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Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]

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Abstract

Aroma extract from dried clove buds [*Syzygium aromaticum* (L.) Merr. et Perry] was obtained by using steam-distillation under mild conditions (55°C and 95 mm Hg). The antioxidant property of the aroma extract was evaluated in two different assays. The aroma extract isolated from clove buds inhibited the oxidation of hexanal for 30 days at a level of 50 μg/ml. Clove bud extract inhibited malonaldehyde formation from cod liver oil by 93% at the 160 μg/ml level. Twenty-two compounds were identified in the extracts of clove buds by gas chromatography and gas chromatography/mass spectrometry. The major aroma constituents of clove buds were eugenol (24.371 mg/g) and eugenyl acetate (2.354 mg/g). Eugenol, eugenyl acetate, and benzyl alcohol inhibited the oxidation of hexanal by 99, 99, and 82%, respectively, for a period of 30 days at 500 μg/ml. Eugenol, eugenyl acetate, and benzyl alcohol inhibited malonaldehyde formation from cod liver oil by 88, 79, and 63%, respectively, at 160 μg/ml. The antioxidant activity of clove bud extract and its major aroma components, eugenol and eugenyl acetate, were comparable to that of the natural antioxidant, α-tocopherol (vitamin E).

Keywords: Aroma chemicals; Clove bud extract; Eugenol; Natural antioxidants

1. Introduction

Aroma chemicals present in natural leaves and flowers have been widely used in aroma therapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor. Until recently, aroma chemicals have been studied mainly from the aspects of flavor and fragrance chemistry. The medicinal qualities of aroma chemicals, such as antioxidant activities, have been discovered lately. The antioxidant activities of aroma extracts obtained from spices (Nakatami, 1997), herbs (Okuda, 1999), brewed coffee (Singhara, Macku, & Shibamoto, 1998), and beans (Lee, Mitchell, & Shibamoto, 2000) have been investigated in various model systems. Some known natural aroma components, such as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, maltol, and 5-hydroxy methylfurfural have been reported to possess appreciable antioxidant activities (Koga, Moro, & Matsudo, 1998; Singhara et al., 1998).

Cloves are the dried flower buds of *Syzygium aromaticum* (L.) Merr. et Perry — a tree of the myrtle family (Myrtaceae). They are sources of anti-microbial agents against oral bacteria that are commonly associated with dental caries and periodontal disease (Cai & Wu, 1996). In Korea, cloves have been successfully used for asthma and various allergic disorders by oral administration (Kim et al., 1998). Sesquiterpenes, found in clove, were investigated as potential anticarcinogenic agents (Zheng, Kenny, & Lam, 1992). A main aroma constituent of clove buds, eugenol, was reported to have antifungal activity (Martini, Weidenbörner, Adams, & Kunz, 1996). In the present study, the antioxidant activity of aroma extracts isolated from clove buds was examined in two different assays. Some major aroma chemicals identified in the extracts of clove buds were also examined for their antioxidative activities.

2. Materials and methods

2.1. Clove buds

Dried clove buds [*Syzygium aromaticum* (L.) Merr. et Perry] were purchased from a local market.

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2.2. Reagents

Eugenol, eugenyl acetate, benzyl alcohol, hexanal, hexanoic acid, undecane, N-methylhydrazine (NMH), 2-methylpyrazine, sodium dodecyl sulfate (SDS), ferrous chloride, and α-tocopherol (vitamin E) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cod liver oil (approximately 70% ω-3 fatty acid methyl esters), butylated hydroxytoluene (BHT), trizma hydrochloride, and trizma base were bought from Sigma Chemical Co. (St. Louis, MO). Hydrogen peroxide, dichloromethane, and ethyl acetate were bought from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). The standard stock solution of 2-methylpyrazine was prepared by adding 100 mg of 2-methylpyrazine to 10 ml of dichloromethane and was stored at 5°C. Authentic 1-methylpyrazole (1-MP) was synthesized from malonaldehyde (MA) and N-methylhydrazine according to a previously developed method (Umano, Dennis, & Shibamoto, 1988).

2.3. Isolation of aroma chemicals by steam-distillation under reduced pressure (DRP)

Dried clove buds (20 g) were placed in a 3-l round-bottom flask with 1 l deionized water. The solution was steam-distilled at 55°C for 3 h under reduced pressure (95 mmHg). The distillate (900 ml) was extracted with 100 ml dichloromethane, using a liquid-liquid continuous extractor, for 6 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by a rotary flash evaporator. The distillation was stopped when the volume of extract was reduced to approximately 1 ml, and then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.6 ml.

2.4. Identification of aroma chemicals in the extracts from clove buds

Aroma chemicals obtained by DRP were identified by comparison with the Kovats gas chromatographic retention index I (Kovats, 1965) and by the MS fragmentation pattern of each component compared with those of authentic chemicals.

2.5. Measurement of total aroma compounds in extracts

Solvents from extracts obtained by DRP were removed under a purified nitrogen stream until the total volume of concentrate was reduced to approximately 500 μl. The mass of the concentrate was measured using an analytical balance. The concentrate was then analyzed by a gas chromatograph (GC), equipped with a flame ionization detector (FID), to determine the percent total peak-area of aroma compounds and solvent. The total mass of aroma chemicals was calculated by multiplying the percent representing the total peak-area of aroma compounds with the mass of each extract. Each experiment was repeated three times. The detector response to solvent was determined to be linear over a range of 0.2 to 1.0 μl injected, with a R^2 value of 0.99.

2.6. Aldehyde/carboxylic acid assay

The antioxidative activity of the samples obtained by DRP was tested using their inhibitory effect toward oxidation of aldehyde to acid (Macku & Shibamoto, 1991). Various amounts of aroma extracts and aroma chemicals were added to a 2 ml dichlomethane solution of hexanal (3 mg/ml) containing 0.2 mg/ml of undecane as a GC internal standard. The oxidation of the sample solution was initiated by heating at 60°C for 10 min in a sealed vial and storing at room temperature. The headspace of each vial was purged with pure air (1.5 l/min, 3 s) every 24 h for the first 10 days. The decrease in hexanal was monitored at 5-day time intervals for 30 days. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology.

2.7. Lipid/MA assay

The antioxidative activity of extracts was determined by analyzing MA formed from cod liver oil upon oxidation after derivatizing to 1-MP with NMH (Tamura, Kitta, & Shibamoto, 1991). An aqueous solution (5 ml) containing 10 μl of cod liver oil, 0.25 mmol of trizma buffer (pH 7.4), 5 μmol of ferrous chloride, 10 μmol of hydrogen peroxide, 0.75 mmol of potassium chloride, and 0.2% of surfactant SDS was incubated with various amounts of the aroma extracts and aroma chemicals for 18 h at 37°C in a 20-ml test tube. The oxidation of samples was stopped by adding 50 μl of a 4% BHT solution (Ichinose, Miller, & Shibamoto, 1989). The sample tubes were covered with aluminium foil during incubation to avoid any influence of light on the lipid peroxidation. Known antioxidants, α-tocopherol and BHT, were used to compare antioxidant activity to that of the aroma extracts tested. NMH (30 μl) was added to the above oxidized cod liver oil solutions, and the solutions were stirred for 1 h at room temperature. Each reaction solution was extracted with 10 ml of dichloromethane using a liquid-liquid continuous extractor for 3 h. The solution was saturated with NaCl prior to extraction to prevent the formation of an emulsion. The extract was adjusted to exactly 10 ml by adding dichloromethane and 20 μl of a 2-methylpyrazine solution as a GC internal standard. To avoid damage to the nitogen-phosphorus detector (NPD), the solvent, dichloromethane, in the adjusted solution was removed under a purified nitrogen stream and then the volume

was adjusted to exactly 10 ml with ethylacetate. The solution was analyzed for 1-MP by a GC with a NPD.

2.8. Instrumental analysis

The quantitative analysis of hexanal and 1-MP was conducted according to an internal standard method (Ettre, 1967). A Hewlett-Packard (HP) model 5890 GC equipped with a 30 m×0.25 mm i.d. (d_f=0.25 μm) DB-1 bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and a FID was used for analysis of hexanal. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1. The injector and the detector temperatures were 300 and 280°C, respectively. The oven temperature was programmed from 40 to 180°C at 4°C/min and held for 10 min.

To determine total aroma compounds in extracts, a HP model 6890 GC equipped with a 30 m×0.25 mm i.d. (d_f=0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and a FID was used.

A HP model 6890 GC, equipped with a 30 m×0.25 mm i.d. (d_f=0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA), and a NPD was used for analysis of 1-MP.

A HP model 6890 GC, interfaced to a HP 5791A mass selective detector (GC/MS), was used for mass spectral identification of the GC components at MS ionization voltage of 70 eV. A 30 m×0.25 mm i.d. (d_f=0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) was used for a GC. The linear velocity of the helium carrier gas was 30 cm/sec. The injector and the detector temperatures were 250°C. The oven temperature was programmed from 50 to 180°C at 3°C/min and held for 40 min.

3. Results and discussion

The total yield of aroma chemicals from clove buds (relative to the amount of dried clove buds used) was 2.75±0.57% (w/w). The values are mean±standard deviation (n=3). Table 1 shows aroma chemicals identified in extracts from clove buds, along with their calculated concentrations and Kovats indices on a DB-WAX column. Concentration of each chemical was calculated using the following equation:

$$\text{Concentration (mg/g)} = \frac{\text{Weight of extract (without solvent)} \times \text{GC peak area\%}/100(\text{ mg})}{\text{Weight of dried clove buds (20 g)}}$$

A typical gas chromatogram of the aroma extract isolated from clove buds is shown in Fig. 1. The major aroma constituents of this extract were eugenol (24.371 mg/g), eugenyl acetate (2.354 mg/g), and 1-actyloxy-2-propanol (0.162 mg/g). The chemicals marked in italics in Table 1 were tested for antioxidant activity.

Fig. 2 a–d shows the amounts of remaining hexanal in samples containing various amounts of the aroma extract of clove (a), eugenol (b), eugenyl acetate (c), and benzyl alcohol (d) in the aldehyde/carboxylic acid assay

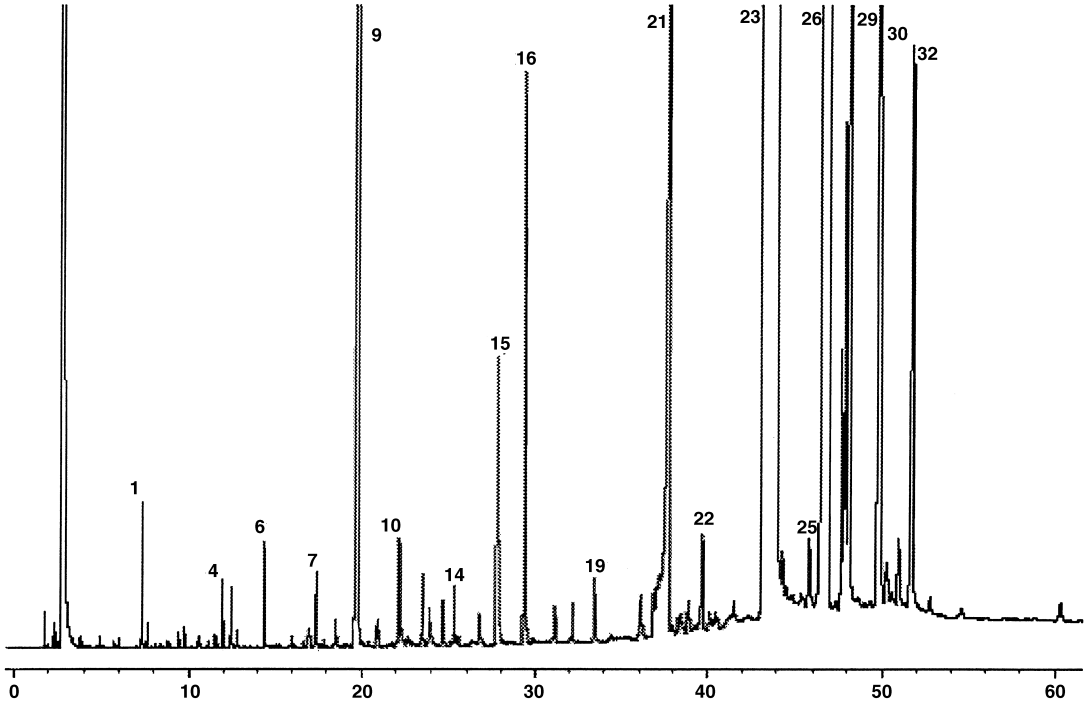

Fig. 1. Typical gas chromatogram of the aroma extract isolated from clove buds. Refer to Experimental Procedure for GC conditions.

Table 1
Aroma chemicals identified in the extract from clove buds

Peak No.	I[a]	Concentration[b] (mg/g)[c]	Component
1	1191	0.004	2-Heptanone
3	1270	0.001	3-Pyrrolidinol
4	1332	0.002	2-Heptanol
5	1346	0.002	2-Butenal
6	1394	0.004	2-Methylpentanal
7	1470	0.003	3(2H)-Pyridazinone
9	1525	0.162	1-Acetyloxy-2-propanone
10	1579	0.004	1-Acetoxy-2-propanol
11	1611	0.003	Methylbenzoate
13	1644	0.002	Benzylaldehyde
14	1660	0.003	Ethylbenzoate
15	1727	0.016	Phenylmethyl acetate
16	1766	0.033	Salicylic acid
19	1874	0.003	*Benzyl alcohol*[d]
21	1992	0.136	2-Methyl-5-(1-methylethenyl)-cyclohexyl acetate
22	2042	0.005	(E,E)-2,4-heptadienal
23	2181	24.371	*Eugenol*[d]
25	2247	0.004	*Trans*-isoeugenol
26	2277	2.354	*Eugenyl acetate*[d]
27	2295	0.019	2-(1,1-Dimethylethyl)-2,5-cyclohexadiene-1,4-dione
28	2300	0.043	2,5-Dimethylanisole
32	2341	0.126	Isophthalaldehyde

[a] On DB-Wax
[b] Solvent peak excluded.
[c] Values are on a dried weight of clove buds in mg/g.
[d] Tested for antioxidant activity

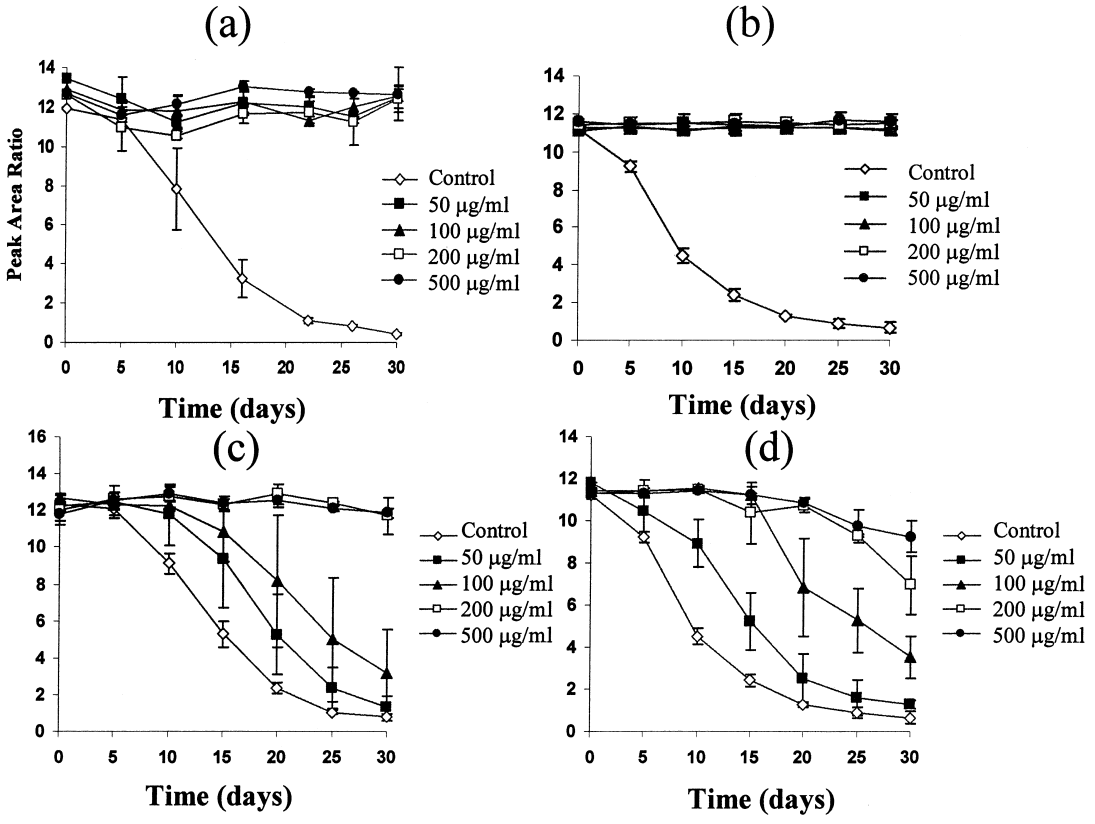

Fig. 2. Relative amounts of remaining hexanal in samples with the aroma extract of (a) clove, (b) eugenol, (c) eugenyl acetate, and (d) benzyl alocohol.

tested for a period of 30 days. The values are mean ± standard deviation ($n = 3$). All extracts exhibited dose-dependent inhibitory activity. In this experiment, the hexanal in a control was completely oxidized to hexanoic acid after 30 days. At extract concentrations of 50, 100, 200, 500 μg/ml, clove buds inhibited the hexanal oxidation by almost 100% for 30 days. Eugenol, a major aroma component in the clove extract, also inhibited the hexanoic acid formation by almost 100% for 30 days at all levels of concentration. Antioxidative activities of pure eugenol were consistent with those of clove extract in the two testing systems. Calculated concentrations of eugenol in 50, 100, 200, and 500 μg/ml of clove extract were 44, 89, 177, 443 μg/ml, respectively. Therefore, antioxidant activity of clove extract was mainly due to the high content of eugenol. Eugenyl acetate inhibited the hexanal oxidation by 99% for 30 days at concentrations of 200 μg/ml and 500 μg/ml. At a level of 50 μg/ml and 100 μg/ml, eugenyl acetate retained the concentration of hexanal by 76 and 85%, respectively, for 15 days. Compared to the other tested aroma chemicals, benzyl alcohol showed a lower antioxidant activity in the Aldehyde/Carboxylic Acid Assay. Benzyl alcohol inhibited the hexanal oxidation by 82 and 62% at concentrations of 500 μg/ml and 200 μg/ml, respectively, for 30 days.

Fig. 3 shows the percent of hexanal remaining in samples containing different amounts of the aroma extract of clove, eugenol, eugenyl acetate, benzyl alcohol, and 50 μg/ml of BHT or α-tocopherol at the end of a storage period of 30 days. The values are mean ± standard deviation ($n = 3$). At a 50 μg/ml level, the inhibitory activity of clove extract and eugenol was comparable to that of α-tocopherol or BHT. Eugenyl acetate showed comparable antioxidant activity to that of α-tocopherol or BHT at a concentration of 200 μg/ml.

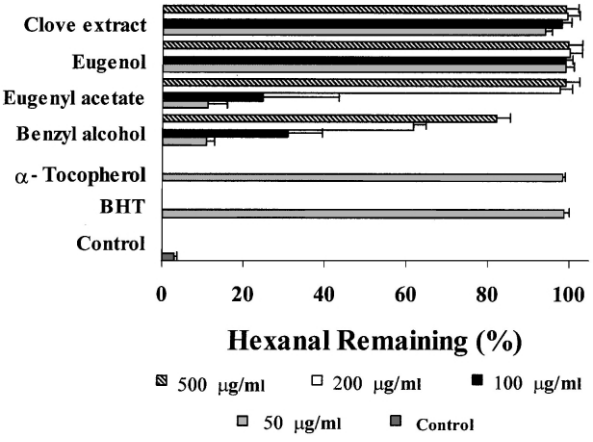

Fig. 3. Percent of hexanal remaining in samples containing different amounts of aroma extract of clove buds, aroma chemicals found in clove buds, and 50 μg/ml of butylated hydroxytoluene or α-tocopherol throughout a storage period of 40 days.

The lipid/MA assay is specific to measure a lipid peroxidation product, particularly MA in samples (Umano et al., 1988). Fig. 4 shows the results of the lipid/MA assay in the presence of clove bud extracts and various aroma constituents, along with those of α-tocopherol and BHT. The values are mean ± standard deviation ($n = 3$). The amount of MA formed from 10 μl of cod liver oil alone was 1422 ± 150 nmol under the conditions used in this study. The results were similar to those obtained in the aldehyde/carboxylic acid assay. Clove bud extract, eugenol, eugenyl acetate, and benzyl alcohol inhibited MA formation by 93, 88, 79, and 63%, respectively, at the level of 160 μg/ml. The antioxidative activities of clove bud extract, eugenol and eugenyl acetate were comparable to those of α-tocopherol and BHT which inhibited MA formation by 96 and 97% at the same concentration of 160 μg/ml.

Even though the antioxidative activity of clove buds has been reported several times (Farag, Badei, Hewedi, & El-Baroty, 1989; Kramer, 1985), the antioxidative activity of an aroma extract of clove buds has not been investigated prior to the present study. The antioxidative activity of eugenol was consistent with previous reports (Nagabubu & Lakshmaiah, 1992; Satoh, Ida, Sakagami, Tanaka & Fusisawa, 1998). However, the antioxidative activity of eugenyl acetate has not been investigated prior to the present study. A significant relationship between the antioxidant efficiency and the chemical composition of aroma chemicals was observed in the present study. Phenolic groups play an important role in antioxidative activity (Huang & Frankel, 1997). The strong antioxidant activities of eugenol in two assays are due to the presence of the phenolic group. However, eugenol acetate does not have

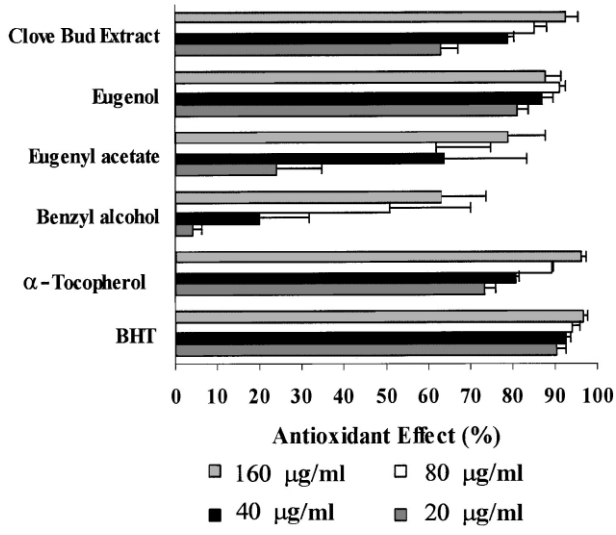

Fig. 4. Inhibitory effects (%) of aroma extract of clove buds, aroma chemicals found in clove buds, α-tocopherol, and butylated hydroxytoluene toward malonaldelhyde formation from cod lover oil at a various levels.

a phenolic group; therefore, its antioxidant activity is presumably due to the stability of the radical formed by abstraction of H from the CH_2 para to the ester group.

4. Conclusion

Varying degrees of antioxidative activity were exhibited by the aroma extract and major aroma chemicals found in clove buds. The aroma extract of clove buds and its two major aroma chemicals, eugenol and eugenyl acetate, showed obvious antioxidative activity in two different assays. Although their activity is not as strong as a-tocopherol and BHT, tremendous numbers of these aroma compounds are present in natural plants, so their combined activities might be comparable to those of known antioxidants. It is nearly impossible to test the possible synergism between the more than 50 compounds observed in a gas chromatogram of clove bud extract. However, the present study suggests that the antioxidative activities of clove buds are due in part to the contributions of aroma chemicals such as eugenol and eugenyl acetate. The presence of various aroma chemicals may explain the improvement of food stability. Moreover, ingestion of these compounds may help to prevent in vivo oxidative damage, such as lipid peroxidation, which is associated with many diseases, including cancer, arteriosclerosis, diabetes, and immune deficiency.

References

Cai, L., & Wu, C. D. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *Journal of Natural Products*, *59*, 987–990.

Ettre, L. S. (1967). Interpretation of analytical results. In L. S. Ettre & A. Zlatkis, *The practice of gas chromatography* (pp. 402–440). New York:Interscience Publishers.

Farag, R. S., Badei, A. Z. M. A., Hewedi, F. M., & El-Baroty, G. S. A. (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *Journal of American Oil Chemists' Society*, *66*, 792–799.

Huang, S. W., & Frankel, E. N. (1997). Antioxidant activity of tea catechins in different lipid systems. *Journal of Agricultural and Food Chemistry*, *45*, 3033–3038.

Ichinose, T., Miller, M. G., & Shibamoto, T. (1989). Gas chromatographic analysis of free and bound malonaldehyde in rat liver homogenates. *Lipids*, *42*, 1527–1532.

Kim, H. M., Lee, E. H., Hong, S. H., Song, H. J., Shin, M. K., Kim, S. H., & Shin, T. Y. (1998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. *Journal of Ethnopharmacology*, *60*, 125–131.

Koga, T., Moro, K., & Matsudo, T. (1998). Antioxidative behaviors of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone against lipid peroxidation. *Journal of Agricultural and Food Chemistry*, *46*, 946–951.

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

Kramer, R. E. (1985). Antioxidants in clove. *Journal of American Oil Chemists' Society*, *62*, 111–113.

Lee, K. G., Mitchell, A. E., & Shibamoto, T. (2000). Determination of antioxidant properties of aroma extracts isolated from various beans. *Journal of Agricultural and Food Chemistry*, *48*, 4817–4820.

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

Martini, H., Weidenbörner, M., Adams, S., & Kunz, B. (1996). Eugenol and carvacrol: the main fungicidal compounds in clove. *Italian Journal of Food Science*, *1*, 63–67.

Nagababu, E., & Lakshmaiah, N. (1992). Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochemical Pharmacology*, *43*, 2393–2400.

Nakatami, N. (1997). Antioxidants from spices and herbs. In F. Shahidi, *Natural antioxidants: chemistry, health effects, and applications* (pp. 64–75). Champaign, Illinois: AOAC Press.

Okuda, T. (1999). Antioxidants in herbs: polyphenols. In L. Packer, M. Hiramatsu & T. Yoshikawa, *Antioxidant food supplements in human health* (pp. 393–410). San Diego, California: Academic Press.

Satoh, K., Ida, Y., Sakagami, H., Tanaka, T., & Fusisawa, S. (1998). Effect of antioxidants on radical intensity and cytotoxic activity of eugenol. *Anticancer Research*, *18*, 1549–1552.

Singhara, A., Macku, C., & Shibamoto, T. (1998). Antioxidative activity of brewed coffee extracts. In T. Shibamoto, J. Terao & T. Osawa, *Functional foods for disease prevention II: medicinal plants and other foods, ACS symposium series 701* (pp. 101–109). Washington, DC: American Chemical Society.

Tamura, H., Kitta, K., & Shibamoto, T. (1991). Formation of reactive aldehydes from fatty acids in a Fe^{2+}/H_2O_2 oxidation system. *Journal of Agricultural and Food Chemistry*, *39*, 439–442.

Umano, K., Dennis, K. J., & Shibamoto, T. (1988). Analysis of free malonaldehyde in photoirradiated corn oil and beef fat via a pyrazole derivative. *Lipids*, *23*, 811–814.

Zheng, G. Q., Kenny, P. M., & Lam, K. T. (1992). Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *Journal of Natural Products*, *55*, 999–1003.