Antioxidant Properties of Aroma Compounds Isolated from Soybeans and Mung Beans

Kwang-Geun Lee and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California at Davis, One Shields Avenue, Davis, California 95616

Aroma compounds contained in the extracts of soybean and mung bean that possess antioxidant activity were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major aroma constituents of soybeans were 1-octen-3-ol (13.699 ppm), maltol (1.662 ppm), phenylethyl alcohol (1.474 ppm), hexanol (1.430 ppm), and γ-butyrolactone (1.370 ppm). The major aroma constituents of mung beans were hexanol (3.234 ppm), benzyl alcohol (2.060 ppm), γ-butyrolactone (1.857 ppm), 2-methyl-2-propenal (1.633 ppm), and pentanol (1.363 ppm). The major aroma chemicals of soybeans and mung beans were examined for antioxidative activities in two different assays. Eugenol, maltol, benzyl alcohol, and 1-octen-3-ol showed potent antioxidative activities in two different assays. Eugenol, maltol, benzyl alcohol, and 1-octen-3-ol inhibited the oxidation of hexanal by 100%, 93%, 84%, and 32%, respectively, for a period of 40 days at the 500 μg/mL level. Eugenol, maltol, benzyl alcohol, and 1-octen-3-ol inhibited malonaldehyde (MA) formation from cod liver oil by 91%, 78%, 78%, and 78%, respectively, at the 160 μg/mL level. The antioxidative activity of eugenol was comparable to that of the natural antioxidant α-tocopherol (vitamin E).

Keywords: *Aroma chemicals; soybean extract; mung bean extract; natural antioxidants*

INTRODUCTION

Much work has been done to find safe and potent natural antioxidants from various plant sources. Many natural antioxidants are found in the seeds, beans, and the nuts of plants (Namiki, 1990). Plant-based antioxidants such as tocopherols, vitamin C, cartenoids, and phenolic compounds are known to protect the plants against oxidative stresses (Packer et al., 1999). Among plants, various beans have been investigated to determine whether they possess antioxidative activity (White and Xing, 1997). Soybeans and soy products (Pratt and Birac, 1979), pea bean extract (Tsuda et al., 1993), mung bean hulls (Duh et al., 1997), and phenolic fractions of pea and fava bean (Amarowicz et al., 1996) have been reported to have antioxidative activity in various model systems. These studies were focused on the antioxidant activity of various bean parts extracted by solvents such as methanol, acetone, and water. There have been few studies available on the antioxidant potential of aroma compounds found in beans.

Buttery et al. (1975) characterized volatile aroma compounds of dry red beans. Aroma compounds of soybean oil have been described several times (Kao et al., 1998; Lee et al., 1995; Wu et al., 1990).

In our laboratory, aroma extracts isolated from soybeans and mung beans have been shown to have potent antioxidant activity comparable to that of the known natural antioxidant vitamin E (Lee et al., 2000). In this study, the aroma compounds of soybeans and mung beans were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), and then the identified aroma compounds were examined for their antioxidant activities in two different assays.

* Corresponding author. Phone: 530-752-4523. Fax: 530-752-3394. E-mail: tshibamoto@ucdavis.edu.

MATERIALS AND METHODS

Beans. Fresh soybeans (*Glycine max*) and mung beans (*Vigna radiata)* were purchased from a local market.

Chemicals. Eugenol, maltol, benzyl alcohol, 1-octen-3-ol, γ-butyrolactone, δ-valerolactone, phenylethyl 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 and ethyl acetate were bought from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). The standard stock solution of 2-methylpyrazine was prepared by adding 10 mg of 2-methylpyrazine to 1 mL of dichloromethane and stored at 5 °C. Authentic 1-methylpyrazole (1-MP) was synthesized malonaldehyde (MA) and *N*-methylhydrazine (NMH) according to a previously developed method (Umano et al., 1988).

Isolation of Aroma Chemicals by Steam Distillation under Reduced Pressure (DRP). Soybeans or mung beans (200 g) were placed in a 3 L round-bottom flask with 1 L of 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 of 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 0.2 mL.

Identification of Aroma Chemicals in the Extracts from Soybeans and Mung Beans. Aroma chemicals obtained by DRP were identified by comparison with the Kovats

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

Aldehyde/Carboxylic Acid Assay. The antioxidative activity of aroma chemicals was tested using their inhibitory effect toward oxidation of aldehyde to acid (Macku and Shibamoto, 1991). Various amounts of aroma chemicals were added to a 2 mL dichloromethane 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 stored 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. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology.

Lipid/MA Assay. The antioxidative activity of aroma chemicals was also determined by analyzing MA formed from cod liver oil upon oxidation after derivatizing to 1-MP with NMH (Tamura et al., 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, 0.5 μmol of hydrogen peroxide, 0.75 mmol of potassium chloride, and 0.2% of surfactant SDS was incubated with various amounts of the 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 et al., 1989). The sample tubes were covered with aluminum 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 ethyl acetate. The solution was analyzed for 1-MP by a GC with a NPD.

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.

An 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.

An HP model 6890 GC interfaced to an 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/s. 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.

RESULTS AND DISCUSSION

The total yields of volatile chemicals from soybeans and mung beans (relative to the amount of fresh beans used) were 0.0033% (w/w) and 0.0023% (w/w), respectively. Table 1 shows the compounds identified in extracts obtained from soybeans and mung beans, along with their calculated concentration and Kovats index on a DB-Wax column. Concentration of each chemical was calculated using the following equation:

$$\text{concentration (ppm)} = \frac{\text{weight of extract (without solvent)} \times \text{GC peak area \%} /100\ (\mu\text{g})}{\text{weight of beans (200 g)}}$$

Over 100 GC peaks were observed both in chromatograms of soybean and mung bean extracts. Among those, 54 aroma chemicals were identified in the soybean extract and 37 aroma chemicals were identified in the mung bean extract. Aroma chemicals identified in the soybean extract were 26 alcohols, 10 aldehydes and ketones, 8 heterocyclic compounds, 3 lactones, and 6 miscellaneous compounds. The major aroma constituents of soybeans were 1-octen-3-ol (13.699 ppm), maltol (1.662 ppm), phenylethyl alcohol (1.474 ppm), hexanol (1.430 ppm), and γ-butyrolactone (1.370 ppm). The major aroma constituents of mung beans were hexanol (3.234 ppm), benzyl alcohol (2.060 ppm), γ-butyrolactone (1.857 ppm), 2-methyl-2-propenal (1.633 ppm), and pentanol (1.363 ppm).

The chemicals marked with an asterisk (∗) in Table 1 were tested for antioxidative activity. The aldehyde/carboxylic acid conversion assay (Macku and Shibamoto, 1991) is a relatively simple assay that can be used to measure the antioxidant potential of a chemical in an organic system. Figure 1 shows the percent of hexanal remaining in samples containing different amounts of aroma chemicals and 50 μg/mL of BHT or α-tocopherol throughout a storage period of 40 days. The values are mean ± standard deviation (n = 3). All chemicals exhibited dose-dependent inhibitory activity. In all experiments, the hexanal in a control was completely oxidized to hexanoic acid after 30 days. Among the chemicals identified in the extracts from soybeans and mung beans, eugenol exhibited the most potent activity; it inhibited hexanal oxidation by 99% at a 100 μg/mL over 40 days, which is comparable to that of α-tocopherol or BHT. Both α-tocopherol and BHT inhibit hexanal oxidation by 100% at 50 μg/mL. At a level of 500 μg/mL, maltol, benzyl alcohol, and 1-octen-3-ol inhibited the hexanal oxidation by 93%, 84%, and 32%, respectively, over a period of 40 days.

The lipid/MA assay is specific for the determination of MA in samples. MA formed upon the oxidation of lipids is derivatized to 1-MP, and then the 1-MP is analyzed by a GC equipped with a nitrogen−phosphorus detector (NPD) (Umano et al., 1988; Nishiyama et al., 1994; Ogata et al., 1996). Figure 2 shows the results of the lipid/MA assay in the presence of aroma chemicals found in extracts from soybeans and mung beans. BHT and α-tocopherol were used as standard antioxidants. The values are mean ± standard deviation (n = 3). The amount of MA formed from 10 μL of cod liver oil alone was 1864 ± 27.19 nmol under the conditions used in the experiment. Among the aroma chemicals tested, eugenol exhibited the most potent antioxidant activity, which is consistent with the results obtained by the aldehyde/carboxylic acid assay. The antioxidant activity of eugenol was similar to α-tocopherol and BHT. Eugenol, α-tocopherol, and BHT inhibited MA formation by 91%, 96%, and 97%, respectively, at the level of 160 μg/mL. The antioxidant activity of eugenol has been reported several times tested on various systems (Satoh

Table 1. Aroma Chemicals Identified in the Extracts from Soybeans and Mung Beans

compd	I[b]	concn (ppm[a]) soybeans	mung beans
Alcohols			
3-buten-2-ol	1022	0.033	0.344
3-methylbutanol	1035	1.001	0.716
3-buten-1-ol	1036	0.081	*d*
2-methyl-1-pentanol	1040	*d*	0.240
isobutanol	1041	0.024	*c*
2,3-dimethyl-1-butanol	1088	*d*	0.308
butanol	1115	0.070	0.095
1-penten-3-ol	1129	0.023	0.104
2-methylbutanol	1167	0.027	0.052
pentanol	1217	0.501	1.363
2-ethyl-1-butanol	1259	*d*	0.138
4-methyl-1-pentanol	1261	*d*	0.163
3-heptanol	1290	0.178	*d*
hexanol	1306	1.430	3.234
4-pentyn-1-ol	1333	0.162	*d*
(Z)-3-hexanol	1358	0.025	*c*
3-octanol	1379	0.081	*d*
3-hexene-1-ol	1400	0.117	*d*
1-octene-3-ol*	1463	13.699	*d*
heptanol	1466	0.972	*d*
6-methyl-5-hepten-2-ol	1473	0.188	*d*
1-heptene-4-ol	1495	0.026	*d*
2,3-diethothy-1-propanol	1520	0.116	*d*
2,4-hexadienol	1523	0.059	*d*
octanol	1562	0.145	*d*
2,7-dimethyl-1-octanol	1625	*c*	0.685
nonanol	1685	*d*	0.179
benzyl alcohol*	1878	0.890	2.060
phenylethyl alcohol*	1913	1.474	1.159
phenol	1951	0.048	*d*
2-phenyl-2-propanol	1972	0.062	0.135
maltol*	2017	1.662	0.583
methyleugenol	2061	*d*	0.087
eugenol*	2169	*d*	1.255
5-(2-propynyloxy)-2-pentanol	2273	*d*	0.566
2-hexadecanol	2312	*d*	0.391
Aldehydes			
hexanal	1089	0.117	*d*
2-methyl-2-propenal	1215	*d*	1.633
2-butenal	1225	0.042	*d*
2,3-dimethyl-pentanal	1258	0.065	*c*
2-methyl-2-propenal	1490	0.092	*d*
benzylaldehyde	1510	0.795	*d*
(E)-2-decenal	1597	0.038	*d*
Ketones			
acetone	823	0.053	*c*
1-methoxy-2-propanone	1137	*d*	0.043
1-hydroxy-3-methyl-2-butanone	1178	*d*	0.070
2-methyl-1-propen-1-one	1215	0.070	*d*
3-hydroxy-2-butanone	1288	0.079	0.357
2-hydroxy-2-methyl-4-pentanone	1390	*c*	0.258
3-hydroxy-3-methyl-2-cyclopenten-1-one	1807	0.142	*d*
Lactones			
δ-valerolactone*	1609	0.770	*d*
γ-butyrolactone*	1652	1.370	1.857
γ-hexalactone	1664	0.463	*d*
Heterocyclic Compounds			
pyridine	1190	0.173	*d*
2-methyl-pyrazine	1276	0.360	*d*
pyrrole	1277	*d*	0.209
2,5-dimethyl-pyrazine	1300	0.264	*d*
2,5-dihydrofuran	1333	*c*	0.131
1-methyl-2-pyrrolidone*	1343	0.690	*d*
2,3-dimethyl-pyrazine	1345	0.098	*d*
2,4,5-trimethyl-thiazole	1390	0.722	*d*
trimethyl-pyrazine	1416	*d*	0.078
dihydro-3-methyl-2,5-furandione	1440	0.070	*d*
3-methyl-1H-pyrazole	1590	*d*	1.046
2,6-dimethyl-pyrazine	1638	0.034	*d*
Miscellaneous Compounds			
undecane	1100	0.067	0.361
dodecane	1200	*d*	0.104
3-butanoic acid	1412	0.107	*d*
2-methylthioethanol	1533	*d*	0.199
heptadecane	1700	1.051	0.171
hexanoic acid	1733	0.074	*d*
octadecane	1800	*d*	0.226
nonanoic acid	2167	0.119	*d*
diethyl-phthalate	2312	0.564	*d*
decanedioic acid	2486	*c*	0.445

[a] Solvent peak excluded. [b] On DB-Wax. [c] Value less than 0.001. [d] Not detected. * Tested for antioxidant activity.

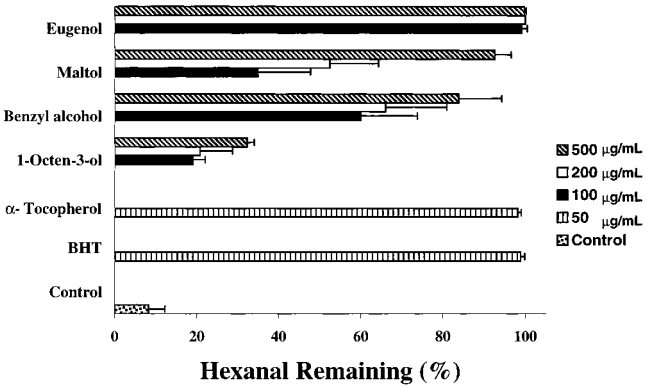

Figure 1. Percent of hexanal remaining in samples containing different amounts of aroma chemicals found in soybeans and mung beans and 50 μg/mL of BHT or α-tocopherol throughout a storage period of 40 days. Note: Four aroma chemicals that did not display any appreciable antioxidant activity are not shown.

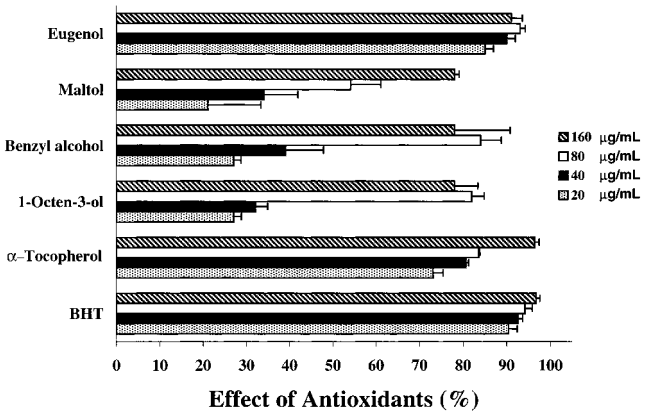

Figure 2. Inhibitory effects (%) of aroma chemicals found in soybeans and mung beans, α-tocopherol, and BHT toward MA formation from cod liver oil at a various levels.

et al., 1998; Nagabubu and Lakshmaiah, 1992). However there has not been any report of the antioxidant activity of eugenol as an aroma constituent in mung beans. The antioxidant activity of maltol in the present study is consistent with a previous report (Singhara et al., 1998). Maltol, benzyl alcohol, and 1-octen-3-ol all inhibited MA formation by 78% at the level of 160 μg/mL. Except for the aroma chemicals shown in Figures 1 and 2, the other aroma chemicals tested did not show any appreciable antioxidant activity.

CONCLUSION

Major aroma compounds found in soybean and mung bean extracts exhibited varying amounts of antioxidative activity. In particular, eugenol found in mung beans exhibited potent antioxidant activity comparable to those of known antioxidants, BHT and α-tocopherol. In addition to eugenol, maltol and benzyl alcohol found in both soybeans and mung beans contributed to the antioxidant potential of aroma extracts of soybean and mung bean. 1-Octen-3-ol, the major aroma constituent in soybeans, showed reasonable antioxidant potential. The antioxidant activities of these aroma chemicals, as well as BHT and α-tocopherol, were consistent in both the aldehyde/carboxylic acid assay and the lipid/MA assay. Our preliminary experiments indicated that antioxidative activities of aroma constituents were not as potent as those of known antioxidants, BHT and

α-tocopherol. However, these aroma chemicals are present in natural plants in tremendous numbers. Therefore, the total activity may be comparable to those of known antioxidants. Furthermore, ingestion of these aroma compounds may help to prevent in vivo oxidative damage such as lipid peroxidation which is associated with cancer, premature aging, atherosclerosis, and diabetes.

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