, furfural increased with increasing the temperature. It is suggested that furfural is formed mainly during the drying process. Thermal processing seems to be necessary to obtain a caramel-like aroma (Kroh 1994). As to the terpenes, the content of linalool decreased with increasing temperature, while the amount of other terpenes changed irregularly. Because of the complex nature of these compounds, we conceded that oven drying leads to the formation of thermally transferred terpenoid aroma and also leads to the loss of some of them. In all samples, only furanoid-type linalool oxides could be found. This reveals that linalool oxides might originate from acid and heat degradation rather than biogenic pathways (Nagy *et al.*, 1989; Williams *et al.*, 1986). No obvious change of eugenol (phenolic derivatives) from all samples was noticed.

Causative Components of Roselle Volatiles

All volatiles were further analyzed by PCA to find out the causative compounds in the roselle volatiles. Each principal component or axis was a linear combination of the original variable. In the volatiles extracted by L-N, first five principal components (eigenvalue greater than 1.0) accounted for approximately 86.41% of the total variance. Figure 2 represents a plot of values for the first two principal components, which together account for 66.84% of the variation in the data Along the positive direction of factor 1 axis, peaks were terpene derivative, such as limonene, -terpineol and linalool oxide, while along the negative direction, peaks were related to sugar derivatives (furfural). For the volatiles extracted by

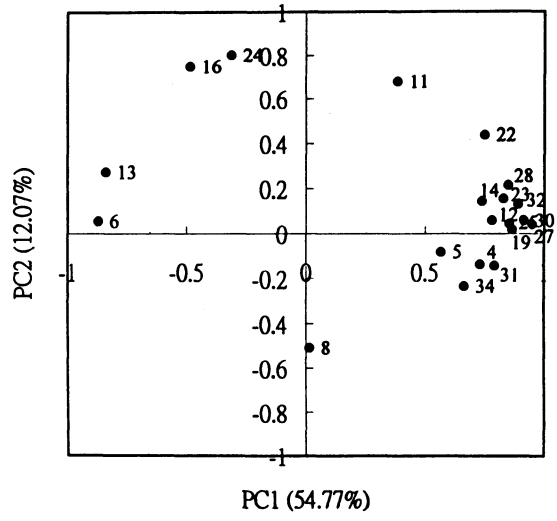

Figure 2. Distribution of the 20 volatiles in the canonical plot extracted by L-N.

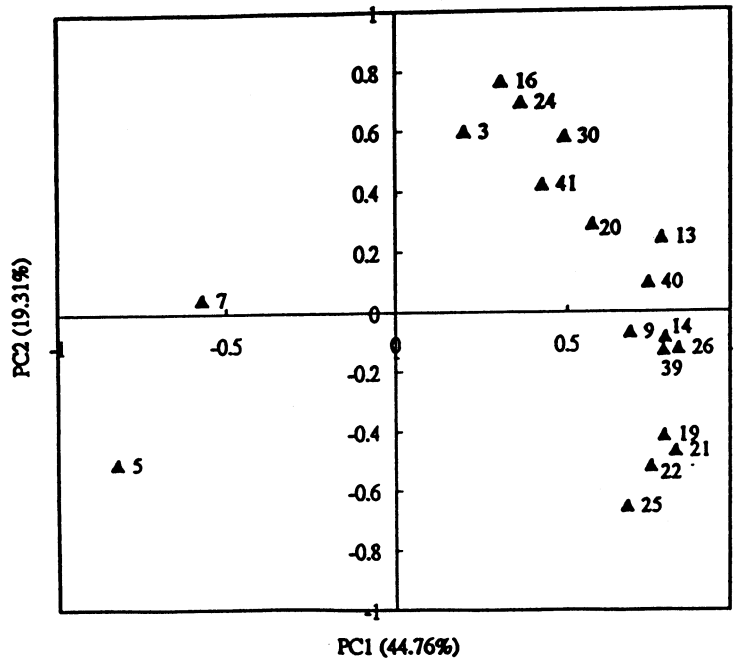

Figure 3. Distribution of the 18 volatiles in the canonical plot extracted by TD-3.

TD-3, the eigenvalues, their cumulative proportion and all the distribution of volatiles in canonical plots were quite similar to the ones obtained by L-N method. The first four principal components accounted for about 81.39% of the total variance. Figure 3 shows a plot of values for the first two principal components, which together accounted for 64.07% of the variation in the data. Along the positive direction of the factor 1 axis, peaks were terpene

Table 3. Percent correct classification of roselles according to their volatiles extracted by L-N or TD-3 for separation into different groups of drying temperature in cross-validation

		Description of analysis			
		Peak		PC	
Method	Processing mode	37	20	37	20
L-N	Fresh roselle	88.89	88.89	77.78	77.78
	Frozen roselle	87.50	93.75	64.71	82.35
	50 dried	77.78	100	62.50	75.00
	70 dried	55.80	88.89	55.56	77.78
		Peak		PC	
		43	18	43	20
TD-3	Frozen roselle	92.86	92.86	80	85.71
	25 dried	77.78	66.67	77.78	88.89
	50 dried	70.00	80.00	70	100.00
	75 dried	91.67	100.00	60	100.00
	85 dried	100.00	100.00	100	100.00

derivatives, while along the negative direction, peaks were related to sugar derivatives (furfural). As the result, over 37 compounds in L-N method or 43 compounds in TD-3 method were characterized, yet none of the volatiles with a unique odor of typical roselle tea has been evidenced. It is likely that the characteristic roselle tea aromas depend upon a subtle quantitative balance of various components. Therefore, roselle volatile might come from a combination of volatiles primarily having terpene and sugar derivatives.

Classification of Roselle According to Thermal Generated Volatiles

The drying temperature significantly affected the thermal generation of volatiles in roselle. That is, the roselle volatiles could be quite different if treated under different drying temperature. In this study, volatiles were used to discriminate the subtle changes in roselle flavor due to oven drying. Discriminate analysis was performed according to the volatiles extracted by L-N or TD-3 method, using peaks or principal component, respectively. The results of roselle drying temperature classification as to its volatiles are summarized in Table 3. Using the principal components, which accounted for the accumulated variation up to 96% (20 pc in l-N and in TD-3), it was possible to obtain a higher level of

Table 4. Highly significant (p0.0001) volatiles identified in the discrimination of roselle into Fz, c25, d50, d75, and d85 groups (model with 18 peaks)

Peak No.	Volatile components	R^2	F	PrF
Univariate				
A5	Furfural	0.96	298.7796	***
A21	trans-Linalool oxide	0.87	79.1940	***
A19	3-Carene	0.80	47.3705	***
A13	β-Pinene	0.69	26.1692	***
A25	Undecane	0.69	25.7538	***
A22	Colrius	0.68	25.2147	***
A30	β-Terpineol	0.67	24.1733	***
A24	Linalool	0.61	18.3054	***
A16	D-Limonene	0.59	16.9486	***
A14	alpha-Phellandrene	0.59	16.9080	***
A3	Hexane	0.58	15.9817	***
A8	1-Hexanol	0.56	15.0305	***
A9	Heptanal	0.50	11.7381	***
A20	γ-Terpinene	0.47	10.2695	***
A39	Tetradecane	0.43	8.9715	***
A17	(E)-Ocimene	0.39	7.3725	***
A26	2,4,6-Octatriene,2,6-dimethyl,(E,Z)-	0.38	7.2318	***
A31	Decanal	0.37	7.0316	
A37	Tridecane	0.35	6.2613	
A28	Naphthalene	0.33	5.8914	
A12	Furan,2-pentyl-	0.30	4.9189	
A7	3-Hexen-1-ol,(Z)-	0.28	4.6684	
A43	Pentadecane	0.26	4.1313	
A11	5-Hepten-2-one,6-methyl-	0.14	1.9277	
A1	Hexane	0.13	1.7780	
Multivariate				
Wilks' Lambda			10.9037	***
Pillai's Trace			3.78397	***
Hotelling-Lawley Trace			88.0149	***

***: 0.0001

correct classification rate than using the total principal components (37 pc in L-N and 43 pc in TD-3). This situation also occurred in the classification derived for the model using 'peaks' instead of principal components. Discrimination analysis carried out on highly significant ($p<0.001$) peaks avoid the redundant variables and performed a better classification for both methods. There is a lower correct classification percentage occurring in the 25 or 50°C oven dried samples. The volatiles in both samples were similar which lead to misclassification if using 18 peaks for TD-3 analysis as variables.

In developing the discriminant model, there were 17 volatiles involved significantly. However, only furfural has the R^2 of up to 0.96 (Table 4). Furfural is the most effective component in classifying roselles by drying temperature. In addition, the first 4 principle components are significant in developing the model, but the R^2 of pc2 up to 0.924 showed the importance of pc2 in contributing to the model (Table 5). This can be clearly demonstrated by projections of samples on canonical plots. As we can be seen in Figure 3, heated and non-heated samples are located on the opposite sides of the pc2 axis. The continuous allocation of D50, D75 and D85 samples with increasing drying temperature also evidences the effect of heat on the volatiles when drying proceeds.

Further assignment of the commercially dried roselles to the discriminant model showed that their volatiles are quite similar to those of samples dried at 75. This can be evidenced by the low classification correct percent(<50%) between them (Table 5). The

Table 5. Univariate and multivariate statistics of discriminant analysis for roselle classification grouped by drying temperature (into Fz, c25, d50, d75, d85 group)

Variable	R^2	F	PrF
Univariate			
P1	0.720	32.1500	***
P2	0.924	152.9475	***
P3	0.224	3.6041	*
P4	0.562	16.0254	***
P5	0.039	0.5037	
P6	0.043	0.5662	
P7	0.067	0.8952	
P8	0.033	0.4234	
P9	0.121	1.7205	
P10	0.065	0.8635	
P11	0.032	0.4085	
P12	0.021	0.2684	
P13	0.004	0.0446	
P14	0.017	0.2178	
P15	0.012	0.1485	
P16	0.153	2.2623	
P17	0.013	0.1693	
P18	0.136	1.9708	
P19	0.067	0.8991	
P20	0.043	0.5574	
Multivariate			
Wilk's lambda		20.1564	***
Pillai's trace		7.35662	***
Hotelling-Lawley trace		53.058	***

*: 0.05
**: 0.01
***: 0.001

Table 6. Comparison of percent in dried roselle from different sources

	Description of analysis	
Sample	Peak	Pc
Market	50.00	58.33
D75	33.33	41.67

D75: roselle being hot-dried at 75°C.

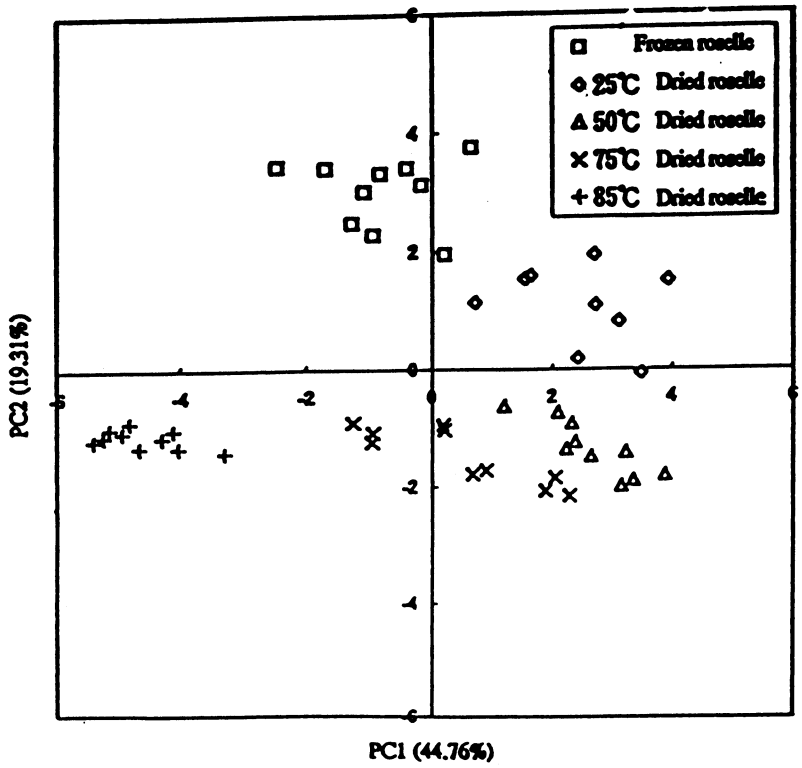

Figure 4. Classification of the different samples on the basis of their volatile compounds extracted by TD-3.

canonical plot further showed that the distribution of samples bought from the market was near to D75 samples, thus suggesting that the volatiles in the samples dried at 75°C resemble those involved in the commercially dried roselles which have been accepted for a long time in the market.

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19

PHENOLIC COMPOUNDS AS ASTRINGENT FACTORS IN BLACK TEA LIQUORS

E. Ponce[1] and A. J. Taylor[2]

[1]Departamento de Biotecnología
Universidad Autónoma Metropolitana-Iztapalapa
Apartado Postal 55-535, México, D.F., 09340, México
[2]Department of Applied Biochemistry and Food Sciences
University of Nottingham, Sutton Bonington Campus
Leics, LE12 5RD United Kingdom

Black tea manufacturing involves an enzymatic oxidative reaction of the green tea polyphenols and subsequent polymerization reactions leading to complex polyphenols called theaflavins and thearubigins which are known to be astringent and responsible for the overall quality of the tea extract. The quality of the tea liquor is described using five parameters, namely color, strength, flavor, brightness and astringency and these depend on several factors like genetic strain, manufacturing process, brewing conditions, size of the tea leaf, etc. Changes during the manufacturing process are discussed, along with methods for analysis of phenolics in black tea liquors, including liquid chromatography, fast atom bombardment and thermospray mass spectrometry.

INTRODUCTION

Tea ranks as the most popular beverage in the world and second to coffee in commercial value. It is made from the infusion of processed leaves of the tea plant *Camellia sinensis*. The tea plant is a small evergreen shrub that belongs to the family Theaceae. Leaves are dark green, elliptical in shape and have serrated edges, with a high content of caffeine and flavonoids.

The tea plant is a native of Southeast Asia in the area encompassing Tibet, western China, and northern India. From very early times, tea leaves were processed and used in the form of the dried leaf itself, as a powder, or as a cake of steamed leaves. Chinese Buddhists introduced the plant into Japan, and after the European discovery of the beverage in the early 1600s, the Dutch and British East India companies spread tea cultivation to Java and India, respectively. Tea began to be sold in England in 1657 and rapidly supplanted coffee in popularity.

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Plants are pruned periodically in order to maintain a height of about 1 m and to encourage the growth of new leaves. Plants grown at low altitudes yield leaves for commercial use after two and a half years, while those grown at high altitudes are ready in 5 years.

The best teas are produced at altitudes of 1,000 to 2,200 m, because tea plants grow more slowly in cooler air and their leaves yield a better flavor. The tea produced on the lower slopes of the Himalayas near Darjeeling, India, is considered to be among the finest in the world.

High quality tea is made from the young shoots consisting of the tender bud and the first two leaves; very often however, three or even four leaves are taken. There are three general types of tea and they are named according to their manufacturing process as Green (unfermented), Oolong (partially fermented) and Black tea (fully fermented). They can be further classified as 'plain' or 'flavory' teas, however, the term 'plain' does not necessarily imply lack of quality, but lack of certain flavor components. Teas are usually sold as blends and the price is determined according to their quality described by flavor, strength, color, brightness and briskness (McDowell *et al.*, 1991; Owoner *et al.*, 1986). Among the unblended teas, Assam and Darjeeling are, produced in India; the Ceylon teas, from Sri Lanka, which have a smooth, flowery flavor; and Keemun, a dark China black tea also known as English Breakfast tea. Popular blended teas include Irish Breakfast (high-grown Ceylon and Assam teas); Russian style, which is a China Congou sometimes containing other teas or scents; and Earl Grey, a black tea flavored with bergamot or lavender oil.

India, China, Sri Lanka, Kenya, Indonesia, Japan, and the republics of Georgia and Azerbaijan are among the leading producers of tea. Of the approximately 2.5 million metric tones that were produced annually in the early 1990s, India accounted for about one-third of the total.

The manufacturing process of black tea involves a series of enzymatic oxidative reactions of the green tea polyphenols to their respective O-quinones by the enzyme polyphenol oxidase (PPO), and subsequent polymerization reactions leading to formation of complex polyphenols called theaflavins and thearubigins. The overall quality of black tea depends on the thearubigin and theaflavin contents (Mahanta and Boruah, 1986), however, the exact chemical nature of these compounds remains uncertain. The overall "tea flavor" appears to be determined by a combination of aroma and astringent phenolics. Theaflavins contribute to the astringency (briskness) and brightness, while thearubigins play an important role towards taste, color and body of the black tea brew.

BLACK TEA MANUFACTURE

The manufacture of tea involves various stages (withering, rolling, fermentation and firing) in which the leaves undergo several chemical reactions, but it is the chemical composition of the tea leaf and the products of these reactions that determine the final quality of black teas (Mahanta and Boruah, 1986).

Withering

This stage is conducted to reduce the moisture content of the tea leaf. Tea shoots are plucked from the bush and spread into layers. Then warm air is forced to circulate through the beds, and the moisture content is reduced by evaporation from 75–80% to 65–70%, over a period of 12–24h. During the withering period, the leaf becomes flaccid, and partial breakdown of proteins to amino acids, among other reactions, takes place. It is thought that these reactions contribute to the aroma formation in the final product (Takeo, 1994).

Rolling

The leaf is then rolled, and this stage produces mechanical cellular damage. Rolling can be either Orthodox or Unorthodox. Orthodox processing is the oldest form and is still widely used. It consists of rolling the leaf on a rolling bed by stretching and tearing the leaf. Unorthodox methods (Rotorvane, Cutting-Tearing-Curling and Lowrie Tea Processor) are quick and more severe, leading to the production of smaller leaf fragments and more oxidation (Mahanta and Boruah, 1992).

Fermentation

The penultimate stage is known as fermentation, although this term is now considered to be misleading since the major changes involved are enzymatic rather than microbiological. The rolled leaf is piled in layers (5–7.5cm thick) and allowed to stand exposed to the atmosphere at room temperature (30°C) with a RH=96–98% and a constant oxygen supply for a period up to 3 h, depending on the type of leaf and the manufacturing process. Among the many reactions that occur, the most important are those that involve the production of volatiles responsible for the aroma of black tea (Co and Sanders, 1970) and the development of the color, strength and quality of the tea brews, from the production of non-volatiles. These reactions consist of a series of oxidative reactions of the simple flavan-3-ols (catechins) to more complex polyphenol theaflavins (TF) and thearubigins (TR) catalyzed by the enzyme PPO in the presence of molecular oxygen (Bailey *et al.*, 1993). It is believed that reactions occur both on the surface of the leaf and within the leaf.

Firing

Fermentation is stopped by drying (firing) the leaf with hot dry air. The process deactivates the enzymes and reduces the moisture content to 2–4%. During drying, further reactions occur in which theaflavins are oxidized to thearubigins, and low molecular weight thearubigins react to form high molecular weight thearubigins (Hazarika *et al.*, 1984). Also, other changes, like the conversion of chlorophyll to pheophytin and subsequent pheophorbides, contribute to the appearance of the black tea leaf.

Prior to firing, the taste is harsh and metallic, but the subsequent combination of polyphenols with leaf proteins decreases the astringency. Firing is essential for the development of black tea flavor and aroma (Kawakami *et al.*, 1995).

Green tea manufacturing process involves a rapid steaming of the harvested leaves to inactivate enzymes, followed by rolling and firing.

Grading

The dried tea leaf is then sorted by size (grading) and weight, before storing. The major tea grades usually fall into four groups called leaves, broken, fannings and dust. Orange pekoe, for example, is a fairly large leaf; if it includes the leaf bud as well, it is called flowery orange pekoe. Broken teas, those with leaves broken during processing, are graded as broken orange pekoe; the largest tea segments; broken pekoe; pekoe fannings; and dust, the smallest leaf particles. Fannings fill the world's tea bags. Instant tea is made, like instant coffee, by spray-drying a strong black-tea brew.

The overall quality of black tea is assessed by highly skilled testers who have developed a glossary of terms to describe the quality attributes of tea liquor. In the simplest case it could be described using five parameters *i.e.* color, strength, brightness, briskness, and flavor.

CHEMISTRY OF BLACK TEA MANUFACTURING

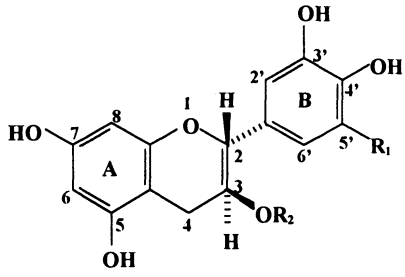
The manufacturing of black tea involves an intensive oxidation of green tea flavan-3-ols (catechins) by the enzyme polyphenol oxidase, resulting in formation of catechin quinones. Further reactions and condensation of these quinones lead to the production of colored complex polyphenols known as theaflavins and thearubigins. However, the extent of these reactions depends on the composition of the fresh green tea leaf (Robertson and Bendall, 1983).

Green Tea Polyphenols

Green tea leaves are particularly rich in polyphenolic compounds that belong to the flavonoid group, but their concentration will vary as a function of the genetic strain, climate and growing conditions (Owour and Langat, 1988; Reeves *et al.*, 1987).

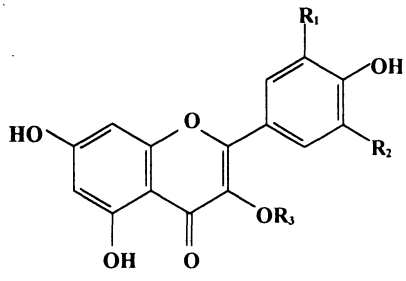
The term flavonoid describes those compounds whose structure is based on that of the flavone structure, consisting of two benzene rings 'A' and 'B' joined together by a three-carbon link which is formed into a g-pyrone ring (Figure 1). The various classes of flavonoids differ by the oxidation of the 3-C link from flavan-3-ols (Catechins), flavanols (3-hydroxyflavones) and anthocyanins. Also included are the flavanones, dihydroflavonols and the flavan-3,4-diols (proanthocyanidins). Other compounds (*i.e.* dihydrochalcones, chalcones, isoflavones, neoflavones & aurones) do not actually possess the 2-phenylchrome skeleton, but are closely related to them and have also been included in the flavonoid group.

The 'A' ring of the majority of flavonoids is hydroxylated either at C-5 and/or C-7, or only at C-7. The 'B' ring is usually substituted by either one, two or three hydroxyl or methoxy groups at C-3', C-4' and C-5'. Flavonoids usually exist as glycosides. The sugars found in the flavonoid glycosides include hexoses, pentoses, di- and trisaccharides; D-glucose is the most common sugar whether alone or as a part of a disaccharide (Swain, 1976; Harborne, 1976).



CATECHINS

R_1= H, R_2=H (+) Catechin
R_1= OH, R_2=H (+) Gallocatechin
(oposite configuration at C-3)
R_1, R_2=H (-) Epicatechin
R_1= OH, R_2=H (-) Epigallocatechin
R_1= H, R_2=gallate (-) Epicatechin gallate
R_1= OH, R_2=gallate (-) Epigallocatechin gallate



FLAVONOLS
R_1, R_2, R_3=H Kaempferol
R_1= OH, R_2,R_3=H Quercetin
R_1, R_2=OH, R_3=H Myricetin
R_3=glucosyl, etc = flavonol glucoside

Figure 1. Structure of green tea flavan-3-ols (Catechins) and flavonols.

Flavonoids are widely distributed in higher plants. They contribute to the color of fruits and flowers either as colorants or as co-pigments, but also, they are present in higher concentrations in young leaves (Geissman, 1963). Their presence in leaves appears to have a protective role against insects, however, it has been suggested that leaf flavonoids also have a protective action against UV radiation, and that, they may take part in photosynthesis. Some of them have biochemical and pharmacological applications as they have anti-inflammatory, anti-allergic and anti-carcinogenic effects (Hertog *et al.*, 1993).

Phenolics constitutes approximately 15–30% of the dry weight of green leaf; the most important of which are the flavan-3-ols (catechins), flavonols and flavonol glycosides, their structures are presented in Figure 1. These phenolic constituents are the key reactants involved in the manufacture of black tea. Catechins represent about 80% of the total polyphenols and are responsible for the metallic taste and astringency of green tea. Organic acids (caffeic acid, gallic acid, cinnamic acid and their quinic acid esters) and methyl xanthines (caffeine and theobromine) are minor constituents but not less important in tea quality.

The biosynthesis of the green tea flavonoids has been discussed by Wong (Wong, 1976). Cinnamic and coumaric acids are the precursors of the green tea polyphenols. Both organic acids are produced from carbohydrates through the shikimic acid pathway, in which, the amino acid phenylalanine is produced from glucose. Deamidation forms cinnamic acid, then hydroxylation of cinnamic acid forms coumaric acid. Co-enzyme A esters of these acids react with malonyl-CoA to form chalcones, then ring closure of chalcones results in dihydroflavonols, which then react to form flavonols through ketoreduction of ring B and hydration of ring A, or flavan-3-ols through ring A hydration and ring B reduction.

Flavonoid Oxidation

A series of oxidative reactions whereby the simple catechins are converted to more complex polyphenols (theaflavins and thearubigins) are catalysed by the enzyme polyphenol oxidase in the presence of oxygen.

It is known that theaflavins are produced from the catechins present in the leaf, and that their concentration increases as the tea fermentation proceeds, but smaller amounts are present at longer fermentation periods, comprising 1–2% of the dry weight of the black tea. On the other hand, thearubigins comprise 10–20% of the dry weight and their concentration not only increases during fermentation, but firing causes additional increase (Sanderson *et al.*, 1972). It is likely that about 10% of the catechins are converted to theaflavins and 90% to the thearubigins or to other condensation products.

The ratio between theaflavins and thearubigins is an important factor for the flavor of tea (Robertson, 1983b). The proportion of simple to gallate catechins in the green tea leaf is important in determining the final theaflavin level, rather than the total concentration of catechins. A high proportion of catechins tends to produce tea with a high theaflavin level. The optimum temperature for theaflavin and thearubigin formation is 30ºC but a decrease to 20°C had little effect on theaflavin concentration, and optimal pH values for theaflavin and thearubigin formation are 5 and 6, respectively (Robertson, 1983a). Oxidative degradation of amino acids and carotenoids also occur during fermentation and is related to the development of the tea aroma (Sanderson and Graham, 1973).

Activity of the polyphenol oxidase determines the tea quality. It has a molecular weight of 140kDa and is located in the leaf microsomes, but during the rolling and crushing stages, the cellular structure of the tea leaf is altered, thus allowing the enzyme to react with the cytoplasmic catechins (Cloughley *et al.*, 1981; Coggon *et al.*, 1977).

Several isoforms of PPO have been identified in tea leaves by Coggon *et al.* (1973) and Takeo and Baker (1973). Both workers proposed that there are at least three copper containing isoenzymes that may have a different activity for different substrates, and that the proportion of each isoform changes with the age of the leaf. On the other hand, the enzyme peroxidase and gallate esterase also affect the fermentation process, because they promote oxidation and oxidative degallation of phenolics and formation of high molecular weight protein-phenol complexes, as well as decreasing the level of the flavonol glycosides (Finger, 1994; Mahanta *et al.*, 1993).

BLACK TEA CONSTITUENTS

Black tea infusion consists of a complex mixture of polyphenols which account for 48% of the dry weight, caffeine 7%, organic acids, amino acids, proteins, carbohydrates, and other minor constituents. Black tea is low in catechins but has significant quantities of complex polyphenols (theaflavins and thearubigins) that are crucial elements for color, astringency and "tangy" taste, while caffeine accounts for the bitterness.

Theaflavins

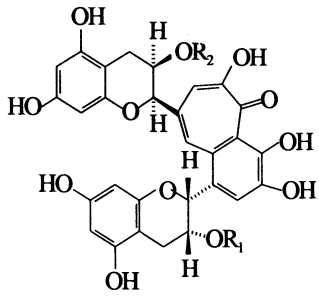
These compounds (Figure 2) are formed during fermentation from the flavan-3-ols by condensation of a simple catechin and a gallocatechin (Collier *et al.*, 1973), resulting in a flavonol with a benzotropolone ring system. Theaflavins are responsible for the characteristic red color, astringency (briskness), and the brightness of black tea liquors (Sanderson *et al.*, 1975). Several studies have demonstrated the relationship between the theaflavin levels and quality. Results showed that the quality of tea is related to the ratio of the individual theaflavins as each one has a different astringency. Also, concentration of theaflavins varies with the clone, time of fermentation and type of processing (Owour *et al.*, 1986; Owour, 1994; Owour and McDowell, 1994). The theaflavin fraction represents only 1–2% of the total solids in black tea, and is composed of theaflavin (18%), theaflavin-monogallate (8%), theaflavin-3'-monogallate (20%), theflavin-3-3'digallate (40%) and other minor theaflavins (4%), varying with the conditions during fermentation.

Epitheaflavic Acids and Bisflavonols

Epitheaflavic acids are minor components, also produced through the same basic mechanism as theaflavins from a reaction between a gallic acid quinone and the quinone of epicatechin or epicatechin gallate (Berkowitz *et al.*, 1971; Balentine, 1992). Bisflavonols are another group of polyphenols, also produced during the manufacturing of black tea. These compounds are dimmers of the catechins produced from flavan-3-o-quinones by covalent linkage of ring B. Structures of theaflavins, epitheaflavic acids and bisflavonols are presented in Figure 2.

Thearubigins

These are a heterogeneous and probably unstable group of complex polyphenolic compounds, with molecular weights ranging from 700 to 40000, formed by the oxidative condensation of simple flavonols during the fermentation process. They constitute 10–20% of the dry weight of black tea, and contribute significantly towards taste, color



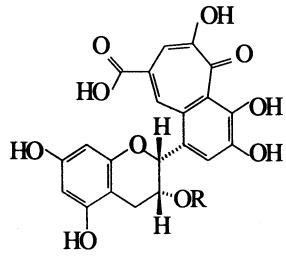
THEAFLAVINS

$R_1 = R_2 = H$ Theaflavin

R_1=gallate, R_2= H Theaflavin gallate A

R_1=H, R_2= gallate Theaflavin gallate B

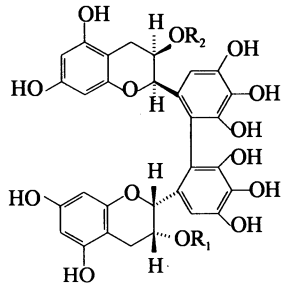
$R_1 = R_2$=gallate Theaflavin digallate



EPITHEAFLAVIC ACIDS

R=H Epitheaflavic acid

R=gallate Ephiteaflavic gallate



BISFLAVONOLS

$R_1 = R_2$=gallate Bisflavonol A

R_1=gallate, R_2=H Bisflavonol B

R_1=H R_2= gallate Bisflavonol C

Figure 2. Structures of theaflavins, epitheaflavic acids, and bisflavonols.

and body of tea brew. Originally, these compounds were classified as brown, weakly acid pigments, and classified according to their solubility in ethyl acetate as the S_I and the aqueous fraction as S_{Ia} and S_{II} thearubigins.

There is still little progress in elucidating the thearubigin structures and their exact contribution to black tea quality. Attempts to separate thearubigins by paper, thin layer and open column chromatographies failed to obtain material that was of any use for subsequent structure elucidation. Not only there is the possibility of artifact formation, because of the time taken for sample preparation, but there is also, an irreversible binding of some thearubigins to the stationary phase. More effective separation of the thearubigin fraction was achieved using HPLC (Opie *et al.*, 1990; Robertson and Hall, 1989; Bailey *et al.*, 1990; 1992, 1994a).

Brown *et al.* (1969) classified the thearubigins, extracted from aqueous solutions by organic solvents, as polymeric proanthocyanidins (procyanidin, procyanidin-3-gallate, prodelphinidin and prodelphinidin-3-gallate), where those in the aqueous phase were the

protein-polyphenol type. Bailey *et al.* (1991, 1994a,b) have studied fractionated tea liquors using liquid chromatography and plasmaspray mass spectrometry. Only low molecular weight species corresponding to flavonols, flavonol gallates, chlorogenic acid, caffeine, quinic acid, and flavonol glycoside fragments were detected in whole tea liquors. Isolation and analysis of the thearubigin fractions gave gallic acid, quinic acids, flavonol gallates and theogallin ions, suggesting that thearubigins are anthocyanidin-like polymers. However, acid hydrolysis of a thearubigin fraction called theafulvin, is a flavonol polymer with other linkages different to the proanthocyanidin polymers thus supporting the theory that thearubigins are flavonol polymers. Also, Ozawa *et al.* (1996), extracted butanol-soluble neutral and acidic thearubigins present in black tea by a combination of solvent extraction, fractional precipitation and column chromatography using Toyopearl; and suggested that thearubigins are heterogeneous polymers of flavan-3-ols and flavan-3-ol gallates which have a bond at C4, C6, C8, C2', C5' and C6' in the flavan-3-ol units; in addition to the C4-C8 or C6 interflavanoid linkage with a C6'-C6' linkage.

Aroma Constituents

Many volatiles have been detected in green tea, but about 30 of them have been identified, being benyl, phenylmethyl alcohols, hexenol, linalool, geraniol methyl salicylate as the main components. In black tea, however, 300 compounds have been detected, but just half of them have been identified. None of them possess the unique black tea aroma alone, and tea aroma is considered to be due to a mixture of aroma compounds present. Amino acids, fatty acids and carotenoids are aroma precursors (Co and Sanders, 1993; Kawakami *et al.*, 1995). Their degradation take place during the flavonoid oxidation mainly due to the action of pro-oxidant products. Aldehydes are derived from amino acids, for instance, theanina an ethyl amide of the glutamic acid is the main amino acid in the green leaf and contributes from 1–2% of the dry weight. Trans-2-hexenal is formed from linolenic acid and is one of the main components of the tea aroma (Cloughley *et al.*, 1981).

CHANGES DURING BREWING

Tea is made from the infusion of leaves in boiling water, thus, size of the tea leaf, nature and accessibility of the solute, time and temperature of infusion, water composition, etc., affect the quality of this beverage. Tea solids have classified into three groups according to solubility as: instantaneously soluble, rapidly soluble and slowly soluble (Long, 1997, 1979). The instantaneously soluble components must be immediately accessible to water and be outside the tea leaf; while the rapidly soluble must be extracted from inside the tea leaf, where the cellular structure retards both solvent penetration and outward diffusion. The slowly dissolving components may be either high molecular weight material which diffuse more slowly through the leaf matrix or products which are formed during brewing. On the other hand, temperature of extraction also affects extraction of tea solids, for instance, theaflavins and thearubigins have maximal diffusion rates at 79° and 94°C, respectively, and it is possible that thearubigins are relatively inaccessible and become fully extractable only at high temperatures (Spiro and Siddique, 1981,a,b). In addition, the presence of salts slightly increases the rate of extraction, but higher pH does not increase the extractability of theaflavins (Spiro and Price, 1987,a,b; Spiro *et al.* 1987). Moreover, an insoluble film called tea scum appears on the surface of a hot tea infusion if tea has been brewed in water which contains calcium and dihydrogencarbonate ions.

It is known that tea liquors darken in color even over short periods, Mellon *et al.* (1987) indicated that there is a substantial increase in higher molecular weight compounds, which is favored when oxygen is present. It was also observed that the amount of theaflavins is redueed during brewing. When the theaflavin fraction was removed by extraction with ethyl acetate, prior to brewing, similar color darkening took place upon ageing brews. Therefore, Mellon *et al.* (1987) concluded that higher molecular weight compounds were due to the continuing polymerization of phenolic compounds and due to chemical combination of proteins with polyphenols. They also pointed out that low molecular weight acids like Theogallin which is water soluble, may be involved in the formation of these compounds. Kuhrs *et al.* (1994) have also reported a decrease of theaflavins, thearubigins and other pigments in brews left at 90°C for periods of more than 2h, suggesting possible reaction. However, the smaller amounts of extracted thearubigins are more likely to be the result of incomplete extraction rather than degradation, as this fraction contains less hydrolysable bonds.

Moreover, it is known that caffeine self associates in aqueous solutions, thus, phenolic association with other solutes or with the internal surface of the leaf has to be taken into consideration (Price and Spitzer, 1993) in order to explain the rapid reduction of phenolics observed in tea brewed for longer periods.

Several changes occur when tea liquor cools down to room temperature (Spiro and Jaganyi, 1994,a,b), like formation of tea cream which is an insoluble opaque precipitate and contains caffeine-polyphenol complexes. Powell *et al.* (1992) reported that removal of caffeine by chloroform extraction reduced formation of caffeine-polyphenol complexes. Some authors suggest that proteins and lipids also intervene in the tea cream formation (Bee *et al.*, 1987; Ramachandran and Nagalakshimi, 1988).

ANALYSIS OF TEA POLYPHENOLS

Flavonoids in foods have not been extensively investigated, because of the lack of good analytical methods. Most identification procedures for flavonoids involve controlled hydrolysis to their aglycone, sugar and possibly acyl components followed by chromatography with identification based on Rf values, or absorbance behavior in the 300–400 nm range or fluorescence with aluminum chloride.

There are many approaches involving chromatographic separations in the determination of the phenolic composition of black tea. Early work on the analysis employed thin layer chromatographic techniques. Lately, gel filtration and HPLC reversed phase liquid chromatographic (Bailey *et al.*, 1990, 1993; Opie et al;, 1990; Taylor and Clydesdale, 1987; Galleti *et al.*, 1992) methods were developed for analysis of both simple and complex polyphenols), but none has been entirely satisfactory. HPLC methods generally employ reverse phase C_{18} chromatography columns using acetonitrile and acetic acid buffers as a mobile phase. The main problems involved in polyphenolic analysis are the need for authentic standards and the fact that these compounds have high affinities for the stationary phases and become strongly adsorbed (Balentine, 1992). Other spectrometric techniques like electron impact mass spectrometry (EI-MS) have provided molecular and structural information, however, flavonols, flavonol-glycosides and bisflavonoids are not sufficiently volatile and must be derivatised to improve their volatility. Permethylation and trimethylation are the methods commonly used, but they increase the molecular mass and often produce mixtures of partially derivatised compounds which require a subsequent purification step. Recently, phenolic standards have been analyzed using FAB and ther-

mospray mass spectrometry; and there is general agreement that phenolic compounds might be characterized by mass spectrometry using soft ionization techniques which require no chemical derivatisation prior to analysis.

Thermospray mass spectrometric analysis, coupled to HPLC, was reported by Galleti *et al.* (1992), who examined various standard phenolic compounds. Protonated $[M+H]^+$ species were observed, but intense solvent cluster ion series $[(MeOH)_n + H_2O]^+$ and solvent adducts $[M+ MeOH + H]^+$ derived from the HPLC solvent were also detected. Ions derived from aldehydes were more intense than those obtained from acids when equimolar mixtures were analyzed, thus suggesting differences in proton affinity between these compounds. Fragmentation was also evident in acids, with losses of 44 (-COO) and 18 (H_2O) units. Lin *et al.* (1993) also characterized some flavonoids extracted from the tea plant using thermospray liquid chromatography mass spectrometry and MS-MS. Flavonols were recognized by their $[M+ H]^+$ ions, and MS-MS gave simple fragmentation patterns, showing three types of ring cleavages at the pyran ring.

Self *et al.* (1986) applied positive and negative ionization FAB mass spectrometry coupled with HPLC to characterize vegetable tannins (proanthocyanidins and esters of gallic acids). $[M+ H]^-$ ions were visible, but glycerol adducts were very intense and dominated the spectra, and the positive ionization mode gave inferior spectra. Mellon *et al.* (1987) and Bailey *et al.* (1994a) compared thermospray and FAB mass spectrometry for phenolic compounds analysis, using negative and positive ionization modes, respectively, although Mellon *et al.* (1987) suggested that molecular weight information was only obtained in the negative ionization mode. However, both authors concluded that the FAB spectrum is more complex because of the presence of glycerol adducts, as samples are mixed with glycerol prior to ionization. Also, FAB produces more fragmentation than thermospray ionization but it ionizes a wider range of compounds.

Black tea fractions were studied using FAB and thermospray mass spectrometry (Bailey et al., 1992, 1994a,b). Abundant ions were observed between *m/z* values 100 to 980, like catechol (*m/z* 110.8), protocatechuic acid (*m/z* 152.6), gallic acid (*m/z* 168.6), caffeic acid (*m/z* 178.7), quinic acid (*m/z* 190.8), epicatechin (*m/z* 289), epigallocatechin (*m/z* 304), chlorogenic acid (*m/z* 353.1), 4-coumarylquinic acid (*m/z* 337) and theogallin (*m/z* 343) , but none of them resembled the complex theaflavins and thearubigins, and it was suggested that they are probably flavonol fragments. On the other hand, Sakata *et al.*, (1986) reported quinic acid as the major tea component in samples from green and black tea liquors by NMR spectroscopic analysis. However, it may be the result of the hydrolysis of chlorogenic acid and theogallin.

Problems derived from the LC-MS analyses are the different ionization efficiencies of each compound, giving rise to ions with different intensities, even if they were present in the mixture at equimolar concentrations. There may also be competition for the ionization potential and this effect might be greater in the complex composition of tea liquors such that some components may not be fully ionized and thus produce low intensity values. On the other hand, phenolics contain oxygen and they are regarded as being highly reactive with regards to ionization. New developments in LC-MS will undoubtedly assist in improving the analysis of phenolics.

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20

TEQUILA AROMA

Mercedes G. López

Unidad de Biotecnología e Ingeniería Genética de Plantas
Centro de Investigación y de Estudios Avanzados del IPN
Apartado Postal 629, 36500 Irapuato, Gto., México

Tequila (*Agave* sp.) is one of the most consumed Mexican liquors worldwide. Commercial tequilas (blanco, reposado, and añejo) were purchased in shops and supermarkets. Determination of major volatiles in all samples was carried out by adding 100 μL of 1-nonanol as an internal standard to 1 mL of each tequila. However, minor volatiles were examined by liquid-liquid extraction. Fifteen milliliters of tequila were extracted with 15 mL (3 × 5 mL) of dichloromethane. The internal standard in this case was 3-octanol. The extracts were concentrated in a Kuderna-Danish apparatus to 50 μL. GC-MS analyses were performed in two capillary columns HP-FFAP and HP-5 MS. Among the major volatiles, 3-methylbutanol and phenylethyl alcohol were most abundant in all types of tequila, however, some fatty acids were also detected in most of the reposado and añejo types. Minor volatiles, on the other hand, varied widely between tequilas. Alcohols (C_3-C_{16}), esters (C_6-C_{18}), and acids (C_2-C_{12}) were the most common volatiles. Various furans and nitrogenous compounds were also present. Significative differences, both qualitative and quantitative were found among all tequilas. Major and minor volatiles were sniffed in a GC-O. The evaluation of potent odorants demostrated that volatiles with sweet, floral, woody, and tepache-like notes are the impact character compounds of tequila. According to this study most of the volatile contributors to the aroma of tequila are similar to those reported for wine and sake. However, mezcal (*Agave angustifolia*) was used as a negative control.

INTRODUCTION

Blue *Agave tequilana* Weber is native of Mexico, specifically from the state of Jalisco. An alcoholic beverage called Tequila is obtained from the fermentation of the cooked "piña" of the agave. Tequila might be made of a 100% agave or at least 51% agave and the rest of carbohydrates from other sources. Tequilas have been classified into three general types based on the process that they undergo just after distillation. Tequila blanco is bottled after distillation, tequila reposado can be aged from 3 to 12 months in oak casks, and añejo type can be aged from 1 to 5 years.

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Very few studies have been undertaken on tequila aroma. The first reports date from 1969 by Manjarrez and Llama (1969) and by Incitti *et al* (1980). However, recently, Benn and Peppard (1996) published the most potent odorants in some tequilas by instrumental and sensory analysis and Pinal *et al* (1997) reported the parameters affecting the production of higher alcohols in tequila. Nevertheless, there are not still enough information in the 250 different brands of tequilas sold in the markets. Therefore, we decided to establish some of the most abundant components present in blanco, reposado, and añejo tequilas.

MATERIALS AND METHODS

Materials

Commercial tequilas (blanco, reposado, and añejo) and mezcal (negative control) were purchased in shops and supermarkets. Solvents were purified and distilled. Standards were from various suppliers and the hydrocarbon series (C_6-C_{32}) was obtained from Aldrich.

Isolation of Major Volatiles

Determination of major volatiles in all samples was carried out by adding 100 μL of 1-nonanol (1 μL/1 mL CH_2Cl_2) as an internal standard to 1 mL of each tequila. Two microliters of each sample were analyzed by gas chromatography-mass spectrometry (GC-MS) in two columns, HP-5 MS and HP-FFAP.

Isolation of Minor Volatiles

Minor volatiles were isolated by liquid-liquid extraction. Fifteen milliliters of tequila were extracted with 15 mL (3 × 5 mL) of dichloromethane. The internal standard in this case was 3-octanol. The extracts were dried over anhydrous Na_2SO_4 and then concentrated in a Kuderna-Danish apparatus to 50 μL and analyzed by gas chromatography-mass spectrometry (GC-MS) in the same two columns.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was carried out using a Hewlett-Packard 5890 Series II coupled to Hewlett-Packard mass selective detector (MSD). Two capillary columns were used a HP-FFAP (25 m × 0.25 mm) and HP-5 MS (50 m × 0.32 mm). The apolar column was used under the following operating conditions: carrier gas, helium, 1 mL/min; temperature programme, 50°C (2 min), increased at a rate of 4°/min to 260 for 40 min; split ratio, 1:80; detector and injector temperatures 200 and 250°C, respectively. The extracts in the HP-FFAP were analyzed at oven temperature of 80°C, for 2 min, at a rate of 2.5°/min to 220°C for 30 min. The injector and detector temperatures were 180 and 220°C, respectively.

Gas Chromatography-Olfactometry (GC-O)

Hewlett-Packard 5890 Series IIg chromatograph coupled to an Analit olfactometer detector in the HP-FFAP under the same conditions mentioned above was used. Only the original extracts were analyzed by 6 different panelists.

RESULTS AND DISSCUSION

Significative differences, both qualitative and quantitative were found among all tequilas in major and minor volatiles. Table 1 shows the major volatiles found in all tequilas. Among these volatiles, 3-methylbutanol and phenylethyl alcohol were the most abundant components in all types of tequila, however, some fatty acid were also detected in most of the reposado and añejo types, essentially, decanoic and dodecanoic acids. Figure 1 (a and b) shows a typical chromatogram of tequila añejo. Table 2 lists the minor

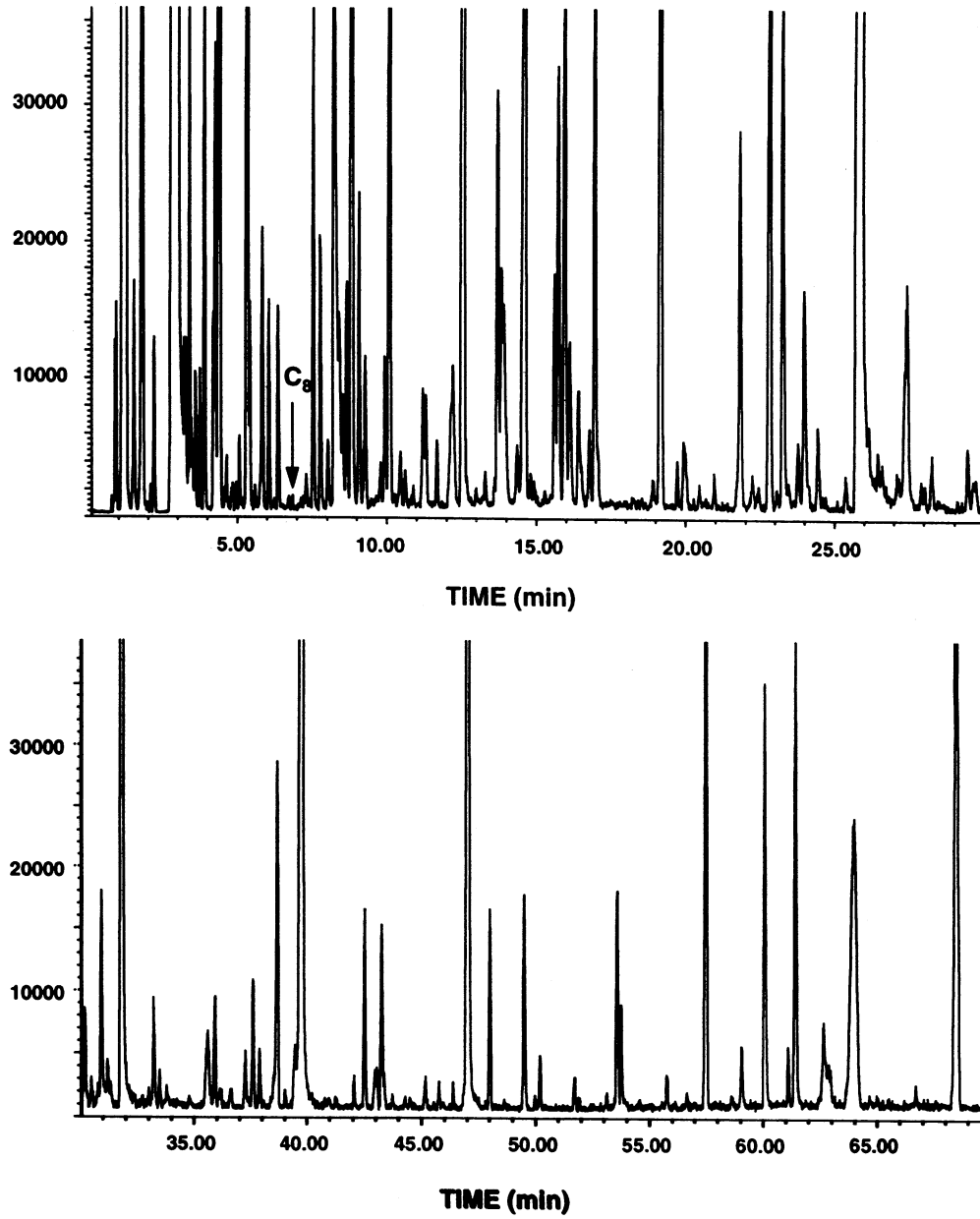

Figure 1. Typical chromatogram of volatiles of tequila (*Agave tequilana*) añejo using a HP-FFAP column.

Table 1. Major aroma components of tequila (ppm)[a]

Compound	Blanco	Reposado	Añejo
Ethanol	9872.00	2606.63	5033.42
2/3-Methylbutanol	34.36	4.96	10.46
Phenylethyl alcohol	1.19	0.31	0.53
Acetic acid			0.40
Decanoic acid		0.47	
Dodecanoic acid		5.17	

[a]ppm, parts per million

Table 2. Minor aroma components of tequila extracts in (ppm)[a]

Rt[b]	Compound	Blanco	Reposado	Añejo	Mezcal
	Alcohols				
1.52	Propanol	1.07	2.42	3.65	+
1.80	2-Methylpropanol	27.99	29.41	24.25	+
2.23	Butanol	0.69	2.39	1.98	
3.08	2/3-Methylbutanol	607.00	770.72	816.12	+
3.42	3-Methyl-3-buten-2-ol	1.31			
3.44	Pentanol	0.17			
4.39	2-Penten-1-ol	2.68			
4.70	2-Heptanol	0.94			
5.41	Hexanol	0.35	0.65		
9.25	3-Ethylhexanol	0.55	1.96		
11.23	2,3-Butanediol	1.62			
11.69	Octanol	0.28	0.41		
13.69	3,3-Diethoxypropanol	0.93			
25.74	Phenylethyl alcohol	76.09	150.00	206.49	+
28.00	Dodecanol	0.39	0.82		
35.88	Hexadecanol	5.20	3.15		
43.14	6-Methylheptanol	0.24			
	Esters				
3.32	Ethyl hexanoate	0.56			
5.32	Ethyl 2-hydroxypropanoate	3.18	9.93	23.95	+
7.56	Ethyl octanoate	2.52	4.09	5.20	+
9.03	6-Methylheptyl-2-propenoate	0.44	3.44		
14.65	Ethyl decanoate	8.77	14.66	19.61	+
21.13	2-Phenylethyl acetate	7.08	10.54	5.13	+
22.81	Ethyl dodecanoate	9.10	11.02	10.27	+
30.84	Ethyl tetradecanoate	0.45			
38.62	Ethyl hexadecanoate	1.91	0.83	6.67	+
42.98	2-Phenylethyl octanoate	0.36	1.89		
47.94	Ethyl linoleate	0.29	0.27	3.63	
	Acids				
8.21	Acetic acid	27.06	27.41	14.32	+
11.20	Propanoic acid	1.56	1.76		
12.24	2-Methylpropanoate	1.04	1.58	3.68	+
18.89	Pentanoic acid	0.30			
23.28	Hexanoic acid	1.58	8.48	9.82	+
31.87	Octanoic acid	41.12	123.26	82.00	+
39.78	Decanoic acid	49.46	109.32	150.49	+
46.95	Dodecanoic acid	15.14	30.53	84.90	+
53.24	Tetradecanoic acid	1.87			
57.15	Hexadecanoic acid	0.24	0.36		

(*Continued*)

Table 2. (*Continued*)

	Furans				
3.88	2-Methyldihydrofuran-3-one	0.94	2.79	4.13	+
8.62	5-Ethenyltetrahydro-2-furan[c]	0.59	3.09		
8.83	Furfural	0.88			
10.12	1-(2-Furanyl) ethanone	5.98	16.13	9.01	+
12.49	5-Methylfurfural	1.63			
13.58	2-Furfuryl alcohol	0.37			
13.95	2-Acetyl-5-methylfuran	0.55	0.62		
15.98	3-Furfuryl alcohol	14.66	5.32		
30.12	5-Pentyldihydrofuran-2-one	0.48	3.07		
47.83	5-(Hydroxymethyl)furfural	0.52			
	Ketones				
3.19	2-Methylcyclopentanone	0.64	1.29	1.25	+
4.25	3-Hydroxybutan-2-one	1.91	2.26	3.39	+
16.36	3 Ethyl-2-hydroxy-2-cyclopenene-1-one		0.96		
	Terpenes				
7.78	Linaloxide	0.60	1.52	2.30	+
11.28	Linalool	0.58	0.54		
16.91	Linalyl propanoate	2.55	5.02	8.37	+
30.91	Nerolidol	1.33	4.41		
37.54	Thymol	1.03	0.90	1.41	
	Nitrogenous				
5.82	1-Methylpyrazole	3.25			
12.54	3,4,5-Trimethylpyrazole	1.58			
19.11	1,6-Dimethylpyridinethi-2-one	0.50	18.52		
20.92	3,4-Dihydropyran	0.28			
27.93	3,4-Dimethylpyridine	1.58			
	Others				
9.93	3-Methyl-2-pentene	0.19			
37.19	3,4-Diethylphenol	1.01	0.76	2.47	
43.18	Cyclotetradecane	1.61			
47.87	9-Eicosyne	0.61			
49.41	Vanillin	3.19			
61.32	Syringaldehyde	0.75			

[a]ppm, parts per million; + compound present in mezcal that was found in all types of tequilas.
[b]retention time in the HP-FFAP;[c]compound that coeluted with another.

volatiles identified in all tequilas and mezcal (negative control for the raw material). Twenty seven of the identified compounds were common to all tequilas, and 22 of these were also present in mezcal. Tequila volatiles were 163, 175, and 198 for blanco, reposado, and añejo, respectively. However, only 39, 53, and 43 were completly identified (same order). Alcohols (C_3-C_{16}), esters (C_6-C_{18}), and acids (C_2-C_{16}) were among the most abundant characterized compounds, followed by furans, terpenes, and nitrogenous compounds as well as some phenolic compounds such as vanillin.

Besides the 22 common compounds to tequila, some alkynes, nitrogenous and sulfer compounds were also identified in the mezcal extract, these compounds were not present in tequilas. These minor components might be responsable for the large sensory differences between these alcoholic beverages.

Seventeen alcohols were fully characterized among tequilas, four of which were also present in mezcal. The most abundant alcohols were 2/3-methylbutanol, phenylethyl alcohol, 2-methylpropanol, and propanol. Among esters, ethyl 2-hydroxypropanoate, ethyl decanoate, ethyl dodecanoate, and phenylethyl acetate were the most abundant, mainly in the

Table 3. Impact character components on the original tequila extracts

RI[a]	Descriptor	Compound
1092	Tepache	Unknown
1101	Tequila	Unknown
1223	Sweet	2/3-Methylbutanol
1275	Floral	Phenylethyl acetate
1567	Sweet, floral,lemon	Linalool
1887	Woody, fruit	Phenylethyl alcohol
2248	Butter	Decanoic acid
2277	Warm-phenolic	Unknown
2538	Tequila	Unknown
2552	Very sweet	Vanillin

[a]retention index in the HP-FFAP column

añejo type. The predominance of ethyl esters is due to the esterification of acids with ethanol during storage. Besides, a good correlation was found between acids and esters in almost all tequila types. Furans and terpens were typical of reposado type and phenols and nitrogenous compounds of the añejo type.

The use of a 50 m-long HP-5 MS allowed the separation of 3-methylbutanol and 2-methylbutanol; the proportion of these two isomers in tequila was about 5 to1. This column also allowed the separation of phenylethyl alcohol and phenylmethylhydrazine.

Table 3 shows the main impact character compounds identified in the original extracts detected by 6 people in a HP-FFAP column. The predominant descriptors are tepache, tequila, sweet, floral, woody, phenolic, and vanillin.

The qualitative and quantitative variations found in this study might be due to parameters like yeast strain used during fermentation, cooking temperature and time of the heart agave, distillation, location, and harvest time of agave (7 to 12 years). Studies controlling some of these parameters are being pursued.

CONCLUSIONS

Most of the flavor differences found in tequilas (blanco, reposado, and añejo) may be due to their elaboration process, since all of them come from the same raw material (*Agave tequilana*), however, the specific geographic location and harvest time are not known most of the time. These might also be important in the final flavor profile of the product. On the other hand, similarities found with volatiles of mezcal may be due to the raw material (*Agave angustifolia*).

The evaluation of potent odorants demostrated that volatiles with sweet, floral, woody, and tepache-like notes are the impact character compounds of tequila. According to this study most of the volatile contributors to the aroma of tequila are similar to those reported for many wines and sake. However, their unique overall aroma might be due mainly to the synergism of multicomponents (minor volatiles).

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THE FORMATION AND RELEASE OF ODOR ACTIVE COMPOUNDS DURING OXIDATION OF VEGETABLE OILS

J. P. Roozen, P. A. Luning, and M. A. Posthumus

Department of Food Technology and Nutrition Sciences
Laboratory of Food Chemistry
Wageningen Agricultural University
P.O. Box 8129, 6700 EV Wageningen, The Netherlands

The formation of volatile secondary lipid oxidation products during storage and processing has detrimental effects on the quality of lipid containing foods. Release of volatile compounds is diffusion controlled, and is affected by temperature and physical state of lipids. In this study, the development of odor active compounds was investigated in sunflower and olive oils, and their I and 50% oil-in-water emulsions during storage at 55'C. Volatile compounds were analyzed by dynamic headspace analysis, and their odor activity was determined by a trained GC sniffing port panel. The number of odor active compounds detected in the I and 50% o/w emulsions was higher than in the bulk oils. The amounts released of pentanal (buttery note) and hexanal (grassy note) were highest in the 1% o/w emulsions.

INTRODUCTION

Autoxidation of unsaturated fatty acids causes deterioration of the quality of edible oils and lipid-containing foods. An important problem is the formation of volatile secondary lipid oxidation products during storage and/or processing (Mistry and Min, 1992). The majority of these volatile compounds are lipophilic and therefore mostly present in the lipid phase of a foodstuff. The release of volatiles in the mouth is controlled by diffusion into and mixing with saliva (McNulty, 1987). This process is affected by the physical state of lipids and by the temperature (Kinsella, 1990). In preparation of low-fat foods decrease of the fat content can affect the release of volatile off-flavors (Roozen *et al.* 1994). It has been demonstrated that lowering of the oil fraction in emulsions increases the relative amount of hexanal released from emulsions.

This study intended to analyze the development of primary and secondary oxidation products of sunflower and olive oils, and their corresponding 1 and 50% o/w emulsions

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during storage at 55°C. In addition the odor active compounds were determined by sniffing port analysis, as well as the effect of emulsification on their composition.

EXPERIMENTAL PROCEDURES

Vegetable Oils and Their Emulsions

Sunflower oil (fatty acid C18 composition: C18:3 0.3%; C18:2 63.5%; C18:1 23.3%; C18:0 4.3%), and olive oil (fatty acid C18 composition: C18:3 0.6%; C18:2 7.8%-C18:1 73.5%; C18:0 2.7%) were obtained from Unilever (Vlaardingen, The Netherlands). Fifty percent o/w emulsions were prepared from both oils by dispersing 50 ml of oil in 50 ml of water using a 2% (v/v) Tween-20 solution (polyoxyethylene sorbitan monolaureate, Sigma Chemical Co., St. Louis, MO). The mixture was homogenised under pressure (650 bar). The 1% o/w emulsions were prepared by diluting the 50% o/w emulsion with 3% (v/v) Tween-20 solution. All samples (65 ml, n=4) were stored in 350 ml glass jars in the dark at 55°C and gently stirred.

Peroxide Value (PV)

Five gram samples were weighed in 200-ml Erlenmeyer flasks, and 10 ml of chloroform, 15 ml of acetic acid (Merck), I ml of saturated potassium iodide and 75 ml of water were added prior to titration of the mixture with sodium thiosulfate.

Conjugated Diene Hydroperoxides

UV measurement was carried out in the oils and the oils extracted from the emulsions. For the extraction 5g of emulsion were added to 25 ml of methanol (Merck). After 15 min the methanol-water layer was removed and the remaining oil was used for analysis of the dienes. An aliquot of pure oil or extracted oil was dissolved in 5 ml of cyclohexane (Merck), the absorbance measured at 234 nm, and calculated per mg of oil.

Static Headspace Analyses

Volatile compounds of 0.5 ml of oil or o/w emulsion were analyzed by static headspace capillary gas chromatography (SHGC) in special 10-ml headspace bottles, which were inserted into a HS 800 Fisons instrument headspace sampler at 10°C. The HRGC 5300 Mega series gas chromatograph (Carlo Erba Instruments) was equipped with a capillary DB-wax (30 m × 0.542 mm, 1.0 gm thickness; Chrompack) and programmed as follows: 1 min isotherm at 50°C, 2.5°C/min from 30 to 120°C, 40°C/min from 120 to 180°C, and 1 min isotherm at 180°C. Headspaces of two alkane mixtures (C7–C10; C11–C14) were analyzed to calculate Kovats indices.

Dynamic Headspace Analysis

An aliquot of 2 ml of vegetable oil or emulsion were transferred to a 20-ml glass bottle for dynamic headspace purge and trap isolation of volatile compounds using Tenax TA as adsorbent. The samples were kept at 30°C in a water bath during isolation. Purified nitrogen was flushed through the samples with 60 ml/min for 15 min.

GC-Sniffing Port Analysis

Volatile compounds were desorbed from the Tenax tubes for 5 min at 260°C (TDAS 5000 Carlo Erba, Interscience, Breda, The Netherlands) and after cryofocussing, injected on a capillary Supelcowax 10 column (60 m x 0.25 mm i.d., 0.25gm film thickness, Supelco Inc., Bellafonte PA). The oven temperature was progranuned from 40 to 230°C at 5°C/min and subsequently heated to 272°C at 10°C/min. The gas chromatograph (Carlo Erba CiC vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionisation detector (FID) and two sniffing ports (SGE, Milton Keynes City, Great Britain) receiving column effluent at a 1:2:2 ratio. An experienced GC-sniffing port panel was trained and used for the evaluation of the smell of the effluent. The with portable computers, which were programmed as described by Linssen *et al.* (1993). The background noise of the sniffing port panel was determined by analyzing dummy Tenax tubes.

GC-Mass Spectrometry

Volatile compounds were identified by using a GC/MS which was equipped with a thermal desorption unit (TCT injector 16200, Chrompak bv, Middelburg, The Netherlands). Thermal desorption and GC column conditions were identical to those for gas chromatography. Electron impact mass spectral (ElMS) analysis was carried out at an ionization energy of 70 eV.

RESULTS

Peroxide Value

The changes in PV during storage at 55°C are shown in Figure 1. PV was highest in sunflower oil after 25 days of storage at 55°C. The coefficients of variation (CV(%)) of replicates (for each bottle) ranged from 0.2–3.3.

Conjugated Diene Hydroperoxides

The absorbance at 234 nm of the oils and emulsions increased during storage at 55°C; the highest values were observed in the sunflower samples (Figure 2). Most of the samples show a steady state after approximately 15 days of storage. The oils extracted from the emulsions appear to have higher absorbance values than the corresponding pure oils.

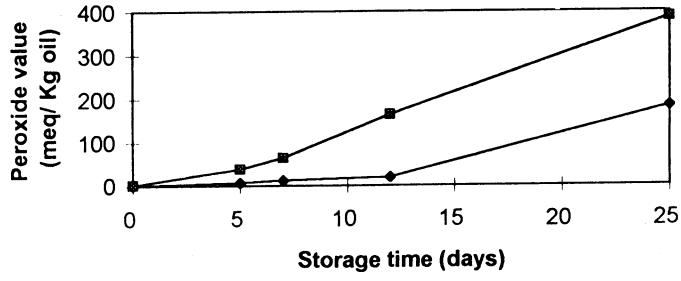

Figure 1. Increase of peroxide value of olive (◆) and sunflower oil (■) during storage at 55°C.

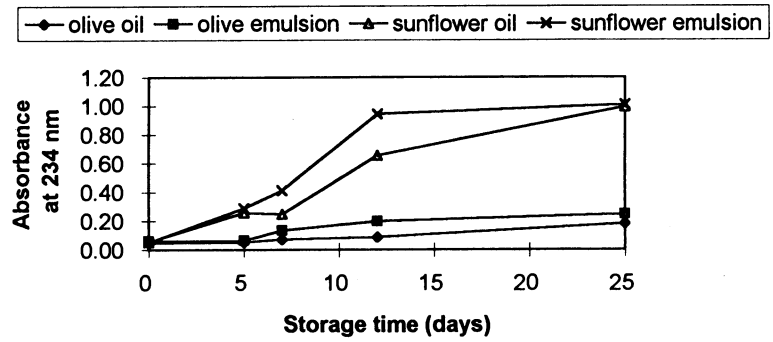

Figure 2. Increase of conjugated hydroperoxide dienes in olive and sunflower oils and their 50% oil in water emulsions during storage at 55°C.

Table 1. Identified and quantified compounds detected in sunflower and olive oil and their corresponding 50% and 1% o/w emulsion after 5 and 10 days storage at 55°C, respectively

No.	KI[1] sniffing interval	Odor description	Identified compound (KI)[2]	FID peak areas[3] in sunflower oil samples (mV s)			FID peak areas[3] in olive oil samples (mV s)		
				Oil	50% o/w	1% o/w	Oil	50% o/w	1% o/w
1	696-720	Fruity/sharp/glue	Heptane (700)	3	20	400	50	623	571
2	808-819	Sharp/glue/fruity	Octane (800)	5	17	<1	73	780	935
3	883-819	Chemical/cacao	Butanal (877)	5	32	42	37	254	875
4	933-938	Sharp/glue	Not identified	—[4]	—	—	—	—	—
5		Sharp/grassy	Not identified	—	—	—	—	—	—
6	80-900	Butter/caramel	Pentanal (983)	53	363	1,996	267	800	6,234
7	1017-1027	Sharp/leather	Not identified	—	—	—	—	—	—
8	1024-1040	Sharp/glue/floral	1-penten-3-one (1024)	<1	3	1	4	455	55
9	1062-1070	Butter/almond	2,3-pentadione (1066)	<1	<1	<1	<1	<1	<1
10	1092-1102	Grassy/fruity	Hexanal (1090)	317	2,136	17,895	588	2,505	16,317
11	1110-1113	Grassy/sharp/glue	Not identified	—	—	—	—	—	—
12	1164-1166	Rancid/leather	1-penten-3-ol (1163)	24	51	438	30	16	181
13		Fish/deep fried fat	Not identified	—	—	—	—	—	—
14	1196-1201	Cacao/sharp	Heptanal (19985)	5	17	435	18	81	4,188
15	1203-1224	Fruity/rancid	20heptanone (1199)	ns[5]	ns	ns	ns	ns	ns
16	1241-1249	Almond/grassy	(E)-2-hexenal (1240)	—	—	—	—	—	—
17	1255-1267	Fish/deep fried fat	Octadienal isomer (1258)	—	—	—	—	—	—
18	1299-1315	Fruity/geranium	Octanal (1297)	11	34	63	28	48	1,179
19	1310-1330	Mushroom	1-octen-3-one (1308)	2	4	65	3	33	111
20	1342-1346	Almond/rancid	(E)-2-heptanal (1338)	66	144	4,568	27	417	452
21	1350-1365	Rancid	1-hexanol (1346)	2	2	108	2	18	101
22	1398-1412	Deep fried fat/leather	Not identified	—	—	—	—	—	—
23		Rancid/deep fried fat	Not identified	—	—	—	—	—	—
24		Deep fried fat	Not identified	—	—	—	—	—	—
25	1626-1630	Floral/deep fried fat	Butanoic acid (1625)	—	—	—	—	—	—
26	1638-1640	Cucumber	6-dodecane (1637)	—	—	—	—	—	—

1. Calculated Kovacs indices for the sniffing interval
2. Kovacs indices of components identified by GC-MS
3. Mean peak rates (n=4); overall coefficient of variation is 26%
4.-: peak areas not quantified, because compound ws not identified or when peak areas of most samples were below 1 mVs
5. ns: peak not properly separated from neighbouring compound; peak area of both components were added.

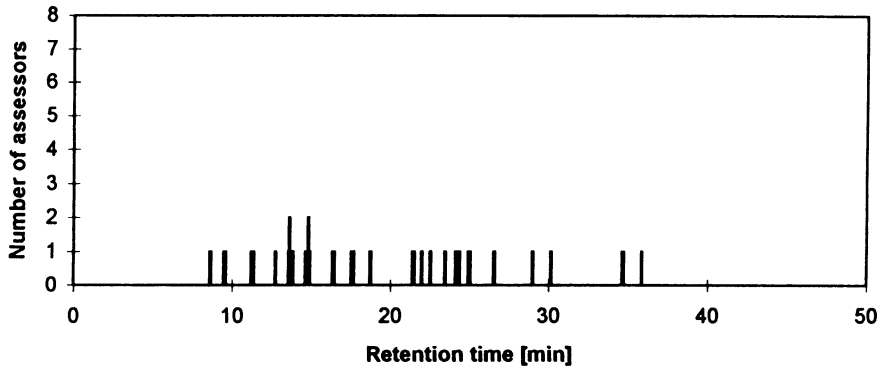

Figure 3. Sniffing chromatogram of a dummy sample.

GC-MS of Volatile Compounds

Identification of the different compounds detected in the oil and emulsion headspace concentrates was based upon mass spectral matching and comparison of calculated KI's with authentic samples. The identified volatile compounds listed in Table 1. The aldehydes, alcohols, ketones and alkanes probably originate from the oxidation of fatty acids, like oleic, linoleic, and linolenic acids.

Noise Level for GC-Sniffing Port Evaluation

Sniffing of dummy Tenax tubes revealed that detection of an odor by less than 3 out of 8 assessors could be considered as background "noise" for our panel (Figure 3).

GC-Sniffing Port Evaluation of the 5O% O/W and L% O/W Emulsions

Fifteen odor descriptors were obtained from the training sessions and were used for odor evaluation of the volatile compounds by the GC-sniffing panel. The odor active compounds were identified by comparing calculated KI's of sniffing responses with these indices of GC-MS identified compounds. A typical gas chromatogram and a sniffing chromatogram of a 50% o/w sunflower oil emulsion are presented in Figures 4 and 5. Table 2 shows the time intervals at which more than 2 assessors simultaneously detected

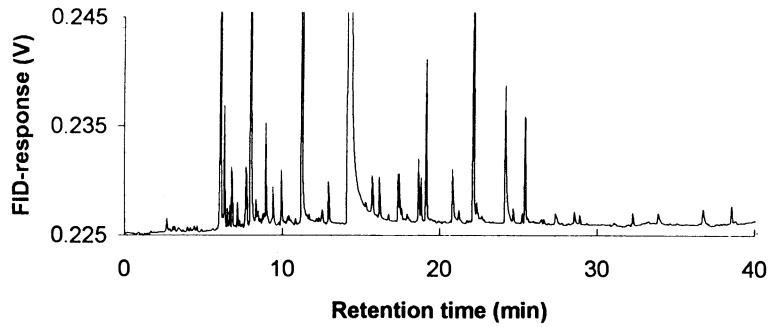

Figure 4. Gas chromatogram of the volatile compounds of the 50% oil in water emulsion of sunflower oil after 5 days of storage at 55°C.

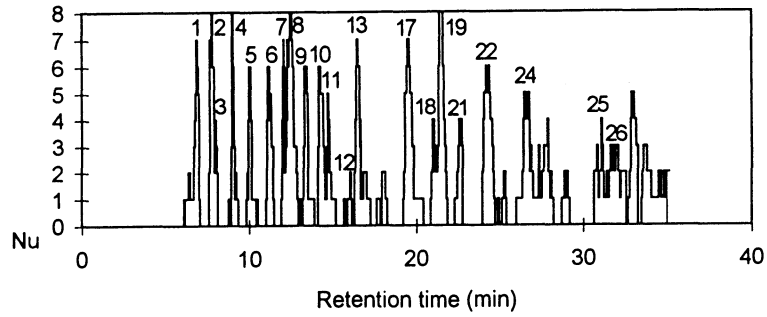

Figure 5. Sniffing chromatogram of the volatile compounds of the 50% oil in water emulsion of sunflower oil after 5 days of storage at 55°C.

odor active compounds. The different oils and emulsions have several time intervals in common, i.e., sniffing response 6 (pentanal), 10 (hexanal), 11 (not identified), and 18–19 (octanal and 1-octen-3-one). The major odor notes of these components were caramel, buttery (6), grassy (10), chemical/glue, grassy (11), and fruity and mushroom (18–19).

Table 2. Odor active components detected in sunflower and olive oil and their corresponding 50% and 1% o/w emulsion after 5 and 10 days storage at 55°C respectively

No.	Sniffing Interval[1]	Odor description	FID peak areas[3] in sunflower oil samples (mV s)			FID peak areas[3] in olive oil samples (mV s)		
			Oil	50% o/w	1% o/w	Oil	50% o/w	1% o/w
1	6.70–7.02	Fruity/sharp/glue	nd[2]	nd	nd	+	+	+
2	7.62–7.91	Sharp/glue/fruity	nd	nd	nd	+	+	+
3	8.94–9.12	Sharp/chemical/cacao	nd	+	nd	+	+	+
4	9.93–10.14	Sharp/glue	+	nd	nd	nd	nd	nd
5	10.49–10.56	Sharp/grassy	nd	nd	+	nd	nd	+
6	11.07–11.42	Butter/caramel	+	+	+	+	+	+
7	11.96–12.14	Sharp/leather	nd	+	nd	+	+	+
8	12.18–12.82	Sharp/glue/floral	nd	+	+	+	+	+
9	13.24–13.50	Butter/almond	nd	nd	nd	+	+	nd
10	14.11–14.44	Grassy/fruity	+	+	+	+	+	+
11	14.65–14.78	Grassy/sharp/glue	+	+	+	+	+	+
12	16.33–16.39	Rancid/leather	nd	+	+	nd	+	nd
13	16.82–17.06	Fish/deep fried fat	nd	+	+	nd	nd	+
14	17.28–17.42	Cacao/sharp	nd	nd	+	nd	+	+
15	17.50–18.25	Cucumber/fruity/rancid	nd	nd	+	nd	+	+
16	18.88–19.16	Almond/grassy	nd	nd	+	nd	nd	nd
17	19.31–19.83	Fish/deep fried fat	nd	+	nd	nd	+	+
18	20.97–21.26	Fruity/geranium[3]	+	+	+	+	+	+
19	21.28–21.79	Mushroom[3]	+	+	+	+	+	+
20	22.32–22.42	Almond/rancid	nd	nd	+	nd	nd	nd
21	22.52–22.77	Rancid	nd	+	+	nd	+	+
22	24.01–24.58	Deep fried fat/leather	nd	nd	nd	nd	nd	+
23	25.79–26.34	Rancid/deep fried fat	nd	nd	+	nd	nd	nd
24	26.40–26.87	Deep fried fat	nd	nd	nd	nd	+	nd
25	31.03–31.14	Floral/deep fried fat	nd	nd	nd	nd	nd	+
26	31.61–31.78	Cucumber	nd	nd	nd	nd	nd	+

1. Time interval at which more than 2 assessors sniff simultaneously a smell.
2. Nd: not detected i.e. number of assessors < 3.
3. The sniffing responses were not properly separated in all samples.

Comparing the sniffing patterns of sunflower oil, 50%, and 1% emulsions (Table 2) shows that, in the 50% and 1% o/w emulsions more odor active compounds (12 and 15 respectively) were detected than in the bulk oil (6). The sniffing response numbers 8 (sharp/glue/floral; 1-penten-3-one), 12 (deep frying fat, 1-penten-3-ol), 13 (fish deep frying fat; not identified), and 21 (rancid; 1-hexanol) were only detected in the 50% and 1% emulsions as compared to the pure sunflower oil. Heptanal (14), 2-heptanone (15), (E)-2-hexenal (16) and (E)-2-heptenal (2) were only detected by sniffing in the 1% o/w emulsion of sunflower; the major odor notes of these components include sharp, cucumber, fruity, and almond and grassy.

Comparison of olive oil, 50%, and 1% o/w emulsions reveals that also in the olive samples more odor active compounds were detected in the 50% and 1% o/w emulsions (17 and 19, respectively) than in the oil (11) (Table 2). Sniffing responses 14 (cacao, sharp; heptanal), 15 (cucumber/ fruity/ rancid; 2-heptanone), 17 (fish deep frying fat; octadienal isomer) and 21 (rancid, 1-hexanol) were only detected in the 50% and 1% emulsions.

GC-FID Results of Several Odor Active Compounds

A selected number of volatile compounds were quantified and the mean peak areas (n=4) are presented in Table 1. Selection of these compounds was based on their retention times, which had to be near or in the range of the time intervals of detected odor active compounds. Assessment of the identity of the quantified volatile compounds was carried out by comparing their retention times (and calculated KI) with those of GC-MS identified compounds and those of sniffing responses. The peak areas were quantified using a standard mixture. Injection of the standard mixture revealed that for hexanal (peak no. 10) 1 mVs corresponded with 2.5 ng. The peak areas in Table 1 range from approximately 1 to 16000 mVs corresponding with 2.5 to 40000 ng. Table 1 shows that the effects of emulsification on the amount of volatiles differed for each compound and each type of oil. However, it is obvious that lowering the percentage of oil in the emulsion resulted in an increase in peak area for the majority of odor active components. The amounts of pentanal (6) and hexanal (10) increased drastically in both emulsions of sunflower and olive oils as compared to the bulk oils. In addition, the peak area of (E)-2-heptenal (20) increased significantly in the sunflower emulsions, whereas the peak areas of heptanal (plus 2-heptanone) (14–15) and octanal (18) increased significantly in the olive oil emulsions.

Odor Active Oxidation Products Generated during Storage

Two odor active autoxidation compounds i.e., pentanal and hexanal were monitored during storage of sunflower, and olive oil and their corresponding 50% and 1% o/w emulsions. Figures 6–9 show the effects of emulsification on the development of pentanal and hexanal during storage at 55°C. Obviously, both volatile compounds revealed a rapid increase in all 1% o/w samples. GC-sniffing port samples for sunflower and olive oils (emulsions) were taken at 5 and 10 days storage, respectively. At these storage times the rapid increase of pentanal and hexanal in the 1% o/w emulsions have been already started, explaining the higher amounts for these compounds in the 1% o/w emulsions (Table 1). Furthermore, Figure 6 and 8 show that after ± 10 days storage the amount of hexanal decreased in the 1% o/w emulsion of olive oil and stabilised in the 1% o/w emulsion of sunflower oil.

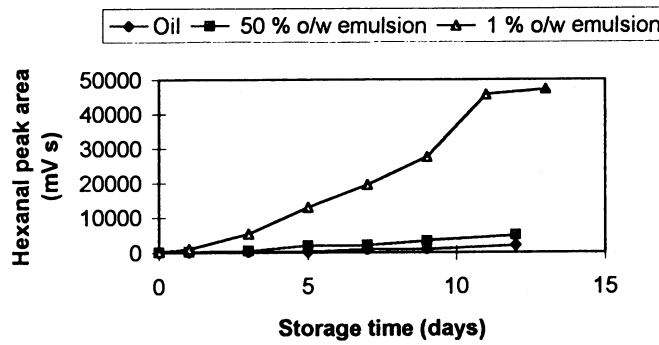

Figure 6. FID peak areas of hexanal in sunflower oil and its 50% and 1% oil in water emulsions after storage at 55°C.

Overall Sensory Screening of the Oils and Emulsions

During oxidation the odor of each vegetable oil was markedly different. Sunflower oil had nutty, almond, and grassy odor notes, while olive oil smelled buttery and alike deep fat drying.

GENERAL DISCUSSION

The objective of this study was to determine odor active compounds of sunflower and olive oil and their 50 and 1% o/w emulsions after storage at 55°C. Additionally, the effect of emulsification on the composition and development of odor active compounds was investigated.

Preliminary studies on the development of primary and volatile secondary oxidation products indicated that autoxidation started at an earlier stage in sunflower oil as compared to olive oil. The overall sensory screening of oils revealed that distinct off-flavors appeared first in sunflower and then in olive oil. Based on these results it was decided to store sunflower and olive oil for 5 and 10 days, respectively. Their 50 and 1% o/w emul-

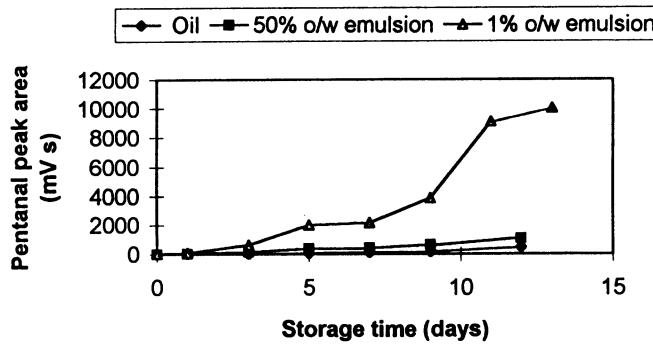

Figure 7. FID peak areas of pentanal in sunflower oil and its 50% and 1% oil in water emulsions after storage at 55°C.

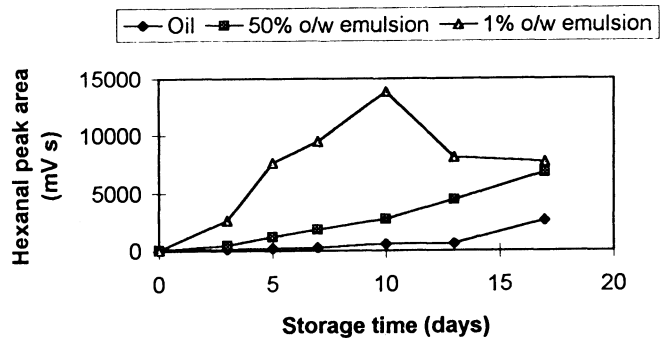

Figure 8. FID peak areas of hexanal in olive oil and its 50% and 1% oil in water emulsions after storage at 55°C.

sions were stored under identical conditions. These samples were analyzed by GC-MS and evaluated by a trained experienced GC- sniffing port panel. The GC-MS identified aldehydes, alcohols, ketones, acids and alkanes probably originating from autoxidation of fatty acids i.e. oleic, linoleic and linolenic acids in the vegetable oils (Badings, 1970).

GC-sniffing port evaluation showed that all oils and emulsions had several odor active compounds in common i.e., pentanal, hexanal, an unidentified compound, octanal, and 1-octen-3-one. The major odor notes of these common sniffing responses were buttery, grassy, chemical/glue/grassy, fruity and mushroom, respectively. The characteristics of the identified compounds are in agreement with odor notes described for these compounds in the literature (Dravnieks, 1985 Sheen *et al.*, 1991). Comparing GC-sniffing port results of the oils and their emulsions revealed that the number of detected odor active compounds increased when the percentage of oil composition was lowered. Mean peak areas of a selected number of volatile compounds indicated that generally the amounts were higher in the 1% o/w emulsions than in the corresponding oils. Apparently, the increase of the number of sniffing responses may be due to quantitative differences of odor compounds more than qualitative differences in the composition.

Additionally, the quantitative differences in the composition of volatiles of the two types of oil seemed to be due to their composition of unsaturated acids. The 1% o/w emulsion of sunflower oil contained relatively higher amounts of (E)-2-heptenal as compared to the 1% o/w emulsion of olive oil; the latter emulsion contained higher amounts of heptanal

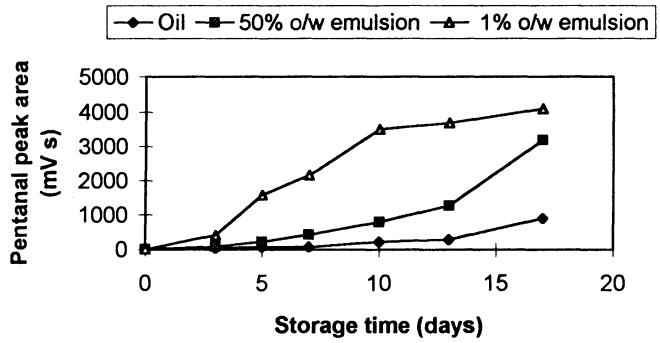

Figure 9. FID peak areas of pentanal in olive oil and its 50% and 1% oil in water emulsions after storage at 55°C.

(plus 2-heptanone) and octanal. Octanal is assumed to be formed from autoxidation of oleic acid which is the major fatty acid (73.5%) of olive oil (Badings, 1970; Frankel, 1991).

The changes of two odor active compounds i.e., pentanal and hexanal, having buttery and grassy odor notes, respectively, were followed during storage of sunflower and olive oils and their 50 and 1% o/w emulsions at 55°C. Emulsification appeared to accelerate the rate of decomposition of unsaturated fatty acids; the increase of pentanal and hexanal was most rapid in the 1% o/w emulsions of both oils. McNulty (1987) and Roozen et al (1994) suggested that, when the fraction of oil in emulsions decreased, the concentration of volatiles in the oil phase increased. Consequently, the equilibrium concentration increased as well as the driving force for the release of volatiles, explaining the higher concentrations in the 1% o/w emulsions. Moreover, it is possible that in the emulsions partition of antioxidant in both the water and oil phase results in lower concentrations in the oil phase as compared to the bulk oil. Furthermore, formation of micelles in the emulsions might result in a higher local concentration of hydroperoxides leading to an accelerated oxidation process (Roozen et al, 1994).

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INFLUENCE OF SEED ROASTING PROCESS ON THE CHANGES IN VOLATILE COMPOUNDS OF THE SESAME (*SESAMUM INDICUM* L.) OIL

Su Noh Ryu,[1] Seong Min Kim,[2] Junwu Xi,[3] and Chi-Tang Ho[3]

[1]National Crop Experiment Station, Rural Development Administration
Suwon, Kyongki Do, 441-100, Republic of Korea
[2]College of Industrial Science, Kongju National University
Yesan, Chungnam, 340-800, Republic of Korea
[3]Department of Food Science, Cook College
Rutgers, The State University of New Jersey
65 Dudley Road, New Brunswick, New Jersey 08901-8520

The volatile component changes of sesame oils prepared at different roasting temperatures (160, 180, 200, and 220°C) and roasting times (10, 20 and 30 min) from sesame seed were evaluated. The recoveries of total volatiles increased as the degree of roasting (temperature and time) increased. The relative amount of pyrazines decreased in contrast to the increase in furans with an increase in the degree of roasting. Pyrazines have important roles in the development of the sweet and roasted flavor of the sesame oil, reaching to maximum amounts after roasting for 30 min at 200°C.

INTRODUCTION

Sesame (*Sesamum indicum* L.) seeds and sesame oils have traditionally been used in eastern Asian countries, especially Korea, China and Japan. Sesame seeds have been prized for their high nutritive value. Sesame oil, prepared from roasted sesame seeds, has a distinctive flavor and long shelflife (Kikugawa *et al.*, 1983; Manley *et al.*, 1974). Several studies have been reported on the flavor components of sesame oil (Yamanishi *et al.*, 1960, 1967; Yoko *et al.*, 1969, Yen, 1990, Shimoda *et al.*, 1996, 1997). The condition of roasting process is the key step in manufacturing sesame oil, significantly influencing the color and flavor quality of sesame oil. Yen (1990) reported that sesame oils prepared at roasting temperatures between 180°C and 220°C have no apparent differences in characteristics, such as acid value, saponification value and refractive index. Yoshida *et al.* (1994) reported that the oxidative stability of the sesame oil was due to the synergism between the endogenous antioxi-

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dants and the browning substances produced during roasting. On the other hand, higher roasting temperatures have usually been employed to obtain a strong flavor but it results in sesame oil of poorer quality (Yen *et al.*, 1986). Thus, in order to make good quality sesame oil, the optimum roasting conditions should be established. This research was designed to investigate the effect of the roasting process, with an emphasis on the roasting temperature and time, on the changes in volatile components of sesame oil.

EXPERIMENTAL

Materials

The sample of sesame *(Sesamum indicum* L.) seeds in this study was a white species which was grown in the summer of 1996 in Suwon, Korea. The seed cultivar (Danbackggae) was selected for its uniformity based on seed weight (i.e. 2.6–3.0 mg), and divided into several groups for storage in stainless-steel containers at 5°C.

Washed and dried sesame seeds were roasted at 160, 180, 200 and 220°C for 10, 20 and 30 min with an automatic roasting machine. The roasted seeds were ground to pass through a 1.40 mm diameter sieve, steamed over boiling water for 7 min and finally pressed (300 kg cm^{-2}) to obtain the sesame oil.

Diethyl ether and anhydrous sodium sulfate were purchased from Nakarai Tesque, Inc. (Kyoto, Japan). 3-Phenyl-1-propanol was obtained from Tokyo Kasei Kogyo Company, Ltd. (Tokyo, Japan).

Isolation of Volatile Flavor Compounds

Three hundred milliliters of deionized water were added to the 100 mL of sesame seed oil. Volatiles were isolated from the dispersion by a combination of steam distillation under reduced pressure (90 mm Hg) and column concentration (Shimoda *et al.*, 1996,1997; Shiratsuchi *et al.*, 1993). Adsorbed volatiles were eluted with 60 mL of diethyl ether, and 10 μL of 1% 3-phenyl-1-propanol in diethyl ether was added as an internal standard. Then, the extract was dried over anhydrous sodium sulfate for 3 hours and concentrated to 200 μL by evaporating the diethyl ether constantly with boiling glass beads in a water bath at 40°C (Shimoda *et al.*, 1996).

Capillary Gas Chromatography (GC) and Capillary Gas Chromatography-Mass Spectrometry

Capillary GC analyses were carried out on a Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector and connected to a Shimadzu chromatopak C-R5A integrator. Separation was achieved on a 60 m × 0.25 mm id fused silica capillary column, coated with cross-linked polyethylene glycol 20M, film thickness 0.25 μ (DB-Wax, J&W Scientific, Folsom, CA). The oven temperature was programmed from 50°C (3 min isothermal) to 230°C at 2°C/min. The temperatures of injector and detector were 230°C. Helium was used as a carrier gas at a column flow rate of 22 cm/s, with an injection splitter at a split ratio of 30:1. Retention indices were estimated by a modified Kovats method (Van den Dool and Kratz, 1963).

A Hitachi 663 gas chromatograph was combined with a Hitachi M-80A mass spectrometer (electron impact mode) with a Hitachi model data system. The column and the

chromatographic conditions were the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 eV and an ion source temperature of 200°C. The mass spectra of the compounds were compared with those in the *Wiley/NBS* Registry of Mass Spectral Data by using a Shimadzu GC-MSPAC 1100 computer system and other published spectra (*Eight peak index of Mass Spectra,* 1983; *Wiley/NBS Registry of Mass spectral Data,* 1989).

RESULTS AND DISCUSSION

The yields of total volatiles were 2198, 9291, 15445 and 20570 ppb from the sesame oils roasted at 160, 180, 200 and 220°C for 10 min, respectively. These quantitative values are only approximate concentrations since they were calculated from peak area to internal standard and then related to the weight of the oil products. Eighty-one compounds were identified: 20 pyrazines, 9 pyrroles, 4 pyridines, 9 thiazoles, 7 furans, 10 aldehydes, 3 alcohols, 2 ketones, 3 fatty acids, 8 aromatic compounds, and 6 miscellaneous compounds. The ratio of the amount of volatiles components in the roasted oils processed at 220°C and 160°C showed that almost all of the volatiles increased by 4–9 folds in the roasted oil processed at 220°C.

Perhaps the most important compounds identified in the roasted sesame oils are 2-furfurylthiol and guaicol. Using aroma extract dilution analysis (AEDA) method, these two compounds have been characterized by Schieberle (1993) to be the most odor-active compounds in roasted sesame seeds. 2-Furfurylthiol, having an intense coffee-like odor (Ohloff and Flament, 1978), increased from 55 ppm in the roasted oil processed at 160 °C for 10 min to 165 ppb in the oil processed at 200°C for 30 min. Guaicol has a burnt and smokey odor with an extremely low odor threshold of 0.02 ppt in water (ASTM, 1978). The amount of guaicol increased from 35 ppb in the roasted oil processed at 160 °C for 10 min to 756 ppb in the oil processed at 220°C for 30 min. The extremely high concentration of guaicol in the high temperature-long time roasting sample certainly contributed to its smoky and over-burnt sensory quality.

Besides furfurylthiol and guaicol, the most important class of compounds could be pyrazines. Pyrazines have been recognized as important flavor components of a large number of cooked, roasted and toasted foods. The occurrence of alkylpyrazines in foods has been extensively reviewed (Maga, 1982, 1992). The alkylpyrazines generally have nutty and roasted aromas. The major pyrazines found in sesame are 2,5- and 2,6-dimethylpyrazines. Shimoda *et al.* (1996, 1997) have considered 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine and 3-ethyl-2,5-dimethylpyrazine to be the most important contributors to the aroma of sesame seed oils. Schieberle (1993) also reported that 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine were among the active-odorants of sesame oil. In the present study, the maximum amounts of pyrazine compounds were obtained in the roasted oil processed at 200°C for 30 min.

The condensation of two molecules of α-amino ketone is the most direct route for pyrazine formation. The α-amino ketone could be generated by the interaction of α-dicarbonyls and α-amino acids through the Strecker degradation. On the other hand, α-amino ketone may also be produced by the interaction of free ammonia with α-hydroxyketones (Ho, 1996). Both α-dicarbonyls and α-hydroxyketones are the retro-aldolization products of the 1-dexoyosones or 3-deoxyosone which, in turn, are the products of the early stage of the Maillard reaction.

Table 1. Changes in the flavor compound contents (ppb) with roasting times (10, 20, 30 min) of sesame seed flavor roasted at 160, 180, 200, and 220°C

Kovats index[a]	Compound	160 °C			180 °C			200 °C			220 °C		
		10	20	30	10	20	30	10	20	30	10	20	30
	Pyrazines												
1271	methylpyrazine	201	384	457	832	913	979	990	1012	1011	1004	989	979
1329	2,5-methylpyrazine	164	282	343	719	813	897	978	1008	996	1003	1002	991
1334	2,6-methylpyrazine	79	186	223	382	414	465	515	565	578	577	570	567
1338	2-ethylpyrazine	38	92	109	149	263	376	421	478	515	503	600	587
1352	2,3-dimethylpyrazine	25	68	87	100	143	178	224	312	356	343	323	321
1390	2-ethyl-6-methylpyrazine	41	74	97	224	242	256	287	298	301	300	302	301
1397	2-ethyl-5-methylpyrazine	39	96	134	208	234	267	289	301	312	307	301	267
1410	trimethylpyrazine	43	73	112	324	343	387	413	465	458	467	453	456
1438	2-vinylpyrazine	10	18	23	34	43	56	79	98	100	103	111	109
1449	3-ethyl-2,5- dimethylpyrazine	87	241	321	497	503	511	623	656	654	654	657	643
1462	2-ethyl-3,5- dimethylpyrazine	43	73	115	148	161	178	220	276	273	280	287	279
1479	2-(1-methylpropyl)pyrazine	14	19	24	19	27	34	56	74	77	74	73	68
1490	2-vinyl-6-methylpyrazine	16	31	37	49	52	54	64	69	75	75	76	81
1494	2,3-diethyl-5- methylpyrazine	9	13	21	23	29	34	46	57	65	54	50	47
1496	3,5-diethyl-2-methylpyrazine	20	42	54	94	103	112	154	168	156	145	139	128
1535	2-methyl-6-(1-propenyl) pyrazine	9	13	31	39	42	45	45	54	66	52	56	57
1543	2-isopropenylpyrazine	8	23	34	45	52	67	76	79	82	80	81	77
1627	2-acetylpyrazine	43	57	151	165	169	178	186	201	202	202	197	187
1671	(E)-2-methyl-6-(1-propenyl) pyrazine	9	27	34	49	63	87	86	98	101	100	98	88
1680	2,3-dimethyl-5-isopentyl pyrazine	8	16	18	23	36	45	45	56	65	54	61	55
	Total pyrazines	906	1828	2425	4123	4645	5206	5797	6325	6443	6377	6426	6288
	Pyrroles												
1183	2-ethylpyrrole	5	6	9	15	19	23	25	31	35	39	65	81
1505	pyrrole	23	31	38	58	65	76	87	103	125	203	234	289
1569	3-methylpyrrole	4	11	17	28	41	56	65	77	81	98	121	143
1598	1- methylpyrrole-2- acetonitrile	11	23	35	48	64	76	78	81	85	91	114	134
1610	1-ethylpyrrole-2- carboxaldehyde	6	12	14	29	37	41	56	65	71	78	112	156
1657	1-methyl-2-acetylpyrrole	4	13	17	25	37	45	49	55	61	69	95	138
1970	2-acetylpyrrole	23	29	34	63	70	76	89	92	95	102	165	189
2030	pyrrole-2-carboxaldehyde	154	151	145	110	104	98	105	123	145	198	232	265
2058	methyl pyrrole-2-carboxylate	5	7	14	28	34	45	51	87	115	123	141	156
	Total pyrroles	235	283	323	404	471	536	605	714	813	1001	1279	1551

Kovats index[a]	Compound	160 °C			180 °C			200 °C			220 °C		
		10	20	30	10	20	30	10	20	30	10	20	30
	Pyridines												
1603	2-acetylpyridine	5	7	9	15	19	31	67	79	110	134	154	176
1643	4-pyridinyl acetate	18	21	34	58	61	67	128	151	154	165	179	205
1782	methyl 4-pyridinecarboxylate	15	23	34	42	49	56	112	134	149	156	178	199
2110	2-pyridinemethanol	13	23	27	42	52	56	132	145	167	176	186	208
	Total pyridines	51	74	104	157	181	210	439	509	580	631	697	788
	Furans												
1228	2-pentylfuran	10	12	19	48	56	67	167	289	311	321	338	345
1456	2-furfural	13	15	23	54	59	71	246	352	412	498	524	612
1521	2-furfuryl acetate	19	23	34	65	72	76	234	312	399	415	449	498
1574	5-methyl-2-furfural	56	65	79	138	156	167	342	423	521	543	597	615
1664	furfuryl alcohol	65	98	134	272	303	343	612	649	735	786	807	817
1686	1-(5-methyl-2-furanyl)-1- propanone	16	34	45	69	79	98	212	315	380	465	497	518
1837	2-furanyl-2-butenal	15	18	21	23	48	67	165	213	276	312	362	497
	Total furans	194	265	355	669	773	889	1978	2553	3034	3340	3574	3902
	Thiazoles												
1281	4-ethylthiazole	6	19	29	34	39	43	45	64	71	75	87	98
1285	2,4-dimethylthiazole	11	25	34	48	51	54	58	58	61	69	76	87
1326	2,5- dimethylthiazole	29	41	69	98	105	112	128	132	154	167	187	198
1400	4,5-dimethylisothiazole	14	25	37	37	39	45	49	56	65	76	98	101
1409	4,5-dimethylthiazole	26	63	92	93	121	143	198	230	340	346	367	401
1467	4-methyl-5-ethylthiazole	9	24	31	45	67	76	78	110	124	156	171	189
1486	2-ethyl-5-methylthiazole	14	18	25	29	33	35	45	87	91	98	110	123
1695	2-propyl-4-methylthiazole	19	42	87	110	134	156	187	201	211	234	276	312
1738	2-butyl-5-methylthiazole	8	19	29	40	59	65	87	134	198	121	135	145
	Total thiazoles	136	276	433	534	648	729	875	1072	1315	1342	1507	1654
	Aldehydes												
987	pentanal	16	19	23	33	39	49	56	67	90	98	100	112
1086	hexanal	49	72	98	274	312	356	453	461	467	521	535	546
1095	2-methyl-2-butenal	28	32	38	43	51	56	78	89	109	113	127	189
1186	heptanal	8	15	19	23	43	67	76	85	90	98	107	112
1291	octanal	9	18	22	25	38	54	67	87	105	112	134	156
1430	(E)-2-heptenal	16	51	65	71	80	89	102	134	154	178	185	198
1528	(E)-2-octenal	19	35	41	40	49	56	78	81	99	109	134	143

(*continued*)

Table 1. (*Continued*)

Kovats index[a]	Compound	160 °C			180 °C			200 °C			220 °C		
		10	20	30	10	20	30	10	20	30	10	20	30
1706	(E,E)-2,4-nonadienal	15	19	26	25	48	67	67	79	98	102	141	167
1766	(E,Z)-2,4-decadienal	9	16	21	28	43	65	78	81	89	113	132	154
1811	(E,E) 2,4-decadienal	39	121	141	143	169	211	256	287	302	312	333	367
	Total aldehydes	208	398	494	705	872	1070	1311	1451	1603	1756	1928	2144
	Alcohol, ketones, and acids												
1185	2-heptanone	8	18	23	28	34	45	67	87	100	112	123	132
1354	hexanol	16	34	59	69	72	87	101	110	117	165	178	198
1393	2-nonanone	15	34	65	68	70	78	98	108	121	134	159	178
1562	octanol	10	19	25	25	56	65	76	83	99	104	167	188
1834	hexanoic acid	9	15	34	36	52	71	87	90	97	102	145	174
1953	heptanoic acid	6	18	32	39	49	56	67	87	91	98	136	165
1977	dodecanol	15	34	65	78	85	95	102	115	121	134	165	195
2062	octanoic acid	6	31	75	83	89	97	121	137	156	178	185	201
	Total alcohols, ketones, and acids	85	203	378	426	507	594	719	817	902	1027	1258	1331
	Aromatic Compounds												
1512	benzaldehyde	58	85	123	214	254	324	389	402	411	423	444	476
1651	acetophenol	26	49	65	139	176	223	324	342	397	401	435	487
1846	guaiacol	35	147	211	334	365	423	567	537	654	687	718	756
1868	phenethyl alcohol	36	82	106	118	145	190	265	323	410	456	498	523
1922	2-phenyl-2-butenal	8	27	35	43	65	78	99	118	156	198	205	210
2169	2-methoxy-5-(1-propenyl) phenol	9	28	34	45	71	87	101	189	235	257	287	312
2200	1-(3-methoxyphenyl) ethanone	36	98	136	165	181	211	256	278	298	321	362	389
	Total aromatic compounds	208	516	710	1058	1257	1536	2001	2189	2561	2743	2949	3153
	Miscellaneous Compounds												
891	ethyl acetate	15	23	31	39	43	51	67	77	99	103	129	145
1194	d-limonene	8	14	24	29	38	43	69	87	96	102	158	165
1617	3,5,5-trimethyl-2cyclopenten- 1-one	6	16	23	42	49	59	87	88	89	114	135	175
1647	2,3-dihydro-1H-indole	9	17	32	48	54	62	78	81	88	115	144	164
1693	3-formylthiophene	9	18	23	39	43	51	78	81	91	112	128	134
1802	2-furfurylthiol	8	16	31	46	51	65	98	105	120	134	158	165
	Total miscellaneous compounds	55	104	164	243	278	331	477	519	583	680	852	948
	Total unknown peaks	169	187	198	986	1020	1121	1243	1323	1543	1673	1878	1945
	Total volatiles	2168	4134	5584	9291	10652	11101	15445	17472	19377	20570	22348	23704

[a] Modified Kovats indices calculated for DB-Wax capillary column on the GC system.

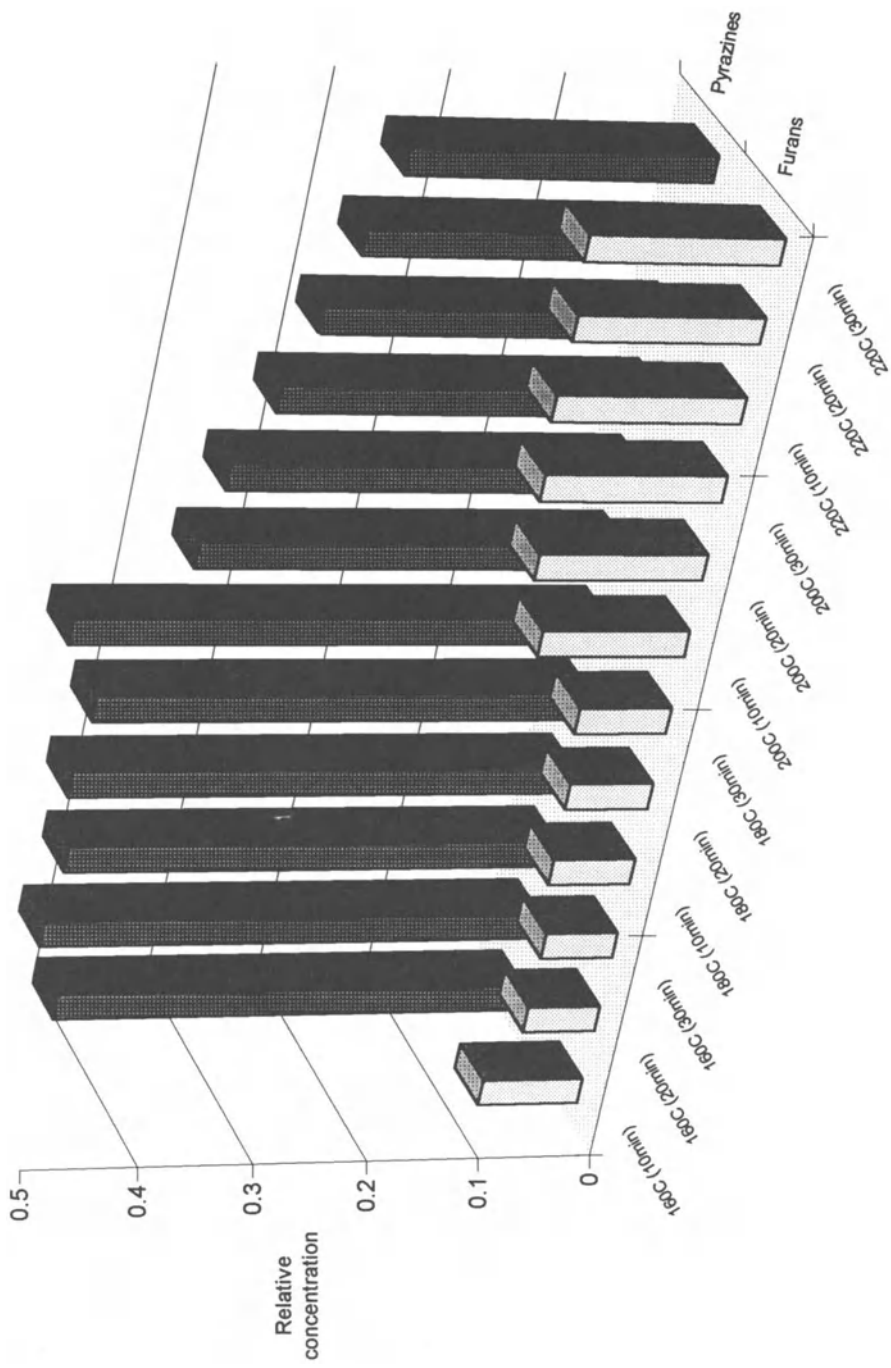

Figure 1. Relative concentrations of pyrazines and furans in roasted sesame oils processed under various conditions.

Yoke *et al.* (1969) have identified popcorn-like aroma as an important sensory attribute of roasted sesame oil. They also characterized acetylpyrazine in sesame oil as having a popcorn aroma. It is interesting to note that in this study the concentration of acetylpyrazine remained relatively constant when the roasting temperature reached 180°C. Another compound having a popcorn-like aroma identified in this present study is 2-acetylpyridine. The concentration of 2-acetylpyridine significantly increased when the degree of roasting was increased.

It is also interesting to note that although the amounts of total pyrazines in roasted oil increased as the degree of roasting increased, the relative concentrations of pyrazines to the total concentrations of volatiles in roasted oil decreased as the degree of roasting increased. The relative concentration of pyrazines in the roasted oil processed at 160 °C for 10 min was 41.8%, it dropped to 26.5% in the oil processed at 220 °C for 30 min.

Pyrroles, which impart burnt and earthy characteristic odors, comprised 8.4% in the roasted oil processed at 160°C for 10 min, but in the roasted oil processed at 220°C for 30 min their relative amounts decreased to 4.8%. Of nine pyrroles, the most abundant one, pyrrole-2-carboxaldehyde, was the only volatile that decreased as the degree of roasting increased.

All thiazoles were alkyl-substituted thiazoles, which have green, nutty, and vegetable-like odors (Ho and Jin, 1985). Nine thiazoles comprised 6.2% of the total amount of volatiles in the roasted oil processed at 160°C for 10 min, on the other hand, their amount increased to 6.5% in the oil processed at 220°C for10 min.

Furans, which generally impart caramel-like and roasted odors (Ohloff and Flament, 1978), comprised 8.9% in the roasted oil processed at 160°C for 10 min and 16.4% in the roasted oil processed at 220°C for 30 min. Shimoda *et al.* (1996,1997) reported that large increases of 1-(5-methyl-2-furanyl)-1-propanone and 2-pentylfuran in sesame oil could contribute to its intense roasted odor. As shown in **Figure 1**, the relative amount of pyrazines decreased in contrast to the increase of furans which increased with the degree of roasting. This could contribute to the burnt and sugary notes of high temperature and long time roasting oils.

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23

VOLATILE COMPONENTS FORMED FROM VARIOUS SUGARS WITH β-ALANINE IN ACTUAL COOKIES

Sukie Nishibori,[1] Toshihiko Osawa,[2] and Shunro Kawakishi[2]

[1]Department of Food Science
Tokaigakuen Women's College
Nagoya 468-8514, Japan
[2]Faculty of Agriculture
Nagoya University
Nagoya 464-8601, Japan

The formation of maltol in a Maillard reaction in a dry system was investigated by the detection of volatile components formed in actual cookies by HPLC and also by the measurement of those formed from the reaction of mono-, di- or trisaccharides added under dry conditions as a model system of cookie processing. 3-Hydroxy-2-methyl-4(*H*)-pyran-4-one (maltol) is reported to be formed by the decomposition of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP). If that is true, DDMP is the prime compound in the pathway to maltol in the Maillard reaction. DDMP was formed in both the actual cookies and model amino carbonyl reactions. However, maltol was not detected in every reaction of these dry systems. Another pathway, not through DDMP, seems to exist in the formation of maltol.

INTRODUCTION

Volatile components in the Maillard reaction (1912) have been investigated by a large number of researchers for a long time. Maltol is one of the best known compounds as the sweet aroma during the processing of foods and agricultural products. Sekiwa (1997) reported that 3-hydroxy-2-methyl-4(*H*)-pyrane-4-one (maltol) was detected in the brew of cooked clams, and the increase in maltol was conspicuous, reaching 4 times the original value within 0–15 min of boiling time. In this study, maltol already existed in the brew of clams before boiling. Therefore, it can be assumed that maltol was produced from other reactions without participation of the amino carbonyl reaction by heating. On the other hand, Ishihara (1996) detected maltol in soybean sauce. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one was attached through an acetal linkage to the C-22 hydroxyl of the aglycones of soybean saponins (DDMP-conjugated soybean saponins) as detected

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by Kudou *et al.* (1992). Maltol in soybean sauce might be formed from these DDMP-conjugated soybean saponins.

Kim and Baltes (1996) described that maltol was produced by the decomposition of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP) in an aqueous solution. Consequently, DDMP is a primary compound in the pathway to maltol in the Maillard reaction.

In the current study, we investigated DDMP and maltol forming in practical foods and model cases of sugars and amino acids by HPLC. Various compounds were identified by gas chromatography (GC) and gas chromatograph-mass spectrometry (GC-MS). However, GC and GC-MS are not suitable for detecting the actual amount of each compound from the reaction. HPLC is suitable for the measurement of the amount of DDMP formed in various reactions without the decomposition and linkage removal.

MATERIALS AND METHODS

Materials and Reagents

Commercial wheat flour (7.2% Protein, Nisshin Milling Co., Tokyo, Japan), potato starch (Katakuriko, Hokuren Rural Organization, Sapporo, Japan), butter (Snow Brand Milk Co., Tokyo, Japan), fresh eggs and reagent-grade sugars were used in the study. Various amino acids were also used in the model system.

Sample Preparation

For the actual baking cookies, the cookie doughs were prepared from wheat flour (45%), eggs (10%), butter (25%), and sugar (20%, mono-, di- or trisaccharides). The doughs were shaped into cookie form (5.0 × 30 mm in diameter) and baked in an electric oven at 150°C for 10 min. For UV and HPLC analyses, 10 g of the baked cookies were crushed and extracted with 50 mL of methanol-water (3:1, v/v) for 30 min at room temperature.

Various combinations of cookie materials placed in test tubes were heated in a block-bath at 150°C for 10 min, and 500 mg of the baked materials were then extracted with 2 mL of methanol-water (3:1, v/v). The mixing ratios of the materials; wheat flour, eggs, butter, sugar and sodium bicarbonate, were the same as those in the cookie doughs. The mixtures of amino acids (10 mg) and proteins (20 mg) with fructose (500 mg) were placed in test tubes and heated at 150°C for 10 min. The baked materials were extracted with 2 mL of methanol-water (3:1, v/v).

For the model system of baking cookies, the reaction mixtures consisted of equimolar amounts of a sugar (either 180 mg of monosaccharides or 360 mg of disaccharides) and β-alanine (89 mg). The components were placed in test tubes, and the mixtures were heated in a block-bath at 150°C for 2–10 min. After cooling, the samples were extracted with 1 mL of methanol-water (3:1, v/v) or dichloromethane, and the extract with dichloromethane was concentrated to 100 μL under a nitrogen stream.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using a Toyo Soda Model HLC-803D pump. Test parameters were as follows: column DEVELOSIL ODS column (4.6 mm i.d. × 250 mm); mobile phase, water-methanol (6:1, v/v) flow rate, 0.8 mL/min; detector, UV 283 nm. A

JASCO model MD-910 photodiode array (PDA) multiwavelength UV/VIS detector was also used for monitoring extraction profiles and chromatograms. A data processing software package DP-910 run on a DELL PC was also used for the post-run data analysis.

Optical Density Spectrometry

For comparison of the coloration, fructose was heated at 150°C for 10 min with β-alanine or arginine and extracted with methanol-water (1:1, v/v). Each extract was diluted 40 times with methanol-water (1:1, v/v) and the absorbance was determined at 420 nm usage JASCO Ubest-30 UV/VIS spectrometer.

RESULTS AND DISCUSSION

Flavor Compounds from Baked Cookies

The cookie doughs containing various sugars were baked at 150°C for 10 min in an electric oven. The mixtures of a sugar and β-alanine were also baked in the model cookies system. The flavor compounds were extracted with methanol-water and analyzed by HPLC.

Figure 1 shows the HPLC profiles of the methanol-water extracts from the above model system. Five main compounds were identified in the reaction mixtures of fructose with β-alanine heated at 150°C for 10 min (Nishibori and Kawakishi, 1990, 1991, 1995). 2,3-Dihydro-3,4-dihydroxy-5-acetylfuran (DDAF), DDMP and 5-hydroxymethylfurfural

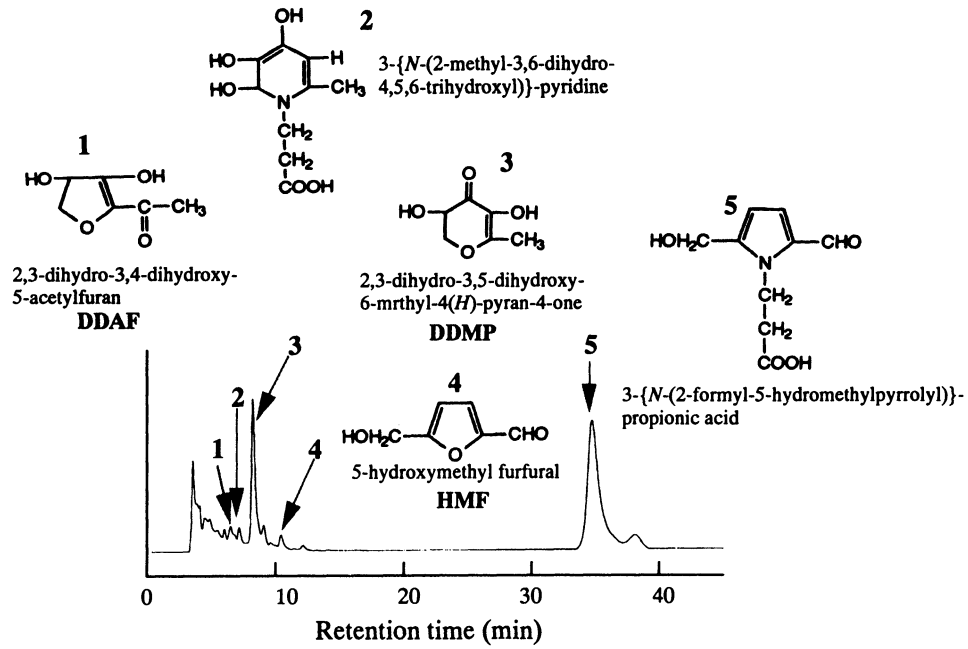

Figure 1. High performance liquid chromatogram of extracts from fructose and β-alanine heated at 150°C for 10 min. Chromatographic conditions: column; DEVELOSIL ODS-5 (4.6 mm i.d. × 250 mm), mobile phase; H_2O-MeOH (6:1 v/v) 0.8 mL/min, UV detection; 283 nm, chart speed; 5 mm/min.

(HMF) were also detected as major compounds in the cookies baked using various sugars. HMF has a bitter taste and a burnt odor, while DDAF and DDMP have a sweet aroma and cookie-like flavor.

Many researchers have reported the odor DDMP, but some have reported that it is odorless (e.g. Kim and Baltes 1996). On the other hand, a few researchers described DDMP as having a sweet aroma. Recently, Cutzach (1997) reported it as having a toasty caramel aroma. It is almost certain that DDMP has a sweet aroma, because DDMP still had a sweet aroma after processing to purify DDMP 3 times by HPLC. However, the odor threshold value of DDMP is very high, so this might explain reports about its odorlessness.

Figure 2–1 shows the formation of DDMP and HMF during baking of the cookies using of fructose or glucose. DDMP was formed more rapidly in both cookies during the heating at 150°C. The content of HMF increased slowly up to 20 min after heating of the cookies, and then increased markedly over 20 min. In the case of the cookies baked at 150, 200 and 250°C, HMF formation increased abruptly at over 200°C, but DDMP formation remained constant at 0.2–0.3 mg (Figure 2–2). From these results, the heating for 10 min at 150°C is considered as providing good conditions for the formation of DDMP without a large amount of HMF formation.

Flavor Compounds from Cookies Baked with Mono-, Di- and Trisaccharides

The main compounds in the cookies baked with monosaccharides (such as mannose, fructose, and glucose) were DDAF, DDMP and HMF (Figure 3). In the cookies baked with mannose, HMF was the major component. In the cookies containing fructose, DDAF and DDMP were generated in large quantities, although the formation of HMF was slight. When glucose was added, the flavor compounds were similar to those when fructose was added although the amounts of DDAF and DDMP were slightly lower than with fructose. These compounds were well-known as important precursors of isomaltol

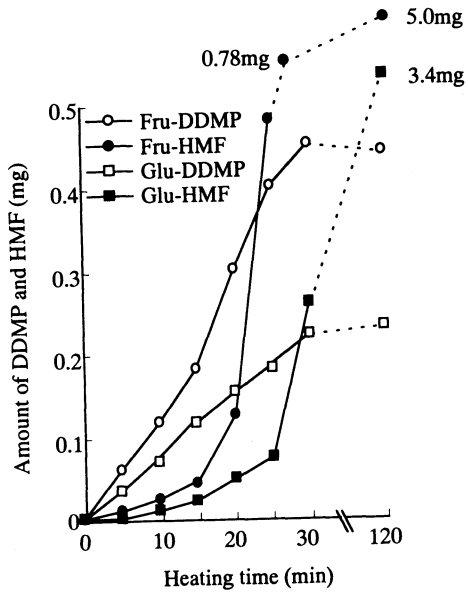

Figure 2. Effects of heating time on DDMP and HMF produced in cookes baked at 150°C for 1–120 min. Fru-DDMP; 2,3-dihydro-3, 5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from fructose, Fru-HMF; 5-hydroxymethyl furfural from fructose, Glu-DDMP; 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from glucose, Glu-HMF; 5-hydroxymethyl furfural from glucose.

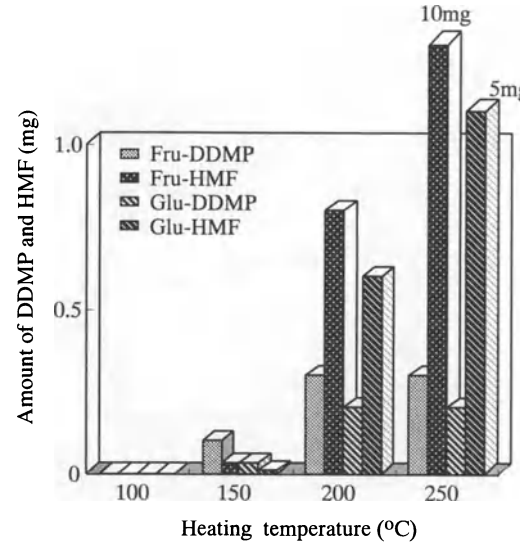

Figure 3. Effects of temperature on DDMP and HMF produced in cookes baked at 100, 150, 200 or 250°C for 10 min. Fru-DDMP; 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from fructose, Fru-HMF; 5-hydroxymethyl furfural from fructose, Glu-DDMP; 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from glucose, Glu-HMF; 5-hydroxymethyl furfural from glucose.

and maltol, respectively (Hodge *et al.*, 1976). In this study, we found that fructose was the favored additive for generation of the sweet flavor preferred as the cookie aroma. When maltose was added to the cookie dough, slight amounts of DDAF and DDMP, high amounts of HMF and an unknown compound were generated (Figure 4). The formation

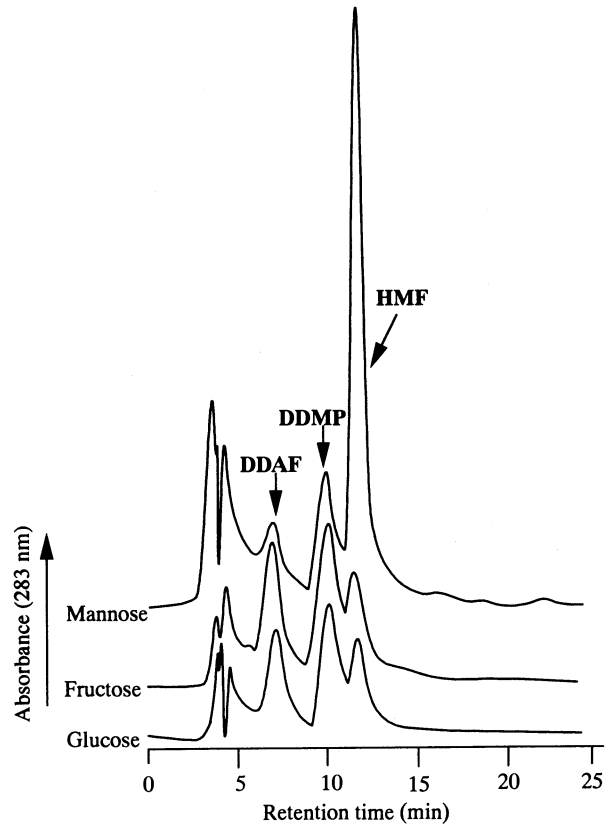

Figure 4. High performance liquid chromatogram of extracts from cookies baked with various hexose at 150°C for 10 min. Chromatographic conditions: column; DEVELOSIL ODS-5 (4.6 mm i.d. × 250 mm), mobile phase; H_2O-MeOH (6:1 v/v) 0.8 mL/min, UV detection; 283 nm, chart speed; 5 mm/min. DDAF was 2,3-dihydro-3,4-dihydroxy-5-acetylfuran, DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

of HMF with the addition of cellobiose was slight. With the addition of sucrose, HMF was not formed in the cookie dough and only a trace amount of DDMP was formed with cellobiose and sucrose.

Flavor Compounds from Sugar Baked with Various Cookie Materials

Eggs, butter and sodium bicarbonate are usually added to the cookie dough to attain a sweet flavor and nice shortness. Formation of DDMP and HMF from cookie materials and their mixtures in a dry state heated at 150°C for 10 min was studied. As shown in Figure 5, DDMP and HMF were not formed from a single material except for HMF from fructose. However, the mixtures of cookie materials with fructose and glucose promoted the formation of DDMP and HMF. In the formation of DDMP, fructose was more effective than glucose. The mixtures of fructose and egg especially promoted DDMP formation more strongly than any of the other materials (Figure 6). Moreover, heating sugar with sodium bicarbonate also generated a small amount of DDMP. This is well-known to be produced in the reactions between alkali (Shaw *et al.*, 1968) or acid (Shaw *et al.*, 1967) and sugar, and amino-carbonyl reactions (Njorge *et al.*, 1989). On the other hand, the formation of HMF was not different with glucose and fructose; however, the amounts of HMF markedly increased on heating of fat-containing materials, e.g., butter and egg, with sugar.

More DDMP was formed from the reaction of fructose and egg white than HMF up to 20 min. Heating over 20 min enhanced the formation of HMF, similar to the reaction of the model system presented in Figure 2–1.

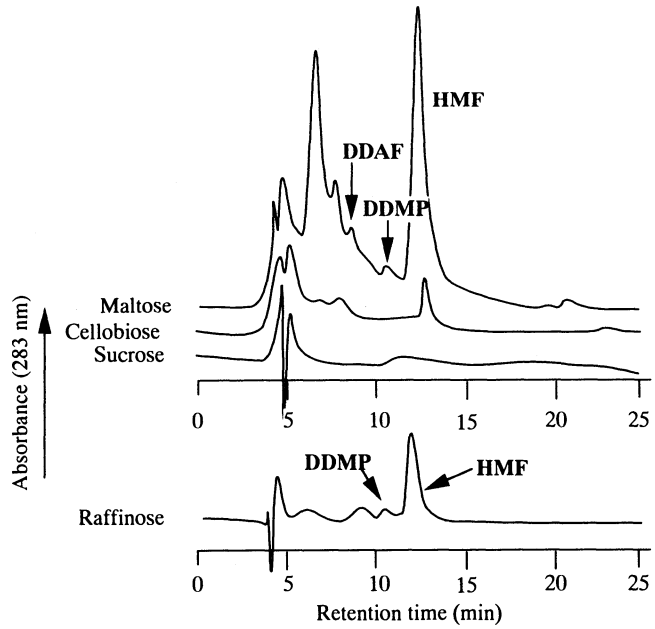

Figure 5. High performance liquid chromatogram of extracts from cookies baked with various di- and trisaccharide at 150°C for 10 min. Chromatographic conditions: column; DEVELOSIL ODS-5 (4.6 mm i.d. x 250 mm), mobile phase; H2O-MeOH (6:1 v/v) 0.8 mL/min, UV detection; 283 nm, chart speed; 5 mm/min. DDAF was 2,3-dihydro-3,4-dihydroxy-5-acetylfuran, DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

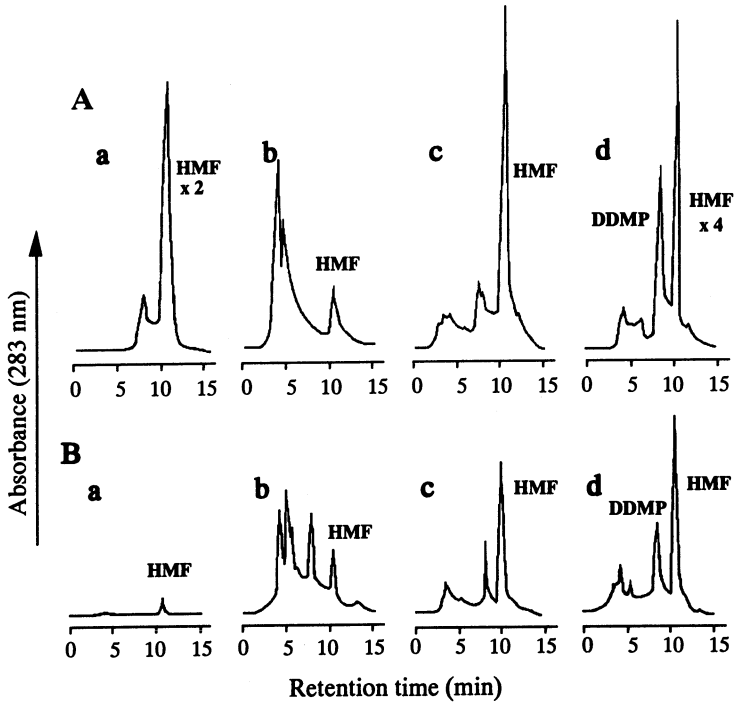

Figure 6. High performance liquid chromatogram of the products from sugar based with various cookies' materials at 150°C for 10 min. Chromatographic conditions: column; DEVELOSIL ODS-5 (4.6 mm i.d. x 250 mm), mobile phase; H_2O-MeOH (6:1 v/v) 0.8 mL/min, UV detection; 283 nm, chart speed; 5 mm/min. A; fructose, B; glucose. a; sugar only, b; sugar and $NaHCO_3$, c; sugar and butter, d; sugar and egg. x 2; double the peak height. x 4; 4 times the peak height. DDMP was 2, 3-dihydro-3,5-dihydroxy-6-methyl-4-(*H*)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

Effects of the Binding Position of Amino Acid Group in Amino Acids on Flavor Compound Formation

Equimolar amounts of fructose with various amino acids were heated in a block-bath at 150°C for 10 min. Figure 7 shows the effects of the binding position of the amino group in alanine on the formation of DDMP and HMF. The formation of DDMP and HMF was promoted by the heating of β-alanine with fructose more than that of α-alanine. As the amino acid group in an amino acid is moved away from the carboxyl group, formation of DDMP and HMF is enhanced through the reaction of the amino acid with the reducing group in a sugar.

As shown in Figure 8, β-amino butyric acid enhanced the formation of DDMP most strongly among three kinds of the binding position of an amino acid, but the difference was small. As a result, the amino group in the β-position was found to be more effective than the α-position for the formation of DDMP.

Effects of Basic Amino Acids on Flavor Compound Formation

The Maillard reaction is that of an aldehyde or a carbonyl group in a reducing sugar and an amino acid. Arginine has two amino groups and one imino group in its structure.

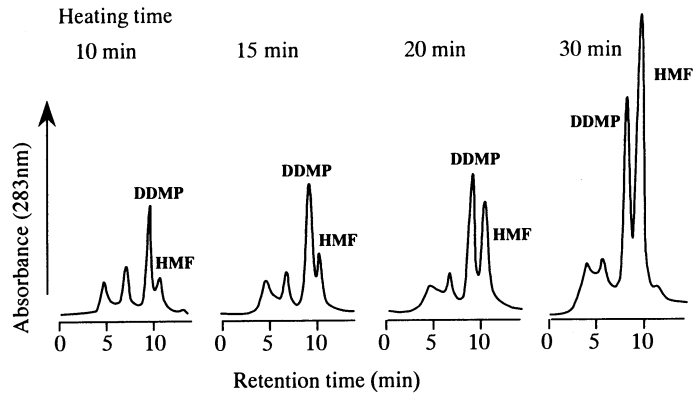

Figure 7. High performance liquid chromatogram of the products from fructose and egg white baked at 150°C for various times. Chromatographic conditions: column; DEVELOSIL ODS-5 (4.6 mm i.d. x 250 mm), mobile phase; H_2O-MeOH (6:1 v/v) 0.8 mL/min, UV detection; 283 nm, chart speed; 5 mm/min. DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

Various amounts of arginine were heated with 1 mmole of fructose at 150°C for 10 min to investigate the effect of the number of the amino groups Maillard reaction, particularly the DDMP and HMF formation, and the color. Various amounts of β-alanine were also heated with 1 mmole of fructose for comparison.

As shown in Figure 9, 0.2 mmole of arginine heated with 1 mmole of fructose showed the most remarkable DDMP formation. On the other hand, 0.6 mmole of β-alanine formed the largest amount of DDMP in the presence of 1 mmole of fructose. HMF was confirmed in the reaction of β-alanine and fructose but not from the reaction of arginine and fructose. HMF formation showed the maximum amount when 1 mmole of β-alanine was added to 1 mmole of fructose. The absorbance of the reaction of arginine and fructose rose rapidly up to 1 mmole of arginine, and then the increase in the optical density continued slowly. The absorbance of β-alanine and fructose rose more slowly, and the speed of the deep coloration of β-alanine and fructose was about half that of arginine and fructose. It has become apparent that more than one amino acid produces the DDMP formation and enhances the coloration. However, DDMP was formed more extensively in the reaction of β-alanine and fructose than in that of arginine and fructose.

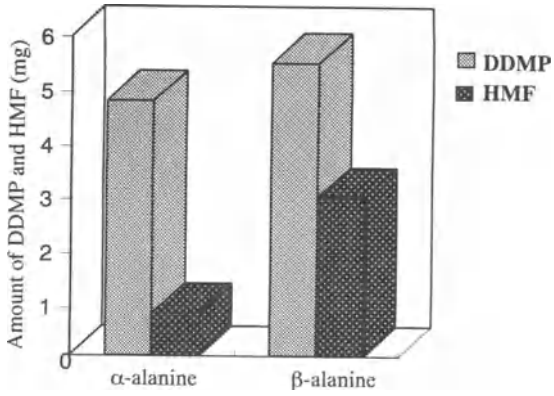

Figure 8. Effects of α- and β-alanine to DDMP and HMF formation from fructose heated at 150°C for 10 min. DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

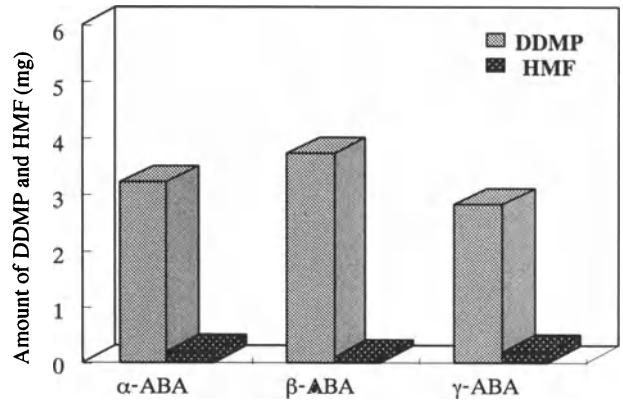

Figure 9. Effects of α-, β- and γ-amino butyric acids on DDMP and HMF formation from fructose heated at 150°C for 10 min. α-ABA; α-amino butyric acids, β-ABA; β-amino butyric acids, γ-ABA; γ-amino butyric acids. DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and HMF was 5-hydroxymethylfurfural.

Effects of Heating Temperature on Flavor Compound Formation

One mmole each of fructose and β-alanine was heated at 150°C for 10 min. The heating at 150°C formed a large amount of DDMP and HMF (Figure 10). Particularly it was shown that the heating at 150°C strongly enhanced DDMP formation.

Flavor Compounds in the Reaction of Fructose, Maltose, and Lactose with β-Alanine

Equimolar amounts of fructose, maltose or lactose with β-alanine were heated on a heater block at 150°C for 10 min. Figure 11 shows the change in DDMP formation from

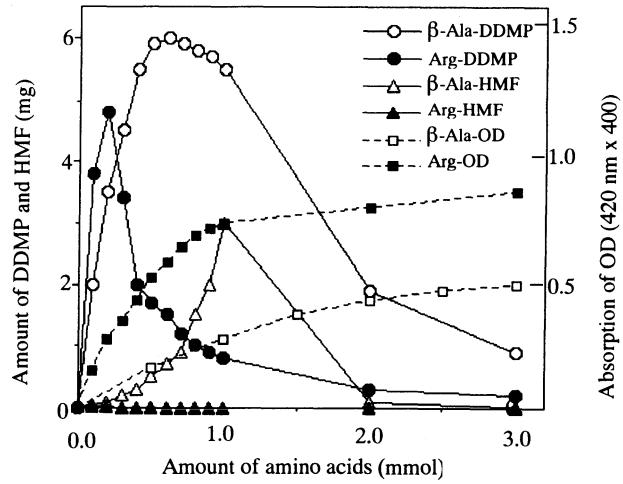

Figure 10. Effects of basic and neutral amino acids on the DDMP formation and the color in the reaction of amino acids and fructose heated at 150°C for 10 min. β-Ala-DDMP; the amount of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from the reaction of β-alanine and fructose, Arg-DDMP; the amount of 2,3-dihydro-3,5-dihydroxy-6-methyl-4-(*H*)-pyran-4-one from the reaction of arginine and fructose, β-Ala-HMF; the amount of 5-hydroxymethyl furfural from the reaction of β-alanine and, Arg-HMF; the amount of 5-hydroxymethyl furfural from the reaction of arginine and fructose, β-Ala-OD; optical density of the extracts from the reaction of β-alanine and fructose, Arg-OD; optical density of the extracts from the reaction of arginine and fructose.

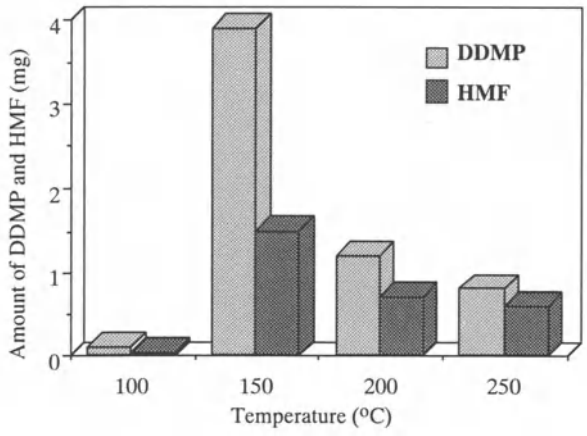

Figure 11. Formation of DDMP and HMF from the reaction of each 1 mmol of fructose and β-alanine heated at 100, 150, 200 and 250°C for 10 min. DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*) -pyran-4-one and HMF was 5-hydroxymethyl furfural.

the reaction mixtures of each sugar and β-alanine with the heating time at 2-min intervals. DDMP from the reaction of fructose with β-alanine was formed more rapidly than that from maltose or lactose with β-alanine, approximately by heating for 2 min. Lactose with β-alanine formed DDMP after 6 min, and maltol formed after 8 min.

Kim and Baltes (1996) and Yaylayan and Mandeville (1991, 1994) reported that maltol was formed by the decomposition of DDMP, the electron impact during mass spectrometry of Amadori products, the reaction under basic neutral conditions of aqueous systems, and the ionization of the ring oxygen in GC analysis. On the assumption that reducing disaccharides can form maltol, they are very useful materials for the formation of aroma in cookies. We (Nishibori and Kawakishi, 1988, 1990, 1992, 1993, 1994) have never detected maltol either in the baking of cookies with maltose or in the model reactions using maltose and amino acids in a dry system. If maltol was formed from DDMP, fructose might be more induced to form maltol through DDMP than maltose. However,

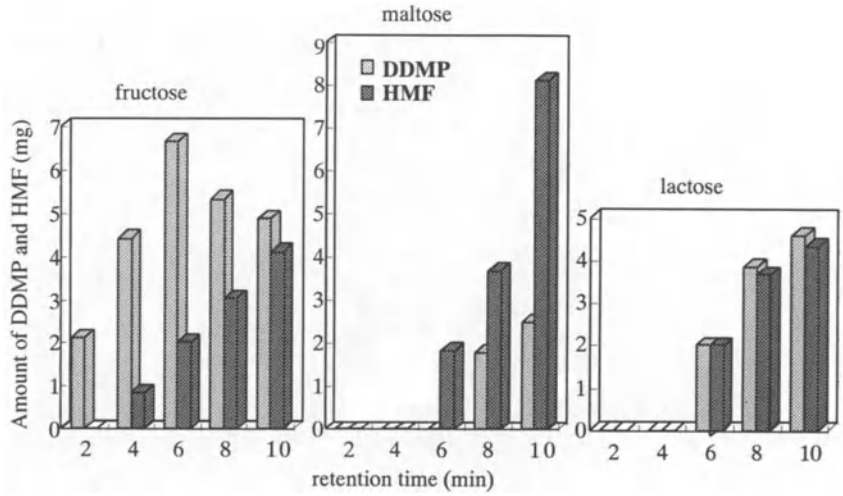

Figure 12. Formation of DDMP and HMF from 1 mmol of selected sugars with 1 mmol of β-alanine heated at 250°C for 10 min. DDMP was 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and HMF was 5-hydroxymethyl furfural.

maltol was not detected in the cookies baked with fructose, that is, maltol did not form as a result of decomposition of fructose.

The Pathway of Maltol from Fructose

It is required to clarify the pathway from sugar to maltol and the origin of maltol. Cutzach (1997) reported the presence of a great amount of DDMP and very little maltol in the toasted oak wood. Sekiwa *et al.* (1997) also showed a large amount of maltol and a slight amount of DDMP in cooked clam extract. The maltol already exists in fresh clams.

On the other hand, Ishihara *et al.* (1996) reported the presence of maltol in raw and pasteurized shoyu of Koikuchi shoyu, which is a kind of soybean sauce. Kudou *et al.* (1992) identified 2,3-dihydro-2,5-dihydroxy-6-methyl-4-(*H*)-pyran-4-one as DDMP. Two hydroxy groups of this DDMP is bonded to C-2 and C-5, but DDMP from amino carbonyl reaction has 2 hydroxy groups attached to C-3 and C-5. Briefly, maltol is formed through some pathways from the mixture of compounds. The mechanism of maltol formation is not yet clear except that it is very hard to produce it from sugars in a normal heating during food processing.

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AROMA FORMATION IN DRIED MULLET ROE AS AFFECTED BY LIPOXYGENASE

Bonnie Sun Pan and Chia-Ming Lin

Department of Marine Food Science, National Taiwan Ocean University
2 Pei-Ning Road, Keelung, Taiwan, Republic of China

The unsaturated fatty acids contributed to over 75% of the total fatty acids in grey mullet roe. In model system, using lipoxygenase (LOX) extracted from mullet gill in place of roe LOX to react with roe lipid resulted in very slight decreases in unsaturated fatty acids and pronounced increase in green and fresh fish-like flavor notes. The intensity of odor increased with increasing LOX activity. Volatile compounds collected from dried mullet roe using simultaneous distillation and solvent extraction, or Tenax-TA, adsorption, followed by GC-sniffing and GC-MS studies. Ethyl acetate or a compound in trace quantity with similar RI was identified with the flavor note of mullet roe. Thirty six to 42 odorous compounds were detected from the 2 aroma concentrates. Compounds, 1-penten-3-ol, 3-penten-2-ol, and 1-pentanol from aroma concentrates with fish-like and green flavor notes were tentatively identified. Other short chain compounds contributing to celery-like, beany, fruity, fishy and seaweed-like flavor notes were also detected.

INTRODUCTION

Dried mullet roe is considered a delicacy in Taiwan and Japan. Mullet roe to Asians is the equivalent of sturgeon caviar to Europeans. The market value of one pair of mullet roe weighing ca 200 g is approximately US $40. Traditionally, mullet roe products are prepared from pairs of roes in sacs, which are taken from ocean-caught grey mullet, salted, then sun dried. In recent years, culturing all-female grey mullet has become possible (Pan *et al.*, 1992; Chang *et al.*, 1995) and serves as an alternate source for mullet roe processing. However, gourmet consumers claim that sensory differences can be detected between the roe products made from wild and cultured mullets. The cultured grey mullet roe products appear darker in color than those from wild mullet. A series of studies has been carried to determine the color and aroma formation mechanism in mullet roe.

Cultured grey mullet roe has a high content of free amino acids, especially those of high browning rate, including lysine, histidine, glutamine, proline, glycine and taurine. Application of trypsin inhibitor or other protease inhibitors greatly reduced the content of

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free amino acids and browning during processing and storage of mullet roe (Pan *et al.*, 1997a). Based on our recent results, a browning mechanism, basically Maillard reaction, and the key parameters responsible for the color formation and possibly aroma formation in mullet roe were proposed (Figure 1) (Huang and Pan, 1998). Lipid oxidation was shown to participate in the reaction mechanism. Since lipoxygenase activities which affect seafood aroma (Kuo and Pan, 1991; Pan *et al.*, 1997b) were found in roe and changed with ovarian development (Liao and Pan, 1997), the role of lipoxygenase in aroma formation in dried mullet roe was studied. The findings on mullet roe may also be of significance to the chemistry and processing of sturgeon caviar and other seafood roe products.

EXPERIMENTAL

Dried Mullet Roe

Fresh grey mullet (*Mugil cephalus*) of 2 year old were harvested from culture ponds in Tai-Nan during November when the gonadosomatic index (GSI) of cultured grey mullet reached a maximum of 15% body weight. Fresh grey mullet ovaries underwent cleaning,

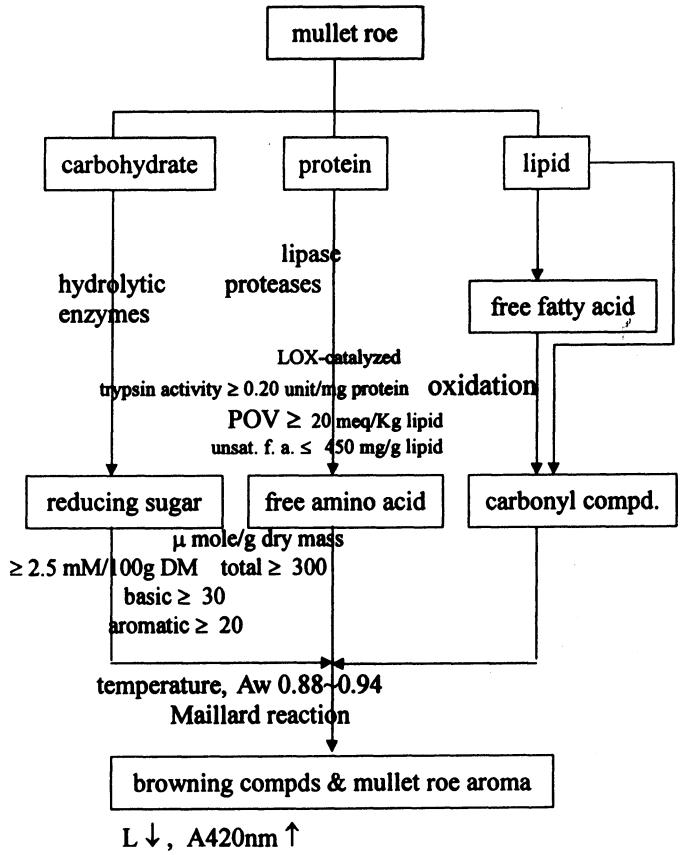

Figure 1. Browning reactions and aroma formation in dried mullet roe (adopted from Huang and Pan, unpublished).

shaping, salting, and then sun drying for about 3–4 days until their water activity (Aw) was reduced to 0.85.

Fatty Acid Analyses

Crude fat was extracted from fresh grey mullet ovary using the method of Folch *et al.* (1957), followed by saponification and methylation with BF_3-CH_3OH (AOAC, 1984). Fatty acid profile was analyzed using a Shimadzu GC-14A (Kyoto, Japan) equipped with a DB-23 fused silica capillary column (60m x 0.252mm, J&W Scientific, Folsom, CA). The initial oven temperature was 170°C and increased to 230°C at 2°C/min, then held for 5min. Both injector and detector temperatures were set at 250°C. The carrier gas was hydrogen at a flow rate of 1ml/min. The fatty acid $C_{13:0}$ was used as an internal standard.

Preparation of Lipoxygenase Extract

Fresh grey mullet gill was homogenized with a 0.05M potassium phosphate buffer (pH 7.0) 1:1.5 w/v, and centrifuged at 24,000xg for 30min at 4°C. The supernatant was the crude lipoxygenase (LOX) extract, which was stirred with hydroxyapatite (HPT) 100:95(v/v) for 20min, centrifuged at 3000xg (Himac CR50L, Hitachi, Tokyo, Japan) for 2 min. LOX was absorbed on HPT, which was then washed with 0.08M potassium phosphate buffer pH 7.0, to remove hemoglobin and protein impurities (Hsu and Pan, 1996). HPT-absorbed LOX was washed with 0.2M potassium phosphate buffer pH 7.0 from HPT. LOX activity was measured with a polarographic method using a biological oxygen monitor (YSI model 5300, Yellow Springs, OH) according to the method of Hsu and Pan (1996).

Simultaneous Distillation and Solvent Extraction of Dried Mullet Roe Aroma

The dried mullet roe was homogenized with distilled water (1:3 w/v) and 135g salt to break the emulsion. The homogenate was extracted with 60ml glass-distilled pentane/ether (1:1 v/v) for 4 h using a Likens-Nickerson apparatus. The extract was dried over anhydrous sodium sulfate and concentrated using a spinning band distillation apparatus followed by gas chromatographic analysis.

Tenax-TA Adsorption

The dried mullet roe was homogenized with 15% brine (1:3 w/v). The volatile compounds were purged with high-purity nitrogen onto a porous polymer Tenax-TA (1.6g, 60–80 mesh, Chrompack, Middleburg, The Netherlands) at 26°C for 4 h at a flow rate of 40–60ml/min. The trapped volatile compounds were eluted with 50ml double-distilled pentane/ether (1:1 v/v), then dried and concentrated using spinning band distillation.

Gas Chromatographic (GC) and GC-Sniffing Analysis

The aroma concentrates were analyzed using a Shimadzu GC-14A (Kyoto, Japan) equipped with a Carbowax-20M fused silica capillary column, 60m × 0.32mm (J&W Scientific, Folsom, CA) and flame ionization detector (FID). The oven temperature was programmed from 50°C for 5min and increased to 200°C at 2°C/min, then held for 60min. Both injector and detector temperatures were set at 250°C. The carrier gas was hydrogen

at a flow rate of 1.6ml/min. Effluents from the outlet of the column were split 8:1(v/v) between an olfactory device (SGE, Austin, TX) and the FID. Odor description of the volatile components was evaluated by 1 to 2 panelists according to Kuo *et al.* (1994).

The retention indices (RI) of the volatile components were calculated using n-paraffin of C6-C25 (Sigma, St. Louis, MO) as reference compounds (Hsu and Pan, 1996; Schomburg and Dielman, 1973).

GC-MS Analysis

A Hewlett-Packard 5890 GC-system equipped with a fused silica capillary column (DB-WAX, J&W Scientific) was used for GC/MS studies. The carrier gas was helium at 1.8ml/min. The oven temperature was programmed as mentioned above. The ionization voltage was 70eV, and the ion source temperature was 200°C. Tentative identifications were based on standard MS library data (Hewlett-Packard Co, 1988). Quantification was expressed by the ratio of the peak area to total peak area.

Sensory Evaluation

Odor was evaluated by a sensory panel consisting of 7 staffs and graduate students who showed consistency in odor description. Odor intensity was ranked from no odor, and odor with increasing intensity from "+" to "++++".

RESULTS AND DISCUSSION

Tentative Identification of Odorous Compounds

About 45 compounds were found in the gas chromatogram of the headspace aroma collected by Tenax TA (Table 1) and 63 compounds in that of the steam distillate (Table 2). Only half of the compounds were tentatively identified by comparison with the MS library data. Characteristic odors of 36 to 42 individual compounds were described.

In some cases, odors were detected when noise-like minor peaks closely associated with other compounds appearing in the chromatogram, and were unable to be identified by GC-MS, i.e., peak No. 2 in Table 1. Other situations were experienced when the odor of a compound was strong and lasting in such a way that probably masked the subsequent compounds in the chromatogram, i.e., peak No. 36 to 39 all were sniffed and reported as painty (Table 2).

Regardless of the fact that some of the compounds identified in dried mullet roe could have been mis-associated with odors detected, these data can still serve as a base for further studies on the aroma generation and characterization in roe products.

Alkane

Hexane present in headspace (Table 1) as well as decane and pentadecane in distillate (Table 2) had no flavor contribution to dried mullet roe.

Alcohols

2-Butoxyethanol was identified in both the headspace (Table 1) and the steam distillate. The characteristic odor detected was green, grassy tea-like (Table 1), and herbal (Table 2). It was described as spicy and woody in cooked crayfish tail meat (Vejaphan *et al.*, 1988).

Table 1. Aroma of unheated dried grey mullet roe collected with Tenax-TA and detected with GC-sniffing method

Peak no.	RI [1]	Compounds [2]	Percentage (%)	Odor [3]
1	747	Unknown	1.99	fruity
2	827	acetic acid ethyl ester	6.31	dried mullet roe
3	894	3-methyl-butanal	3.83	banana essence
4	924	Ethanol	2.86	roasted mullet roe
5	954	3-hexanol	0.71	—
6	983	Unknown	0.40	fruity
7	997	Decanone	0.77	fruity
8	1013	Unknown	0.86	ethanolic
9	1042	Hexanal	0.97	—
10	1051*	Unknown	trace	solvent-like*
11	1088*	Unknown	trace	fruity*
12	1109*	Unknown	trace	reheated sautéed food*
13	1123	2-pentanol	0.52	sautéing
14	1134	3-penten-2-one	0.82	solvent-like
15	1147*	Unknown	trace	sautéing*
16	1171	3-penten-2-ol	1.65	—
17	1178	2-methyl-2-buten-1-ol	1.29	solvent-like
18	1188	Unknown	0.33	squash-like
19	1201	1-pentanol	0.64	beany
20	1205*	Unknown	trace	paint-like*
21	1245	Unknown	6.24	banana-essence
22	1292*	Unknown	trace	fruity*
23	1303*	Unknown	trace	metallic*
24	1314*	Unknown	trace	fruity*
25	1325	2-methyl-2-butan-1-ol	1.39	beany, slightly metallic
26	1342	3-ethyl-2-methyl-1-pentene	5.09	—
27	1357	Unknown	4.50	green
28	1386	Unknown	0.37	celery-like
29	1404	2-butoxy-ethanol	33.21	grassy, tea-like
30	1418*	Unknown	trace	herbal*
31	1429	nonadecane-2, 4-dione	2.74	bleach-like
32	1444	Unknown	0.36	sour
33	1454	Unknown	3.33	sour
34	1467*	Unknown	trace	oily*
35	1484	Trimethyl-nonanol	0.44	solvent-like
36	1493	Unknown	0.79	paint-like
37	1505	Unknown	2.06	paint-like
38	1519	Unknown	4.88	paint-like
39	1524	2-hydroxy-2-methyl-propanoic acid	3.67	paint-like
40	1532	Unknown	0.74	beany
41	1549	Unknown	1.93	beany
42	1570*	Unknown	trace	squash-like*
43	1588*	Unknown	trace	oily*
44	1659	Unknown	0.54	reheated sautéed food
45	1729	Unknown	2.73	dried-bamboo-leave

* Odorous but too dilute to be detected by GC-FID

[1] Retention index on Carbowax-20M

[2] Compounds were tenatively identified based on standard MS library data (Hewlett-Packard Co. 1988).

[3] "—" odorless

Table 2. Aroma collected with simultaneous distillation and solvent extraction of dried grey mullet roe using Linkens-Nickerson apparatus and detected with GC-sniffing and GC-MS method

Peak no.	RI[1]	Compounds[2]	Percentage (%)	Odor[3]
1	781	Unknown	1.06	—
2	856	Unknown	0.78	reheated sautéed food
3	911	Acetic acid ethyl ester	1.14	roasted mullet roe
4	966	Ethanol	0.37	ethanolic
5	1002	Decane	0.17	—
6	1020	Unknown	0.16	garlicky
7	1053	Unknown	0.23	fruity-sour
8	1072	Unknown	0.22	reheated sautéed food
9	1088	3-methyl-heptyl-acetate	0.06	—
10	1103	Unknown	0.10	—
11	1115	Unknown	0.06	garlicky
12	1127	Unknown	0.10	—
13	1142	1-penten-3-ol	0.11	fish-, cooked fish-like
14	1153	3-penten-2-ol	0.06	fish-like
15	1165	Unknown	0.09	grassy
16	1174	Unknown	0.09	—
17	1188	1-pentanol	0.60	roasted mullet roe, green
18	1199	Unknown	0.06	—
19	1207	2-pentyl-furan	0.12	Caramel, preserved dates
20	1232	Unknown	0.17	fish-like, seaweedy
21	1261*	Unknown	trace	fish-like, seaweedy
22	1273*	Unknown	trace	fruity
23	1280	Butenyl-cyclohexene	0.15	metallic
24	1301	Unknown	0.05	—
25	1324*	Unknown	trace	fish-like
26	1334	Unknown	0.12	green, grassy
27	1371*	Unknown	trace	celery-like
28	1378	2-butoxy-ethanol	0.22	herbal
29	1401	Unknown	0.04	tuna-like
30	1408*	Unknown	trace	peanut-like
31	1425	Unknown	0.16	—
32	1441	1-heptanol	0.10	fried, sautéing
33	1473	Pentadecane	0.30	—
34	1495	Unknown	0.07	insect
35	1503	Unknown	0.10	insect
36	1535	5-methyl-2-furancarboxaldehyde	2.33	almond
37	1617	Unknown	0.05	—
38	1654*	Unknown	trace	fishy
39	1703*	Unknown	trace	green, grassy
40	1763*	Unknown	trace	dried bamboo leave-like
41	1790*	Unknown	trace	Chinese-medicinal
42	1840	Unknown	0.07	—
43	1873	4, 6-di(1, 1-dimethyl-ethyl)-2-methyl-phenol	0.08	meaty, dried bamboo leave-like
44	2014	Unknown	0.69	moldy
45	2057	Unknown	0.16	dried bamboo leave-like
46	2091	Unknown	0.07	moldy
47	2151	1-tetradecanol	4.25	seaweedy
48	2185	1-pentadecanol	0.25	—

(*continued*)

Table 2. (*Continued*)

Peak no.	RI[1]	Compounds[2]	Percentage (%)	Odor[3]
49	2241	Unknown	2.21	—
50	2254	Unknown	0.55	—
51	2284	1-hexadecanol acetate	0.44	—
52	2377	Unknown	13.05	—
53	2398	3-hexadecene	30.79	burnt rubber
54	2414	Unknown	6.34	—
55	2441	9-octadecen-1-ol	8.12	—
56	2450	Unknown	1.78	—
57	2467	Unknown	6.10	—
58	2482	Unknown	1.62	—
59	2495	Unknown	3.12	—
60	2514	Unknown	3.93	—
61	2531	Unknown	3.36	—
62	2552	Unknown	1.70	—
63	2562	Unknown	1.88	—

*Possessed odor but too dilute to be detected by GC-FID.
[1]Retention index on Carbowax-20M.
[2]Compounds were tentatively identified based on standard MS library data (Hewlett-Packard Co. 1988).
[3]"—" odorless

Ethanol, 1-pentan-3-ol, and 3-penten-2-ol (fish-like) were also present in both volatile concentrates. Meanwhile 2-pentanol (sautéing), 3-hexanol (odorless), 2-methyl-2-buten-1-ol (organic solvent-like), 2-methyl-2-butan-1-ol (beany, slightly metallic) and trimethylnonanol (slovent-like) were found only in the headspace, and 1-penten-3-ol (fish, or cooked fish-like), 1-heptanol (fried, sautéing), 1-tetradecanol (seaweedy), 1-pentadecanol and 9-octadecen-1-ol (both odorless) only in the steam distillate.

Aldehydes

Only 2 aldehydes were identified in the headspace adsorbed by Tenax TA. 3 Methylbutanal yielded an odor character of banana essence (Table 1). This compound was found in crayfish tail meat and gave a green-plant like aroma (Vejaphan *et al.*, 1988). It was also present in processed shrimp, clam and squid (Pan and Kuo, 1994). In explosion-puffed dehydrated potato, it gave a burnt and aldehyde-like off-odor[15]. The differences in flavor notes detected were probably due to concentration. Hexanal was detected without giving and odor at the concentrations present (Table 1). This compound was also found in other shellfish (Pan and Kuo, 1994; Spares, 1970). No short-chain aldehydes were found in the steam distillate (Table 2).

Ketones

2-Decanone, 3-penten-2-one and nonadecane-2,4-dione were the 3 ketone compounds identified in the headspace (Table 1). 2-Decanone was also present in cooked crayfish, shrimp, krill and corbicular (Pan and Kuo, 1994). Other isomeric forms of pentenone were found in oyster (Josephson *et al.*, 1985) and roasted shrimp (Kubota *et al.*, 1986), but not the 3-penten-2-one detected in dried mullet roe. The C_8-C_{10} ketones generally found in seafoods were not identified in dried mullet roe.

Esters

Ethylacetate ester was detected in both volatile concentrates with odor character of dried mullet roe (Table 1) or roasted mullet roe (Table 2). The odor may come from a different compound that has had a RI closely associated with acetic acid ethyl ester. Since this may be the keynote compound of mullet roe products, confirmation of the structure is being done.

Cyclic Compounds

2-Pentylfuran was found in the steam distillate (Table 2). This compound, or a compound with similar RI, contributed to the odor of caramel and preserved dates that differed from being an off-odor contributor to several fats and oils (Krishnamurthy *et al.* 1967), or not contributing to the flavor quality of crayfish (Vejaphan *et al.*, 1988). 5-Methyl-2-furancarboxaldehyde was detected in the steam distillate and contributed to an almond aroma to the dried mullet roe (Table 2).

Unidentified Odorous Components

In spite of the fact that half of the volatile compounds remained unidentified (Tables 1 and 2), desirable flavor notes, including fruity, beany, herbal, celery-like, squash-like, green and grassy, an odor similar to banana essence, dried bamboo leaves and sautéd-food flavor were found in dried mullet roe volatile.

Undesirable odors resembling organic solvent, paint, bleach, and metallic were also detected. The volatiles collected by Tenax TA consisted of more fruity components (6) than the steam distillate (2), while the latter had more fish-like, tuna-like, seaweedy and roasted mullet roe aroma components indicative of heating in distillation accelerated the seafood flavor formation particularly producing the keynote compounds of dried mullet roe.

Role of Lipoxygenase in Formation of Mullet Roe Aroma

Among the odorous compounds identified in the volatiles of dried mullet roe, alcohols, aldehydes, and ketones were likely derivatives of lipid oxidation. Lipids contributed to 17.6% wet weight of the roe. Polyunsaturated fatty acids contributed 33.54%, (98.19+129.53) / 678.98 to the total and that of unsaturated fatty acids was 75.54% of total amount (Table 3). Fatty acids containing a 1,4-pentadiene moiety can serve as substrates of LOX and result in seafood aroma formation (Kuo and Pan, 1991; Kubota *et al.*, 1986). LOX reacted with unsaturated fatty acids $C_{18:2}$, $C_{18:3}$, $C_{20:4}$, respectively, and produced volatile compounds with green, and fresh fish aromas (Pan *et al.*, 1997b). In addition, 5-, 12-, and 15-LOX activities were found in mullet roe at levels much lower than corresponding activities in mullet gill[7]. Therefore, model systems were developed using gill LOX to test the effect of endogenous LOX activities in mullet roe on its lipids in aroma formation.

The gill LOX was partially purified using 0.2M phosphate buffer (pH 7.0) in order to wash the HPT-adsorbed LOX for 2 times (Table 4). The 2 washings were combined and used to treat the lipids extracted from mullet roe. Roe lipids alone smelled slightly fishy (Table V). Inhibition of the LOX added to the roe lipids yielded a mixture with odor comparable to that of the roe lipids. Increases of LOX in the reaction mixture increased odor

Table 3. Changes in fatty acid composition of lipid extracted from fresh grey mullet roe[1] after reaction with hydro- xyapatite (HPT) partially-purified gill lipoxygenase

		LOX reacted [4]			
Fatty acids [2]	Control	25ml mg/g-lipid [3]	(%) [5]	50ml	(%) [5]
Saturated FA	(166.05)	(166.19)		(169.43)	
C14:0	6.72	6.51		6.95	
C15:0	2.55	2.66		2.50	
C16:0	99.95	100.65		101.55	
C17:0	5.68	5.60		5.72	
C18:0	21.88	21.99		21.85	
C19:0	9.27	9.08		9.91	
C24:0	20.00	19.70		20.95	
Monoenoic acid	(285.21)	(281.75)	(- 1.2%)	(282.44)	(- 1.0%)
C14:1	2.88	2.80		2.81	
C16:1	74.69	73.57		74.51	
C18:1	194.58	192.13		192.18	
C20:1	3.64	3.74		3.53	
C22:1	9.42	9.51		9.40	
Denoic acid	(98.19)	(96.00)	(- 2.2%)	(95.83)	(- 2.4%)
C18:2	95.10	93.13		93.17	
C20:2	3.09	2.87		2.66	
HUFA [4]	(129.53)	(127.31)	(- 1.7%)	(122.87)	(- 5.1%)
C18:3	16.28	15.75		15.33	
C20:3	5.54	5.27		5.11	
C20:4	13.24	12.72		11.28	
C20:5	14.32	14.47		13.61	
C22:6	80.15	79.10		77.54	
Total	678.98	671.25		670.57	

1. Roe was dissected from mullet sampled in Dec. the GSI was 12%; lipid content was 17.6%.
2. Quantification based on internal standard C13:0
3. Highly unsaturated fatty acid (no. of double bond = 3)
4. Lipoxygenase (LOX) extract of 0.55mg protein/ml with a specific activity of 73 O_2 nmole/min-mg protein in 25 ml and 50 ml respectively was reacted with roe lipid at 25° for 2h. In control, the volume of LOX extract was replaced by phosphate buffer.
5. % reduction from the control

intensity with additional flavor notes of green and fresh fish-like. It is thus assumed that the 3 LOX's present in gill extracts were similar to those endogenous in mullet roe catalyzed site-specific oxidation of the polyunsaturated fatty acids in roe lipids forming odorous compounds contributing to green and fresh fish-like aroma. Similar findings showed that fatty acids reacted with LOX produced 1-octen-3-ol possessing grassy and mushroom-like flavor notes in mushroom, yeast, ayu and shrimp (Josephson *et al.*, 1984, 1985; Kuo and Pan, 1992; Hirano *et al.*, 1992). 1-Octen-3-ol and total volatiles increased with increases in LOX activity, and decreased with the addition of LOX inhibitor (Kuo and Pan, 1991).

Changes in Unsaturated Fatty Acids in Lipoxygenase Catalyzed Aroma Formation

It was thought that LOX-catalyzed aroma generation was at the expense of depletion of polyunsaturated fatty acids. Therefore, composition of fatty acids was compared before

Table 4. Protein content and lipoxygenase activity in mullet gill extract treated with hydroxyapatite (HPT)

Treatment	Times of washing	Protein (mg/ml)	LOX	
			Specific activity (O_2nmole/min-mg protein)	Total activity (O_2 nmole/min)
Crude extract[1]		3.80	30	11,699
HPT treated supernatant[2]		0.90	14	2,409
Phosphate buffer washing[3]				
0.08M	1st	0.63	2	667
	2nd	0.21	3	134
0.2M	1st	0.23	105	6,760
	2nd	0.08	79	1,414

[1]In 0.05M phosphate buffer, pH 7 and 1mM glutathione.
[2]HPT:crude extract=95:100 (v/v), mixed and stirred for 20 min, then centrifuged at 3000xg for 2 min.
[3]Washed with 250 ml phosphate buffer (pH 7) 1~2 times, then centrifuged at 3000xg for 2 min.

and after LOX reaction with the roe lipid (Table 3). In this, saturated fatty acids did not show any decrease. Monoenoic acids were reduced by about 1%, while dienoic acids were reduced by about 2%, regardless of the LOX activities that differed by 2 times. The reduction in highly unsaturated fatty acids (HUFA) increased to 5.1% from 1.7% as the LOX activities doubled, indicative of that HUFA's are the prefered substrates for LOX catalysis.

CONCLUSIONS

The endogenous LOX activities in mullet roe are likely involved in generation of the fresh-fish like aroma. The catalysis of LOX on HUFA, possibly followed by mild non-enzymatic reactions, ie Maillard-type browning, leads to the production of the dried mullet roe flavor as proposed in Figure 1. Mullet harvested at different stages of roe development characterized by different physiological and biochemical conditions including different LOX activities may produce roe products of not quite the same flavor quality. However, the HUFA contents of the products may not differ as much, because the keynote compounds are LOX-catalyzed HUFA derivatives of extremely low threshold values.

Table 5. Aroma generated from lipid extracted from fresh grey mullet roe and reacted with HPT-partially-purified gill lipoxygenase

Lipoxygenase extract (ml) [1]	Odor [3]			Overall odor intensity[3]
	Slightly-fishy	Green	Fresh-fish-like	
0	+			+
10[2]	+			+
10	+	+		+++
20	+	+	+	++++
40	+	+	+	++++

[1] 0.28 mg protein/ml, with a specific activity of 88 nmole O_2 consumed /min-mg protein.
[2] LOX was inhibited by addition of 6N HCl 1 ml.
[3] Odor description and intensity was evaluated by 7 panelists and ranked from no odor, to odor with increasing intensity being "+" to "++++" after LOX was reacted with the roe lipid at 25 °C for 2h.

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VOLATILE COMPOUNDS IDENTIFIED IN PRESERVED DUCK EGGS

Jianhong Chen and Chi-Tang Ho

Department of Food Science
Cook College, Rutgers
The State University of New Jersey
New Brunswick, New Jersey 08903

Volatile compounds of preserved duck eggs and cooked duck eggs were isolated by a simultaneous steam distillation/extraction method and analyzed by GC and GC/MS. Flavor profiles in both egg products were compared. The preserved duck eggs have a fresh sulfur, weak roasty, and alkaline odor. Pyrazines, hydrogen sulfide, and ploysulfur compounds were exclusively identified in the preserved duck eggs, while more long chain aldehydes existed in the cooked duck eggs.

INTRODUCTION

Although egg is one of the most popular foods in the world, few studies on the flavor of eggs have been reported and no specific compounds have been determined to be responsible for characteristic egg flavor. MacLeod and Cave are the pioneers to investigate the egg flavor (MacLeod and Cave, 1975, 1976). Using a simultaneous distillation and extraction method, they have identified over 100 volatile flavor compounds in cooked hen's eggs. Those classes of compounds are hydrocarbons, furans, pyrazines, pyrroles, carbonyls, alcohols, indans, and benzenes. A review of egg flavor was later published by Maga (Maga, 1982), where he discussed the previous studies on the flavor of whole egg, egg yolk, fermented egg, and dehydrated egg products, etc. Two papers on scrambled egg flavor were recently published (Matiella and Hsieh, 1991, Warren *et al.*, 1995), and some sulfur-containing compounds such as dimethyl sulfide, thirane, dimethyl disulfide, and tetrahydrothiophene were identified. When the eggs were heated at 200 °C, some sulfides, nitriles, thiazoles, thiophenes, pyridines and more pyrazines were produced from eggs (Umano *et al.*, 1990).

Eggs are mostly consumed as cooked eggs, scrambled eggs, or as an important food ingredient in other food products. Salted eggs and preserved eggs are two traditional egg products in China.

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Preserved duck egg, which is also called pidan, alkalised egg, alkaline-gelled egg, one-thousand-year egg, or century egg, is a traditional and very popular food product in China. Preserved duck eggs have transparent and brown egg white, and semisolid or hard egg yolk. This kind of food has its characteristic aroma, which can be described as fresh sulfur odor with some alkaline sense.

There are several methods to prepare this egg product. Two common processes to prepare preserved duck eggs were described as following (Hou, 1981, Steinkraus, 1995):

"Make a paste of 1500 g lime, 960 g charcoal, 300 g caustic soda, and 240 g salt. Stir to make a paste and used it to cover the shells of about 100 duck eggs. Then cover the eggs with rice hulls to prevent their sticking together and store in an earthware jar sealed with mud. After 2 weeks, the eggs are ready for consumption.

In another procedure, strong tea is mixed with yellow mud to form a thin paste in which the eggs are dipped and covered with the paste. Lime, table salt, and caustic soda are mixed and boiled. The coated eggs are placed in the bubbling mixture and then removed and placed in an earthware jar sealed with mud. The eggs are ready for consumption after 1 month".

Preserved duck eggs can be also prepared by immersing in a water solution containing sodium chloride, sodium hydroxide, or sodium carbonate with other ingredients for around half month (Su and Lin, 1993).

During the preparation process of duck eggs, alkaline chemicals will migrate from the solution or paste through egg shell to egg white and egg yolk. The results of the migration of alkaline chemicals are increasing of the pH value of egg white and egg yolk, denaturation and gellation of egg protein, and other chemical reactions.

The volatiles of preserved duck eggs were studied before (Zhang *et al.*, 1989). The present study used the cooked duck eggs as a control to elucidate the flavor difference between cooked preserved duck eggs and cooked fresh duck eggs.

EXPERIMENTAL SECTION

Material

Preserved duck eggs and fresh cooked duck eggs were purchased in a local Asian food supermarket. The ingredients of the preserved duck eggs were stated as "duck egg, salt water, tea leave, sodium carbonate". Organic solvents were purchase from Fisher Scientific (Springfield, NJ). Antifoam A solution (30 % of concentrate) was a product of Sigma Chemical Company (St. Louis, MO).

Isolation of Volatiles

After removing the shells, the preserved duck eggs or cooked duck eggs were smashed in a food processor. Three hundred grams of egg paste were put in a round flask. After spiking with tridecane as an internal standard, and adding two drops of antiofoam solution to avoid foam generation, the volatile compounds were simultaneously distilled and extracted using a modified Linkens-Nickerson apparatus for 90 minutes. Methylene chloride was used as the extraction solvent. After extraction, organic solvent containing volatiles was then concentrated to 0.2 mL under a gentle stream of nitrogen gas before GC and GC/MS analysis.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The gas chromatography was performed on a Varian Model 3400 equipped with a flame ionization detector (FID) and a nonpolar fused silica capillary column (DB-1, 60 m x 0.32 mm (i.d.), 1.0 μm film thickness, J&W Scientific). The column temperature was programmed from 40°C to 260°C at the rate of 3°C /min. The injector and detector temperature were maintained at 270°C and 300°C, respectively. The flow rate of the helium carrier gas was 1 mL/min. The volume of the injected sample was 1μL, and the spilt ratio was 25:1. GC/MS analysis was performed using an HP Model 5790 GC coupled with a HP 5970A mass-selective detector. The capillary column and temperature program were the same for as GC analysis. Mass spectra were obtained by electron ionization at 70 ev and mass scan from 33 to 300. Compound quantification was based on the GC/FID data, and compound identification was based on mass spectra obtained from the GC/MS.

RESULTS AND DISCUSSION

The odor of the preserved duck eggs paste was like fresh sulfur, weak roasty, and alkaline; while smell of the paste of the cooked duck eggs was dominated by a fatty flavor.

The compositions of identified volatile compounds in preserved duck eggs and cooked duck eggs are listed in Table 1. The compounds are listed according to their elution order. Different aroma profiles were observed, and a total of 26 volatile compounds were identified in the two egg products.

In preserved duck eggs, the largest identified compounds were 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine, 2-butanone, ethanol, hydrogen sulfide, acetaldehyde, 3-hydroxy-2-butanone (acetoin), and 2-butenal in decreasing order. In the cooked duck eggs, the largest identified compounds were hexanal, ethanol, pentanal, and heptanal in decreasing order.

In terms of classification of volatile compounds, sulfur containing compounds and pyrazine compounds are exclusively identified in preserved duck eggs, while many more aldehydes exist in cooked duck eggs.

Sulfur compounds usually have a meaty aroma and possess a very low odor threshold value. Hydrogen sulfide can be a degradation product of sulfur containing amino acids such as cysteine, or peptides such as glutathione or some proteins (Zhang *et al.*, 1988). Hydrogen sulfide was detected in heated eggs, and its generation was increased with heating temperature and pH value (Germ, 1973). The alkaline pH of preserved duck eggs can facilitate hydrogen sulfide. Hydrogen sulfide and acetaldehyde, which were formed in preserved duck eggs, are very active and important precursors of sulfur-containing compounds. Figure 1 shows the formation mechanisms of 3,5-dimethyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine (Boelens *et al.*, 1974). Both compounds have a cooked meaty flavor, and were identified in preserved duck eggs. The compound of 3,5-dimethyl-1,2,4-trithiolane was reported in volatiles of cooked potato, beef, chicken and mutton (Brinkman, 1972, Horvat, 1976, Nixom, 1979), while 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine was identified in volatiles of cooked beef, mutton, shrimp and roasted chicken (Ohloff and Flament, 1978, Brinkman, 1972, Wilson, 1972, Tang, *et al.*, 1983)

Pyrazine compounds have typical roasty and nutty aroma. Conditions that favor pyrazine formation are high temperature, low moisture, and alkaline condition. Pyrazine

Table 1. Volatile compounds identified in preserved duck eggs (PRE) and cooked duck eggs (CDE)

Compounds[a]	RI[b]	Conc. (ppm) PRE	CDE
Hydrogen sulfide	<600	~0.2	
Acetaldehyde	<600	0.15	
Ethanol	<600	0.20	0.40
Acetone	<600	0.02	
2,3-Butanedione	<600	0.06	0.05
2-Butanone	<600	0.34	
2-Butanol	<600	0.03	
2-Butenal	626	0.10	
3-Methylbutanal	634	0.07	
Pentanal	677		0.13
3-Hydroxy-2-butanone	683	0.14	
Pyrazine	710	0.04	
Hexanal	785	0.01	0.98
Methylpyrazine	802	0.07	
2-Heptanone	869	0.02	
Heptanal	883		0.11
2,5-Dimethylpyrazine	891	0.03	
Ethylpyrazine	895	0.04	
2-Ethyl-6-methylpyrazine	879	0.02	
2-Pentylfuran	983		0.06
Octanal	986		0.08
3-Ethyl-2,5-dimethylpyrazine	1061	0.02	
2-Nonanone	1072	0.05	
Nonanal	1089		0.08
3,5-Dimethyl-1,2,4-trithiolane	1126	0.03	
3,5-Dimethyl-1,2,4-trithiolane	1133	0.04	
5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	1191	0.26	
5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	1201	0.11	

[a]Compound identification was referred to computer mass spectra library of Wiley 138.
[b]Retention index, calculated according to the retention time of n-alkanes on DB-1 column.

compounds are normally considered as products of the Maillard reaction. The deamination and deamidation of amino acids, peptides, and proteins is a very important step during pyrazine formation (Wright, 1991, Sohn and Ho, 1995, Riha *et al.*, 1996). Other than the Maillard reaction, pyrazine compounds can be produced from thermal reactions of some amine-hydroxy compounds such as ethanolamine, glucosamine, serine, threonine, etc. (Wang and Odell, 1973). The pleasant odors of pyrazine compounds undoubtedly contribute to the characteristic flavor of preserved duck eggs.

Aldehydes can be the products of sugar degradation or lipid oxidation. Their odor properties can vary from irritant to fatty. Comparing the volatiles of preserved duck eggs and cooked duck eggs, we can see that more long-chain aldehydes such as pentanal, hexanal, heptanal, octanal, and nonanal exist in cooked duck eggs than in preserved ones. These aldehydes are well-known lipid oxidation products. The compound of pentylfuran, which was also identified in cooked duck eggs, can be also derived by lipid oxidation. This compound exists largely in licorice and watermelon as well, and it processes green note.

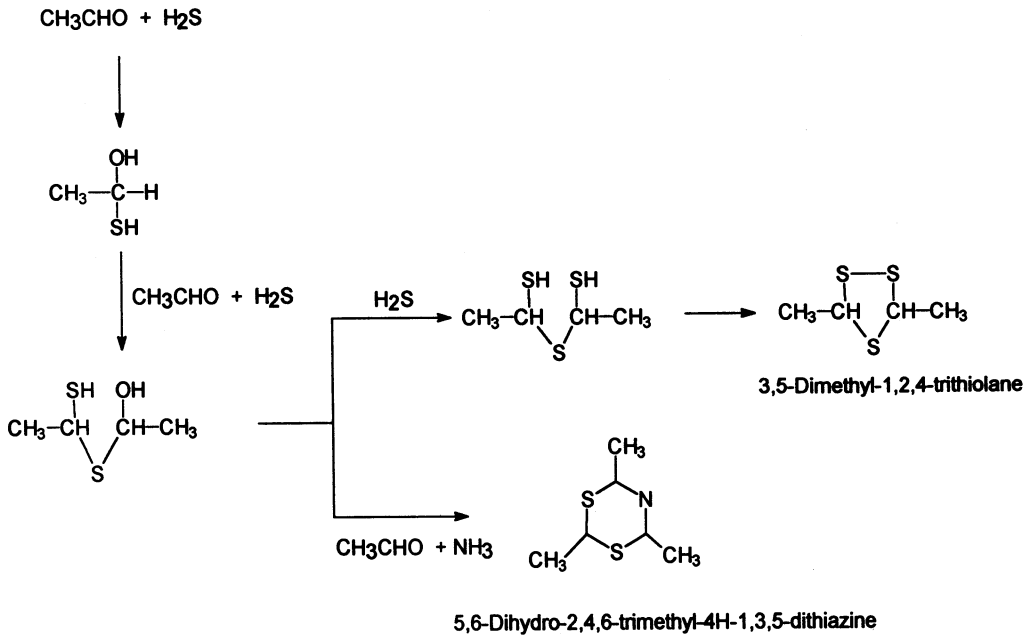

Figure 1. Formation mechanisms of 3,5-dimethyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine (Boelens *et al.*, 1974).

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