Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 522–528
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidative activity of volatile extracts from Maillard model systems

Y. Osada, T. Shibamoto *

Department of Environmental Toxicology, University of California, Davis, CA 95616, United States

Received 7 March 2005; received in revised form 17 May 2005; accepted 17 May 2005

Abstract

Dichloromethane extracts obtained from Maillard model systems consisting of glucose and eight amino acids were examined for antioxidative activity. The extracts from all eight model systems inhibited hexanal oxidation by 100% at levels higher than 50 μg/ml over 40 days. The extracts from the model systems of glucose/asparagine, histidine, or tryptophan inhibited hexanal oxidation by 100% at the level of 10 μg/ml of extract over 40 days. Seven column chromatographic fractions from the extract of a glucose/asparagine model system inhibited hexanal oxidation by 100% at levels higher than 10 μg/ml over 40 days. The fraction eluted with ethyl acetate inhibited hexanal oxidation by 100% at the level of 2 μg/ml over 40 days. 2-Furanmethanol, which has antioxidative activity, was found at a level of 0.022 mg/ml. 2,3-Diydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (pyranone), which may abstract a hydroxyl radical, was found at a level of 7.28 mg/ml in this fraction.

Keywords: Amino acids; Antioxidants; Asparagine; Browning reaction; Glucose; Pyranone

1. Introduction

Maillard reaction products (MRPs) have been shown to inhibit oxidation in model systems (Mastrocola & Munari, 2000; Mastrocola, Murari, Cioroi, & Lerici, 2000) as well as in storage experiments with food products (Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997). The Maillard reaction is the nonenzymatic reaction between reducing sugars and amino acids or proteins, which produces a complex series of compounds (MRPs), including melanoidin-antioxidants (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). There have also been reports of volatile MRPs, which are responsible for flavors in food, possessing antioxidative activities. Volatile compounds, in particular heterocyclic flavor chemicals, obtained from a sugar/amino acid model system have been reported to inhibit the oxidation of lipids (Elizalde, Bressa, & Rosa, 1992; Shaker, Ghazy, & Shibamoto, 1995). These studies clearly indicate that some flavor chemicals possess antioxidative activities (El-Massary, Farouk, & E.-Ghorab, 2003).

Antioxidants have also received much attention among food scientists as inhibitors of lipid peroxidation. Synthetic antioxidants, such as BHA and BHT, have already been used to maintain the quality of foods (Henderson, Slickman, & Henderson, 1999). Although these synthetic antioxidants are efficient and relatively cheap, there are some disadvantages because they are suspected of having some toxic properties and are restricted by legislative rules. BHA and BHT have been reported to have carcinogenic effects (Hocman, 1988). Therefore, natural antioxidants found in plants have begun to receive much attention as nontoxic and safe antioxidants. Recently, volatile chemicals found in natural plants such as beans, herbs, and spices have been reported to possess antioxidative activities (Lee, Mitchell, & Shibamoto, 2000;

* Corresponding author. Tel.: +1 530 752 4523; fax: +1 530 752 3394.
E-mail address: tshibamoto@ucdavis.edu (T. Shibamoto).

0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.05.084

Lean & Mohamed, 1999). Also, heterocyclic flavor chemicals (formed by the Maillard reaction) found in brewed coffee exhibited antioxidative activity (Yanagimoto, Lee, Ochi, & Shibamoto, 2002).

Lipid peroxidation and DNA damage caused by reactive oxygen species are associated with various diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, malaria, arthritis, and aging (Beckman & Ames, 1998; Huang, Sheu, & Lin, 1999). Therefore, it is possible that intake of antioxidants may protect against occurrence of these diseases. For example, antioxidants have a protective role against the oxidation of blood plasma, which leads to coronary disease (Fritz, Seppanen, Kurzer, & Csallany, 2003). Antioxidants, such as α-tocopherols, vitamin C, carotenoids, and phenolic compounds found in fruits and vegetables are known to inhibit the diseases described above (Packer, Rimbach, & Virgili, 1999).

In the present study, the antioxidative activities of volatiles formed in non-enzymatic browning model systems consisting of amino acids and glucose or dietary oils were investigated.

2. Materials and methods

2.1. Chemicals

Asparagine, cysteine, glycine, histidine, methionine, phenylalanine, threonine, tryptophan, butylated hydroxytoluene (BHT), and hexanal were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Undecane was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Preparation of volatile browning mixtures from D-glucose/amino acids model systems

D-Glucose (0.05 mol) and 0.05 mol each of the different amino acids listed above were dissolved in 90 ml of deionized water. The pH of the solution was adjusted to 9 with 6 N NaOH. The solution was then brought to a final volume of 100 ml with deionized water. The solution was heated at 100 °C for 16, 24, or 40 h and the reaction mixture was cooled to room temperature. The solution was extracted with 80 ml of dichloromethane using a liquid–liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate overnight. After removal of the sodium sulfate, the dichloromethane extract was concentrated to 0.3 ml by a rotary flash evaporator and subsequently by a purified nitrogen stream to 0.1 ml.

2.3. Antioxidative test

Antioxidative activities of the samples were examined by quantifying their inhibitory effect on the oxidative conversion of hexanal to hexanoic acid, as has been previously reported (Fuster, Mitchell, Ochi, & Shibamoto, 2000; Shaker et al., 1995; Yanagimoto, Ochi, Lee, & Shibamoto, 2004). Each sample (5, 10, 20, 50, 100, 200, and 500 μg/ml) was added to a 2 ml dichloromethane solution of hexanal (3 mg/ml) containing 0.2 mg/ml undecane as a gas chromatographic internal standard in a vial with a Teflon sealed cap. A sample containing no testing samples was prepared as a control. A solution containing BHT (2, 5, 10, 20, 50, and 100 μg/ml) instead of each sample was prepared as a sample of a known antioxidant. The oxidation of the sample solution was initiated by heating at 60 °C in a sealed vial for 10 min. The headspace of the vial was purged with pure air for 2 s every 2 days for the first 9 days. The decrease in hexanal was monitored by gas chromatography (GC) at 5-day intervals. Each experiment was repeated three times.

2.4. Quantitative analysis of hexanal

The quantitative analysis of hexanal was conducted according to an internal standard method previously reported (Ettre, 1967). A Hewlett–Packard (HP) model 6890 equipped with a 30 m × 0.25 mm i.d. DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used to monitor the relative amounts of hexanal in the samples. The linear velocity of the helium carrier gas was 21.3 cm/s with a split ratio of 1:22. The injector and detector temperatures were 250 °C. The oven temperature was held at 60 °C for 5 min and then programmed to 111 °C at 6 °C/min.

2.5. Fractionation of the samples by column chromatography

The volatile extracts that exhibited high antioxidative activities (glucose/asparagine, glucose/histidine, and glucose/tryptophan) were transferred to a glass column (15 cm × 1 cm i.d.) packed with 160–200 mesh silica gel (J.T. Baker Inc., NJ). The silica gel was slurry packed with pentane. The extract was eluted sequentially with 100 ml solvent mixture of different ratios of pentane and ethyl acetate (100:0, 95:5, 80:20, 50:50, 20:80, and 0:100) and then with 200 ml of acetone. The seven fractions obtained were concentrated to 0.3 ml using a rotary flash evaporator, and subsequently by a purified nitrogen stream to 0.1 ml. Each fraction was tested for antioxidative activity by the method described above.

2.6. Analyses of volatiles in the samples from a glucose/asparagine model system and its fractions

Both the gas chromatographic retention index (Kovats, 1965) and the MS fragmentation pattern of each

component were compared with those of the authentic compound for qualitative analysis. A Hewlett–Packard (HP) model 5890 gas chromatograph equipped with a DB-WAX (30 m × 0.25 mm i.d.) bonded-phase fused-silica capillary column (J&W Scientific) and a flame ionization detector (FID) were used to obtain gas chromatograms of the samples. The injector and detector temperatures were 250 °C. The oven temperature was held at 60 °C for 5 min and then programmed to 180 °C at 2 °C/min and held for 30 min. The linear velocity of the helium carrier gas was 30 cm/s with a split ratio of 1:20.

A HP Model 6890 GC interfaced to a VG Trio mass spectrometer with VG 11-250 computer data system was used for MS identification of the GC components at an MS ionization voltage of 70 eV. The column and oven conditions for GC/MS were as described for the HP 5890 GC. The identification of the GC components was also conducted with the NIST AMDIS version 2.1 software.

3. Results and discussion

The antioxidative test used in the present study was based on the oxidation system of hexanal to hexanoic acid. The aldehyde/carboxylic acid conversion assay was developed for determining the long-term antioxidant potential of a chemical or a group of chemicals in an organic phase (Macku & Shibamoto, 1991). This method is based on the autoxidation of aldehydes to the corresponding carboxylic acids with active oxygen species such as a hydroxyl radical (Nonhebel, Tedder, & Walton, 1979). Antioxidative activities of various substances have been reported using this methods, such as browning reaction products (Shaker et al., 1995), brewed coffee extract (Fuster et al., 2000), and aroma compounds from natural plants (Lee et al., 2000; Lee & Shibamoto, 2001, 2002; Yanagimoto, Ochi, Lee, & Shibamoto, 2003). To validate the aldehyde/carboxylic acid conversion assay in the present study, the antioxidant activity of the known antioxidant BHT was tested at six concentrations of 2, 5, 10, 20, 50, and 100 μg/ml. BHT inhibited hexanal oxidation by 100% even at the low concentration of 2 μg/ml, for 40 days, suggesting that this method is effective for the samples tested in the present study.

Typical results of antioxidative tests on the extracts from glucose/amino acids mixtures are shown in Fig. 1 (glucose/asparagine), in Fig. 2 (glucose/glycine), and in Fig. 3 (glucose/cysteine). The values are mean ± SD ($n = 3$). The extract from a glucose/asparagine mixture exhibited strong antioxidative activity. It inhibited hexanal oxidation at the lowest level of 10 μg/ml over 40 days (Fig. 1). On the other hand, the extract from the glucose/glycine mixture did not have any effect toward hexanal oxidation at the level of 10 μg/ml, whereas it

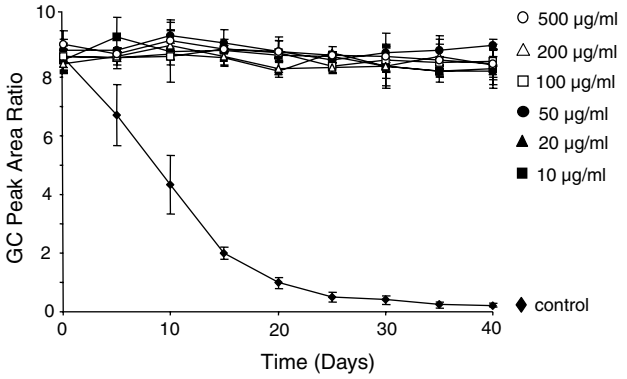


Fig. 1. Inhibitory effects of the extract from a glucose/asparagine model system toward hexanal oxidation.

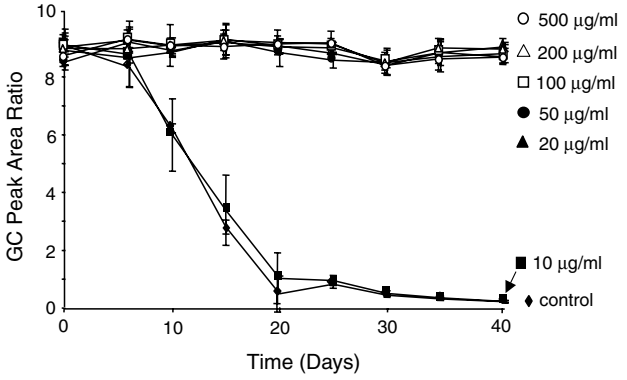


Fig. 2. Inhibitory effects of the extract from a glucose/glycine model system toward hexanal oxidation.

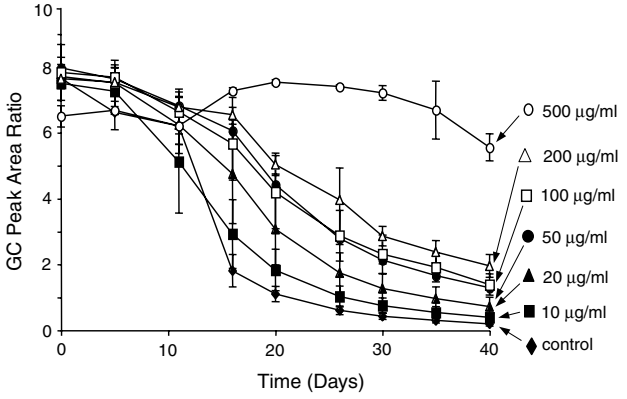


Fig. 3. Inhibitory effects of the extract from a glucose/cysteine model system toward hexanal oxidation.

inhibited hexanal oxidation by 100% at levels higher than 20 μg/ml (Fig. 2). The extract from the glucose/cysteine mixture inhibited hexanal oxidation by 100% and 82% at levels of 500 μg/ml and 200 μg/ml over 20 days, respectively. However, it did not show high activity at levels lower than 500 μg/ml over 40 days. (Fig. 3).

The extracts obtained from the model systems in addition to the three systems described above showed

somewhat similar activities to those shown in Figs. 1–3. The samples inhibited hexanal oxidation by 100% at levels above 50 μg/ml, except the samples from the glucose/cysteine and glucose/methionine systems. Variations of the effect among the samples began to show when the level was lowered below 20 μg/ml. Inhibitory effects of the extracts from eight glucose/amino acid model systems at the level of 10 μg/ml over 20 and 40 days are shown in Fig. 4. The extracts from three model systems consisting of glucose/tryptophan, histidine, or asparagine inhibited hexanal oxidation by 100% over 40 days. However, the extracts from the other five model systems did not show much activity at the level of 10 μg/ml. For example, an extract from the glucose/cysteine model system inhibited hexanal oxidation by only 20% over 20 days. An extract from the glucose/asparagine model system was fractionated into seven fractions and each fraction was tested for its inhibitory effect toward hexanal oxidation.

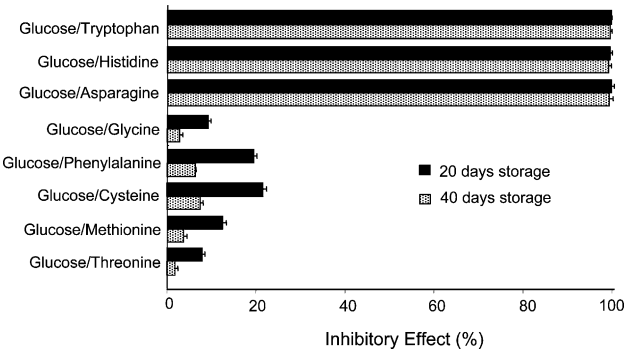


Fig. 4. Inhibitory effects of the extracts from glucose/amino acids model systems toward hexanal oxidation at the level of 10 μg/ml.

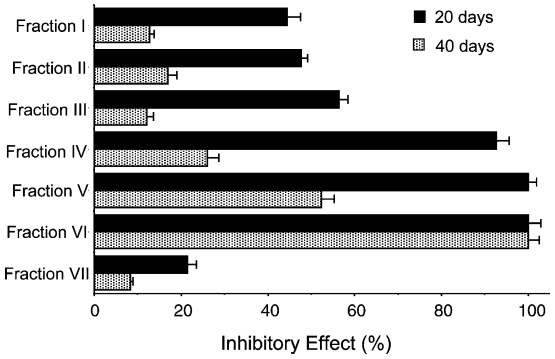


Fig. 5. Inhibitory effects of the fractions from an extract of a glucose/asparagine model system at the level of 2 μg/ml.

All fractions inhibited hexanal oxidation by 100% at levels higher than 10 μg/ml. Fig. 5 shows the results of antioxidative tests on the fractions from the glucose/asparagine model system at a level of 2 μg/ml. Among seven fractions, Fraction VI (eluate of 100% ethyl acetate) exhibited the most potent activity. It inhibited hexanal oxidation by 100% at 2 μg/ml over 40 days, which suggests that there would be compound(s) having very strong antioxidative effects in this fraction. At this low concentration, other fractions did not maintain a 100% inhibition effect on the oxidation of hexanal for 40 days. Fraction V (eluate of 20% pentane and 80% ethyl acetate) maintained a 100% inhibition effect for 20 days; however, the effect declined to about 52% over 40 days. Likewise, Fraction IV (eluate of 50% pentane and 50% ethyl acetate) showed about a 93% inhibition effect for 20 days, which declined to about 26% over 40 days. Fraction I (100% pentane), Fraction II (95% pentane and 5% ethyl acetate), and Fraction III inhibited oxidation by approximately 50% over 20 days, but declined to

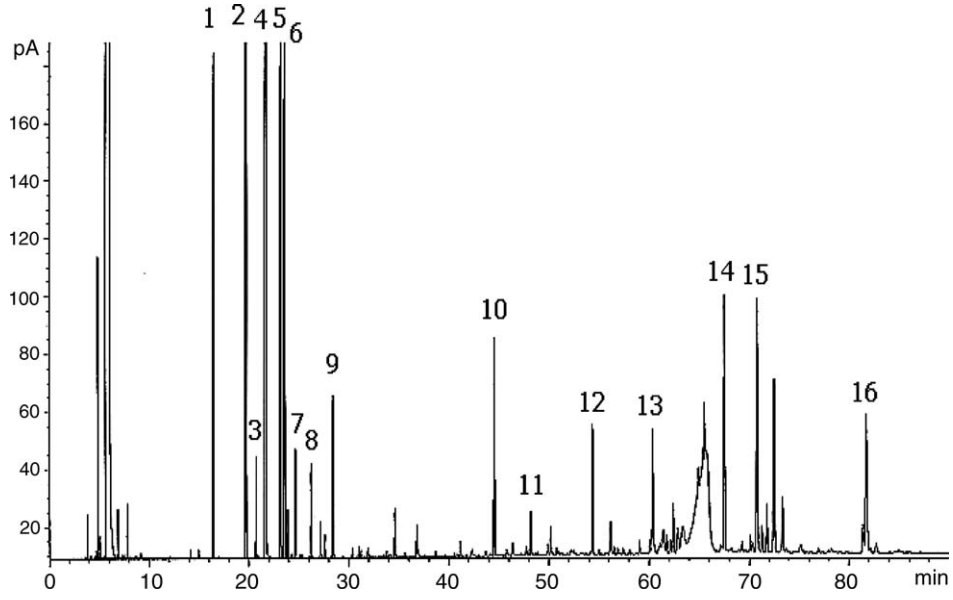


Fig. 6. Typical gas chromatogram of an extract from a glucose/asparagine model system. See Table 1 for peak identification.

less than 17% over 40 days. Fraction VII (100% acetone) exhibited the lowest activity among the seven fractions. A whole extract from the glucose/asparagine model system and an extract from its Fraction VI, which showed the most potent activity, were analyzed by GC/MS for their components to pinpoint antioxidative chemical(s).

Fig. 6 shows a typical gas chromatogram of an extract from the glucose/asparagine model system. Table 1 shows the chemicals identified in this extract. Compounds for which authentic chemicals were not available are listed as tentatively identified in Table 1. The major components are pyrazines, which comprised 17.7 mg/ml of the extract. Pyrazines are the most abundant heterocyclic compounds found in nonenzymatic browning reaction products (Shibamoto, 1983) and are important favor chemicals for a roasted or toasted flavor (Maga, 1982). However, pyrazines do not significantly inhibit hexanal oxidation. For example, 2-methylpyrazine inhibited hexanal oxidation by 20% over 40 days at a level of 500 μg/ml (Yanagimoto et al., 2002). Moreover, addition of an electron withdrawing group, such as an acetyl group, to a pyrazine ring decreased antioxidative activity. Therefore, 5-methyl-2-pyrazinylmethanol (0.495 mg/ml in the extract) may not contribute antioxidative activity to the extract. Antioxidative activity of 2-hydroxy-3-methyl-2-cyclopenten-1-one is not known, but its antitumor activity, which may be associated with antioxidative activity, has been reported (Piazo et al., 1998). This compound also formed in the glucose/alanine browning model system and possessed a caramel-like and burnt sugar-like aroma. 2-Furanmethanol or furfuryl alcohol has been found in various sugar/amino acid browning reactions (Ames, Guy, & Kipping, 2001; Shibamoto, 1983). Various furan derivatives, including 2-furanmethanol, exhibited antioxidative activity (Fuster et al., 2000; Yanagimoto et al., 2002). 2-Furanmethanol inhibited hexanal oxidation by 100% and 95% over 35 days at the levels of 500 μg/ml and 250 μg/ml, respectively (Wei, Mura, & Shibamoto, 2001).

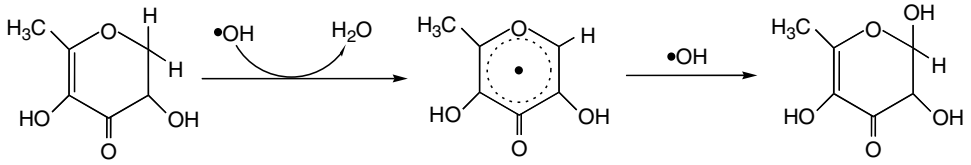
Table 1
Major compounds found in an extract from a glucose/asparagine model system

Peak # in Fig. 6	Compound	I[a]	Concentration μg/ml[b]
1	Pyrazine	1217	0.413
2	Methylpyrazine	1271	2.98
3	3-Hydroxy-2-butanone	1286	9.13
4	1-Hydroxy-2-propanone	1297	2.04
5	2,5-Dimethylpyrazine	1326	1.14
6	2,6-Dimethylpyrazine	1333	1.21
7	2,3-Dimethylpyrazine	1352	0.100
8	Trimethylpyrazine	1410	0.159
9	2,5-Hexanedione	1505	0.034
10	2-Furanmethanol	1666	0.022
11	*N*-methyl-1-butanamine[c]	1728	0.041
12	2-Hydroxy-3-methyl-2-cyclopenten-1-one	1834	0.146
13	Acrylamide	1943	0.177
14	2-Methoxy-6-methylaniline[c]	1969	0.470
15	5-Methyl-2-pyrazinylmethanol	2075	0.495
16	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (pyranone)[c]	2295	0.335

[a] Kovats index on DBWAX.
[b] Solvent peak and concentration less than 0.01 mg/ml are not shown.
[c] Tentatively identified (authentic chemicals are not available).

Acrylamide has recently received much attention as a carcinogen found in cooked starch-rich foods such as potato chips and French fries (Friedman, 2003). A high level of acrylamide (221 mg/mol of amino acid) was found in a heated equimolar mixture of asparagine and glucose at 185 °C (Mottram, Wedzicha, & Dodson, 2002). In the present study, acrylamide (35.4 μg/mol of asparagine) was also found in the extract from the glucose/asparagine model system. The amount found in the present study was much lower than that found in the study cited above. This is due to the fact that the mixture was heated at a much lower temperature (100 °C) than the above study (185 °C). Moreover, it might not be recovered from an aqueous solution with dichloromethane because of its high water solubility (Yasuhara, Tanaka, Hengel, & Shibamoto, 2003). There is no report on the anitoxidative activity of alkyl amides.

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (pyranone; 0.335 mg/ml in the extract) is an interesting compound. An authentic chemical sample of this compound was not available, but its identity was confirmed by the NIST AMDIS software (Yaylayan, Machiels, & Istasse, 2003). Pyranone content in the three extracts (glucose/asparagine, histidine, or tryptophan) ranged from 0.335 to 6.28 mg/ml of the extract. All these samples greatly inhibited hexanal oxidation. Moreover, Fraction VI, which inhibited hexanal oxidation the most among



2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

2,3-dihydro-2,3,5-trihydroxy-6-methyl-4H-pyran-4-one

Fig. 7. Proposed reaction mechanisms of a hydroxyl radical abstraction by pyranone.

the fractions from the glucose/asparagine extract, contained a high level of pyranone (7.28 mg/ml of the fraction), suggesting that this compound plays an important role in the antioxidative activity of extracts obtained from glucose/amino acids model systems.

Fig. 7 shows a proposed reaction mechanism of a hydroxyl radical abstraction by pyranone based on the hypothesis previously reported (Yanagimoto et al., 2002).

4. Conclusions

Amino acids, proteins, sugars, carbohydrates, and lipids are major components of foods and any combinations of these chemical(s) produce Maillard reaction products or nonenzymatic browning reaction products upon heat treatment such as cooking. Dichloromethane extracts obtained from browning model systems consisting of glucose and major amino acids exhibited appreciable antioxidative activities in the present study. Even though the antioxidative activity of each compound present in the extract is not strong, the overall antioxidative effect could be high by the combined effect and the synergistic effect of many compounds produced by the Maillard reaction. Therefore, volatile chemicals, in particular some heterocyclic compounds formed by the Maillard reaction during cooking, may contribute certain beneficial effects to foods.

References

Ames, J. M., Guy, R. C. E., & Kipping, G. J. (2001). Effect of pH and temperature on the formation of volatile compounds in cysteine/reducing sugar/starch mixtures during extrusion cooking. *Journal of Agricultural and Food Chemistry, 49*, 1885–1894.

Beckman, K. B., & Ames, B. N. (1998). The free radical theory of aging mature. *Physiology Reviews, 78*, 547–581.

El-Massary, K., Farouk, A., & E.-Ghorab, A. (2003). Volatile constituents of glutathione – ribose model system and its antioxidant activity. *Amino Acids, 24*, 171–177.

Elizalde, B. E., Bressa, F., & Rosa, M. D. (1992). Antioxidative action of Maillard reaction volatiles—influence of Maillard solution browning level. *Journal of American Oil Chemists' Society, 69*, 331–334.

Ettre, L. S. (1967). Interpretation of analytical results. In L. S. Ettre & A. Zlatkis (Eds.), *The practice of gas chromatography* (pp. 402). New York: Interscience Publishers.

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A Review. *Journal of Agricultural and Food Chemistry, 51*, 4504–4526.

Fritz, K. L., Seppanen, C. M., Kurzer, M. S., & Csallany, A. S. (2003). The in vivo antioxidant activity of soybean isoflavones in human subjects. *Nutrition Research, 23*, 479–487.

Fuster, M. D., Mitchell, A. E., Ochi, H., & Shibamoto, T. (2000). Antioxidative activities of heterocyclic compounds formed in brewed coffee. *Journal of Agricultural and Food Chemistry, 48*, 5600–5603.

Henderson, D. E., Slickman, A. M., & Henderson, S. K. (1999). Quantitative HPLC determination of the antioxidant activity of capsaicin on the formation of lipid hydroperoxides of linoleic acid: a comparative study against BHT and melatonin. *Journal of Agricultural and Food Chemistry, 47*, 2563–2570.

Hocman, G. (1988). Chemoprevention of cancer–phenolic antioxidants (BHT, BHA). *International Journal of Biochemistry, 20*, 639–651.

Huang, Y. L., Sheu, J. Y., & Lin, T. H. (1999). Association between oxidative stress and changes of trace elements in patients with breast cancer. *Clinical Biochemistry, 32*, 1069–1072.

Kovats, E. (1965). Gas chromatographic characterization of organic substances in the retention index system. *Advances in Chromatography, 1*, 229–247.

Lean, L. P., & Mohamed, S. (1999). Antioxidative and antimycotic effects of turmeric, lemon-grass, betel leaves, clove, black pepper leaves and garcinia atriviridis on butter cakes. *Journal of the Science of Food and Agriculture, 79*, 1817–1822.

Lee, K.-G., Mitchell, A., & Shibamoto, T. (2000). Antioxidative activities of aroma extracts isolated from natural plants. *BioFactors, 13*, 173–178.

Lee, K.-G., & Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove bud [*Syzygiun aromaticum* (L.) Merr. et Perry].. *Food Chemistry, 74*, 443–448.

Lee, K.-G., & Shibamoto, T. (2002). Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *Journal of Agricultural and Food Chemistry, 50*, 4947–4952.

Macku, C., & Shibamoto, T. (1991). Volatile antioxidants produced from heated corn oil glycine model system. *Journal of Agricultural and Food Chemistry, 39*, 1990–1993.

Maga, J. A. (1982). Pyrazines in foods: an update. *CRC Critical Reviews in Food Science and Nutrition, 16*, 1–48.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology, 11*, 340–346.

Mastrocola, D., & Munari, M. (2000). Progress of the Maillard reaction and antioxidant action of Maillard reaction products in preheated model systems during storage. *Journal of Agricultural and Food Chemistry, 48*, 3555–3559.

Mastrocola, D., Murari, M., Cioroi, M., & Lerici, C. R. (2000). Interaction between Maillard reaction products and lipid oxidation in starch-based model systems. *Journal of The Science of Food and Agriculture, 80*, 684–690.

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature, 419*, 448–449.

Nicoli, M. C., Anese, M., Parpinel, M. T., Franceschi, S., & Lerici, C. R. (1997). Loss and/or formation of antioxidants during food processing and storage. *Cancer Letter, 114*, 71–74.

Nonhebel, D. C., Tedder, J. M., & Walton, J. C. (1979). *Radicals* (pp. 155–157). London: Cambridge University Press.

Packer, L., Rimbach, G., & Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritime*) bark, pycnogenol. *Free Radical Biology and Medicine, 27*, 704–724.

Piazo, R., Liu, B., Ji, Z., Song, L., Geng, Y. U., & Qi, H. (1998). Synthesis and antitumor activity of Mannich bases of 2-hydroxy-3-methyl-2-cyclopenten-1-one. *Zhongguo Yaowu Huaxue Zazhi, 8*, 157–162.

Shaker, E. S., Ghazy, M. A., & Shibamoto, T. (1995). Antioxidative activity of volatile browning reaction products and related compounds in a hexanal/hexanoic acid system. *Journal of Agricultural and Food Chemistry, 43*, 1017–1022.

Shibamoto, T. (1983). Heterocyclic compounds in browning and browning/nitrite model systems: occurrence, formation mechanisms, flavor characteristics and mutagenic activity. In G. Charalambous & G. Inglett (Eds.). *Instrumental Analysis of Foods: Recent Progress* (vol. I, pp. 229–278). New York: Academic Press.

Wei, A., Mura, K., & Shibamoto, T. (2001). Antioxidative activity of volatile chemicals extracted from beer. *Journal of Agricultural and Food Chemistry, 49*, 4097–4101.

Yanagimoto, K., Lee, K.-G., Ochi, H., & Shibamoto, T. (2002). Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard reaction. *Journal of Agricultural and Food Chemistry, 50*, 5480–5484.

Yanagimoto, K., Ochi, H., Lee, K.-G., & Shibamoto, T. (2003). Antioxidative activities of volatile extracts from green tea, oolong tea, and black tea. *Journal of Agricultural and Food Chemistry, 51*, 7396–7401.

Yanagimoto, K., Ochi, H., Lee, K.-G., & Shibamoto, T. (2004). Antioxidative activities of fractions obtained from brewed coffee. *Journal of Agricultural and Food Chemistry, 52*, 592–596.

Yasuhara, A., Tanaka, Y., Hengel, M., & Shibamoto, T. (2003). Gas chromatographic investigation of acrylamide formation in browning model systems. *Journal of Agricultural and Food Chemistry, 51*, 3999–4003.

Yaylayan, V. A., Machiels, D., & Istasse, L. (2003). Thermal decomposition of specifically phosphorylated D-glucoses and their role in the control of the Maillard reaction. *Journal of Agricultural and Food Chemistry, 57*, 3358–3366.