Antioxidative Activity of Volatile Chemicals Extracted from Beer

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Volatile chemicals obtained from a commercial beer by liquid−liquid continuous extraction were evaluated for antioxidant activity. The inhibitory ability of this extract toward the conversion of hexanal to hexanoic acid was monitored over a 35-day period. The volatile extract demonstrated >99% effectiveness at inhibiting hexanal oxidation at 50 μg/mL, comparable to that of the natural antioxidant α-tocopherol (vitamin E). Volatile compounds contained in the extract were isolated and identified by gas chromatography−mass spectrometry (GC-MS). From the volatile constituents identified in beer extract, phenylethyl alcohol, maltol, and 2-furanmethanol were examined for antioxidative activities. At a concentration of 500 μg/mL, maltol and 2-furanmethanol demonstrated approximately 95 and 100% inhibition of hexanal oxidation over 35 days, respectively. Phenylethyl alcohol did not show any appreciable level of inhibition of hexanal oxidation. Heterocyclic compounds, some of which are known to possess antioxidative activities, were also identified in the volatile extract.

Keywords: *Volatile chemicals; beer extract; natural antioxidants*

INTRODUCTION

Plants have been used for centuries in herbalism, homeopathy, and aromatherapy because of their medicinal qualities. These benefits, in addition to a plant's capability to protect itself against oxidation (*1*), have led to observations about the antioxidant properties of plants (*2*, *3*). Antioxidant activities have also been observed in compounds derived from the volatile constituents (*4*, *5*) and essential oil extracts (*6*, *7*) of plants.

Until recently, volatile chemicals, which are found in many plants as well as in foods and beverages, have been investigated from the viewpoint of flavor and fragrance chemistry (*8*). However, some medicinal activities of volatile chemicals, such as antioxidative activity, have been discovered through the use of essential oils in aromatherapy. Recently, we reported that volatile chemicals extracted from various plants, such as beans (*4*), and beverages, such as brewed coffee (*9*), possess antioxidative activity.

Ingestion of these volatile chemicals helps to prevent in vivo oxidative damage, such as lipid peroxidation associated with diseases including cancer (*10*), atherosclerosis (*11*), aging (*12*), leukemia (*13*), and rheumatoid arthritis (*14*). Therefore, chemical and biological investigation of volatile chemicals is one avenue to assess the health benefits of foods and beverages.

In the present study, analysis and antioxidative tests on the volatile extract isolated from a commercial beer were performed. Beer was chosen because it is one of the most popular beverages in the world. Its popularity as a beverage is second only to that of soft drinks. According to the Beverage Market Index (*15*), U.S. per capita consumption for total beverages for 1999 was 116.3 gal. Of this, per capita consumption for beer was 22.4 gal. In comparison, per capita consumption for other beverages were as follows: soft drinks, 55.9 gal; bottled water, 15.5 gal; fruit beverages, 15.2 gal; sports drinks, 2.3 gal; wine, 1.9 gal; ready-to-drink tea, 1.9 gal; and spirits, 1.2 gal.

MATERIALS AND METHODS

Materials. Commercial glass-bottled stout beer was purchased from a local supermarket. Hexanal, hexanoic acid, undecane, 1-dodecanol, 2-furanmethanol, phenylethyl alcohol, 3-hydroxy-2-methyl-4-pyrone (maltol), and α-tocopherol (vitamin E) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Butylated hydroxytoluene (BHT) was bought from Sigma Chemical Co. (St. Louis, MO).

Extraction of Volatile Chemicals from Beer. Beer (300 mL) was poured into a beaker and placed in the refrigerator (3 °C) for 1 h for degassing. After degassing, 209 mL of beer was mixed with a solution of 10 μL (equivalent to 8.2 mg) of 1-dodecanol internal standard in 1 mL of ethanol. The beer was next extracted by liquid−liquid continuous extraction with 80 mL of dichloromethane for 6 h at 50 °C. The extract was dried over anhydrous sodium sulfate for 12 h, and then the solvent was removed in vacuo with a rotary evaporator RE47 (Yamato Scientific Co. Ltd.). The distillation was stopped when the volume of extract was reduced to ∼2 mL. The extract was transferred to a concentration tube, and then the distillation flask was washed with a minimum amount of dichloromethane. This rinsate was added to the concentration tube. The solvent was further removed from the concentration tube under a purified nitrogen stream until the volume was exactly 0.3 mL. The sides of the concentration tube were washed with dichloromethane until a final volume of 0.5 mL was reached. The extract was transferred to a vial and weighed by an analytical balance. Approximately 56 mg of extract excluding solvent was obtained.

Hexanal/Hexanoic Acid Assay. The antioxidative activity of the beer volatile extract was tested for its ability to inhibit the oxidation of hexanal to hexanoic acid (*16*). Test samples

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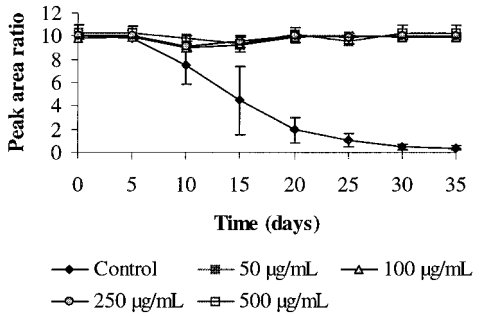

Figure 1. Relative amounts of hexanal remaining in samples containing different concentrations of volatile extracts from beer.

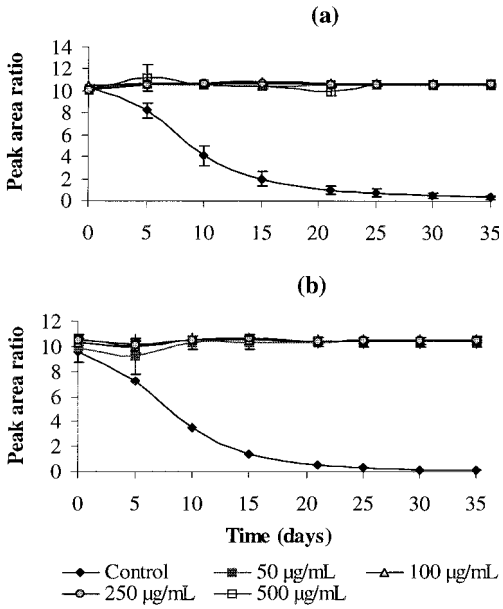

Figure 2. Relative amounts of hexanal remaining in samples containing different concentrations of BHT (a) and α-tocopherol (b).

of beer volatile extracts (50, 100, 250, and 500 μg/mL) were added to a 2 mL dichloromethane solution of hexanal (3 mg/mL) and the GC internal standard undecane (0.2 mg/ mL). To initiate the oxidation process, the sealed sample solution vials were placed in a hot water bath at 60 °C for 10 min. Headspace purging of each vial for the first 10 days, every 24 h, was accomplished with pure air (1.5 L/min, 3 s). The decrease in hexanal levels was monitored at 5-day intervals, beginning with day 0 until day 35. The hexanal/hexanoic acid assay was also performed on the volatile constituents phenylethyl alcohol, 2-furanmethanol, and 3-hydroxy-2-methyl-4-pyrone (maltol). For comparison purposes, α-tocopherol (vitamin E) and BHT standards were also examined for their antioxidative activity using the same methodology. Testing for antioxidative activity was carried out in triplicate for each sample.

Instrumental Analysis. A Hewlett-Packard (HP) model 6890 GC equipped with a 30 m × 0.25 mm i.d. (df = 1 μm) DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and an FID was used to quantitatively analyze the levels of hexanal. The linear velocity of the helium carrier gas was 28 cm/s at a split ratio of 15:1. The oven temperature was programmed from 40 to 120 °C at 8 °C/min. Temperatures for the injector and detector were 300 and 280 °C, respectively.

Volatile chemicals in the beer extract were identified by comparison with the Kovats gas chromatographic retention index *I* (*17*) and by mass spectral analysis. An HP model 5890 series II gas chromatograph equipped with a 60 m × 0.25 mm i.d. (df = 0.25 μm) DB-Wax bonded-phase fused silica capillary column (J&W Scientific) and a 5791A mass selective detector at MS ionization voltage of 70 eV was used for GC-MS analysis. The oven temperature was programmed from 40 to 200 °C at 2 °C/min, with an initial holding time of 2 min. The injector and detector temperatures were 250 °C. The helium carrier gas flow rate was 37 cm/s at a split ratio of 23:1.

RESULTS AND DISCUSSION

The aldehyde/carboxylic acid test is a fast and simple method to assess the antioxidative properties of chemicals or a group of chemicals. This method is based on the autoxidation of aldehydes to carboxylic acids with active oxygen species such as a hydroxy radical (*18*). Fatty aldehydes are converted readily to a corresponding fatty acid in an oxygen-rich dichloromethane solution through a radical-type reaction (*19*). This method has been validated using typical antioxidants such as BHT, α-tocopherol, and caffeine (*9*).

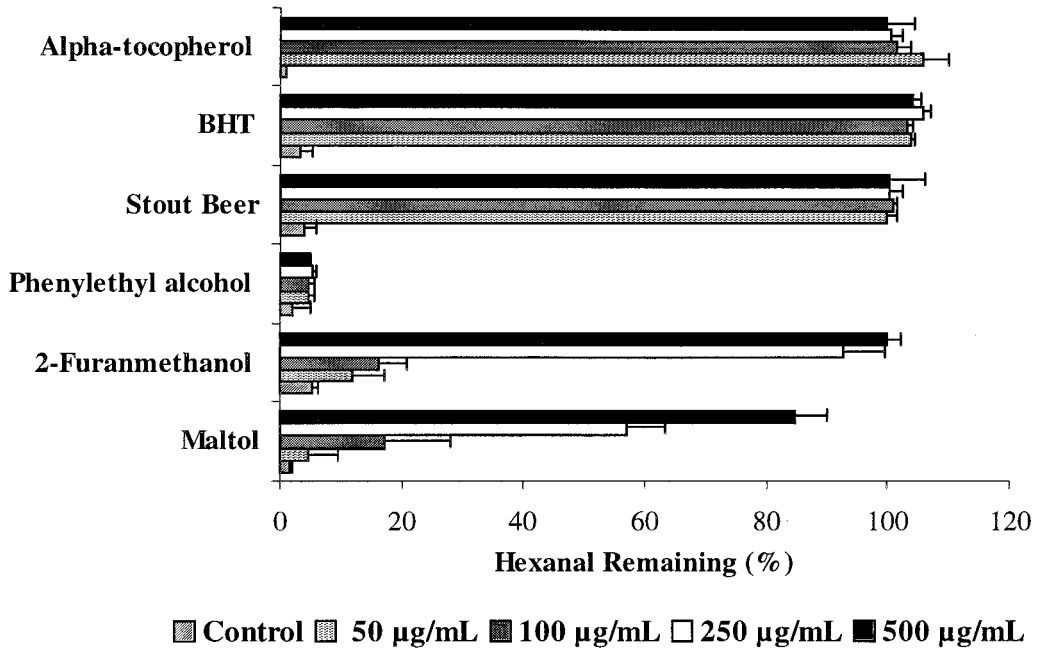

Figure 3. Percent of hexanal remaining in samples containing BHT or α-tocopherol and different amounts of beer volatile extract or volatile chemicals throughout a storage period of 35 days.

Figure 1 shows the antioxidative activities of different concentrations of beer extracts (50, 100, 250, and 500 μg/mL). GC peak area ratios were calculated by dividing the GC peak area of hexanal by the GC peak area of standard undecane. In all extracts, the conversion of hexanal to hexanoic acid was monitored for 35 days. To validate the hexanal/hexanoic acid testing method, the antioxidant activities of known antioxidants BHT and α-tocopherol were also monitored for hexanal oxidation (Figure 2). After 35 days, control samples consisting of hexanal, undecane, and dichloromethane demonstrated >90% hexanal oxidation to hexanoic acid. Figure 3 shows the percent of hexanal remaining for the various concentrations of beer volatile extracts. At a concentration of 50 μg/mL, beer volatile extract was >99% effective at inhibiting hexanal oxidation. This was comparable to the antioxidative activity exhibited by 50 μg/mL of α-tocopherol (Figure 3), which inhibited hexanal oxidation by 100%. For both BHT and α-tocopherol, antioxidative activity was exhibited at 50 μg/mL over 35 days, consistent with values obtained in previous studies (*5*, *20*).

The chemicals in Table 1 designated by footnote *c*—2-furanmethanol, phenylethyl alcohol, and maltol—were evaluated for antioxidative activity. The antioxidative activities for different concentrations of each volatile constituent tested over 35 days are provided in Figure 4. Phenylethyl alcohol, a product of yeast metabolism and a marker for fermentation parameters (*21*), contributes an intense roselike flavor to beers (*22*).

All concentrations of phenylethyl alcohol demonstrated >96% hexanal conversion after 35 days (Figure 4a). In comparison, the control sample showed almost 98% hexanal conversion, thus showing that phenylethyl alcohol does not demonstrate appreciable antioxidant activity. Investigations on the antioxidative activities of 2-furanmethanol (Figure 4b) and maltol (Figure 4c) showed dose-dependent associations between the concentrations at which they were present in the sample and their antioxidative activities. For the 50 and 100 μg/mL concentrations of 2-furanmethanol (Figure 3), the total amount of hexanal that had been converted to hexanoic acid in each sample was approximately 88 and 84%, respectively. At 250 and 500 μg/mL sample concentrations, approximately 93 and 100% of hexanal remained, respectively, suggesting that at these concentrations 2-furanmethanol possesses effective antioxidant activity against hexanal oxidation. In the case of maltol—a product of the roasting process in malt production (*23*)—a more obvious dose-dependent association was observed in inhibiting hexanal oxidation. For maltol concentrations of 50, 100, 250, and 500 μg/mL (Figure 3), the percentages of hexanal conversion were 95, 83, 43, and 15%, respectively. This shows that maltol possesses antioxidative activity. These results were consistent with those obtained by Lee et al. (*5*) and Singhara et al. (*9*).

Table 1 shows volatile compounds identified in beer along with their Kovats index (*I*) and GC peak area percent. Over 100 peaks were observed in a chromatogram of an extract from beer. Of these, 54 volatile compounds were identified and are shown in Table 1. The volatile chemicals identified were 9 alcohols, 16 esters, 11 acids, 3 aldehydes, 5 ketones, 1 sulfur compound, 1 lactone, and 8 heterocyclic compounds.

Heterocyclic compounds, such as 2-furanmethanol and maltol, may be generated from thermal treatment processes or from the raw materials used in beer production. Some of these heterocyclic compounds have also been found to possess antioxidative activities (*20*, *24*, *25*).

Among studies concerning beer antioxidant activity, many reports have examined the antioxidant activity of beer ingredients (*26*−*28*) and of Maillard reaction products formed from malt kilning (*29*) and wort boiling (*30*). Regarding the final beer product, Ghiselli et al.

Table 1. Volatile Chemicals Identified in the Extract from Beer

compound	I^a	GC peak area %[b]
alcohols		
ethanol	934	5.5
propanol	1032	0.32
isobutyl alcohol	1086	1.6
isoamyl alcohol	1206	9.8
2,3-butanediol	1523	0.03
2-furanmethanol[c]	1650	0.76
decanol	1755	0.01
phenylethyl alcohol[c]	1901	5.7
tetradecanol	2164	0.01
esters		
ethyl propionate	945	0.01
ethyl isobutanoate	954	0.01
4-methyl-2-pentanoate	1000	0.01
isobutyl acetate	1005	0.01
2-methyl-2-butanoate	1008	0.03
ethyl butanoate	1025	0.01
isoamyl formate	1070	0.04
isoamyl acetate	1112	0.14
isoamyl isobutanoate	1183	-
ethyl hexanoate	1220	0.02
ethyl lactate	1331	0.09
ethyl octanoate	1421	0.02
phenylethyl formate	1768	0.01
ethyl 4-hydroxybutanoate	1796	0.05
phenylethyl acetate	1798	0.04
succinic acid monoethyl ester	2367	0.09
acids		
acetic acid	1435	0.25
propanoic acid	1521	-
isobutanoic acid	1535	0.58
butanoic acid	1613	0.38
3-methylbutanoic acid	1657	0.24
hexanoic acid	1832	0.19
octanoic acid	2047	0.49
dodecanoic acid	2260	0.13
levulinic acid	2312	0.04
benzoic acid	2410	0.06
phenylacetic acid	2546	0.02
aldehydes		
furfural	1445	0.04
5-methylfurfural	1549	0.01
5-(hydroxymethyl)furfural	2490	0.27
ketones		
acetone	825	0.18
cyclopentanone	1170	0.03
acetoin	1270	0.11
hydroxyacetone	1284	0.04
3-hydroxypentan-2-one	1327	0.01
heterocyclic compounds		
2-methylpyrazine	1252	0.04
5,5-dimethyl-2(5*H*)-furanone	1590	0.18
maltol[c]	1954	0.22
pyrrole-2-carboxaldehyde	2009	0.02
furaneol	2026	0.01
2-furoic acid	2405	0.03
2-ethoxycarbonyl-5-oxopyrroline	2606	0.05
miscellaneous compounds		
dimethyl sulfoxide	1553	0.14
γ-butyrolactone	1608	0.15
γ-butyrolactam	2037	0.08

[a] Kovats index on DB-Wax. [b] Solvent peak is excluded. [c] Tested for antioxidative activity.

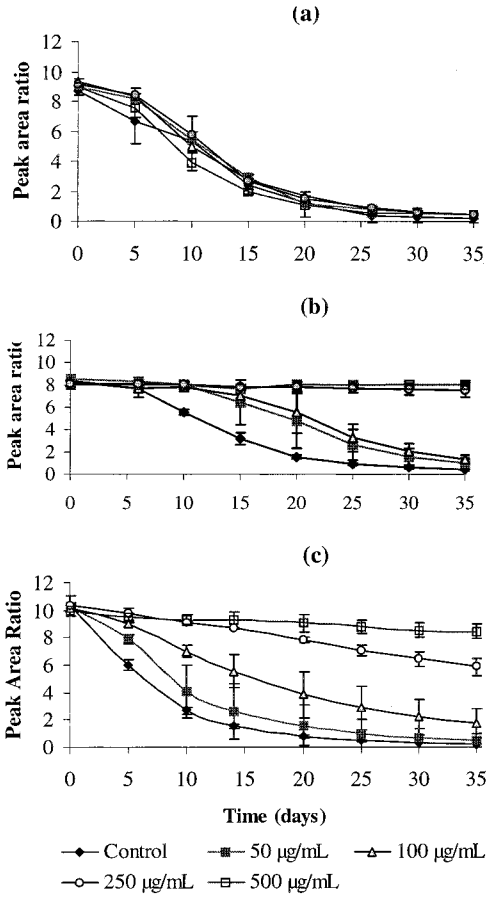

Figure 4. Relative amounts of hexanal remaining in samples containing different concentrations of volatile chemicals identified in stout beer: (a) phenylethyl alcohol; (b) 2-furanmethanol; (c) maltol.

(*31*) found that beer intake induced a significant increase in plasma antioxidant capacity as a result of the transfer of phenolic acids from beer to body fluids. However, research to date establishing the antioxidant activity of the volatile extracts in beer has been lacking.

To investigate the possible presence of antioxidants in beer, it is important to know the antioxidative activities of the extract first. In this study, the levels used to test extract and volatile constituents are considerably higher than those present in actual beer, which range from micrograms per kilogram to milligrams per kilogram levels. However, once activity of the extract is demonstrated, the next step is to investigate the activity of the chemicals at the more relevant low levels shown above. Therefore, investigations on the antioxidant activities of chemicals at the levels of micrograms per kilogram to milligrams per kilogram are in order.

In the present study, the volatile extract from stout beer was found to exhibit antioxidant activity. It is difficult to attribute this antioxidant activity to the individual antioxidant abilities of single volatile constituents. Instead, the likelihood exists that the antioxidative activity of the beer volatile extracts is due to their combined antioxidant activities. Most studies of beer's association with human health have focused on such negative aspects as the toxicity of ethanol. Beer consumption dates back to before 4000 B.C. (*32*). The antioxidant activity of beer's volatile chemicals may be one of its positive effects on human health.

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