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Antioxidative Activities of Volatile Extracts from Green Tea, Oolong Tea, and Black Tea

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Antioxidative activities of volatile extracts from six teas (one green tea, one oolong tea, one roasted green tea, and three black teas) were investigated using an aldehyde/carboxylic acid assay and a conjugated diene assay. The samples were tested at levels of 20, 50, 100, and 200 μg/mL of dichloromethane. The results obtained from the two assays were consistent. All extracts except roasted green tea exhibited dose-dependent inhibitory activity in the aldehyde/carboxylic acid assay. A volatile extract from green tea exhibited the most potent activity in both assays among the six extracts. It inhibited hexanal oxidation by almost 100% over 40 days at the level of 200 μg/mL. The extract from oolong tea inhibited hexanal oxidation by 50% in 15 days. In the case of the extract from roasted green tea, the lowest antioxidative activity was obtained at the level of 200 μg/mL, suggesting that the extract from roasted green tea contained some pro-oxidants. The extracts from the three black teas showed slight anti- or proactivities in both assays. The major volatile constituents of green tea and roasted green tea extracts, which exhibited significant antioxidative activities, were analyzed using gas chromatography−mass spectrometry. The major volatile chemicals with possible antioxidative activity identified were alkyl compounds with double bond(s), such as 3,7-dimethyl-1,6-octadien-3-ol (8.04 mg/kg), in the extract from green tea and heterocyclic compounds, such as furfural (7.67 mg/kg), in the extract from roasted green tea. Benzyl alcohol, which was proved to be an antioxidant, was identified both in a green tea extract (4.67 mg/kg) and in a roasted tea extract (1.35 mg/kg).

KEYWORDS: Black tea; green tea; oolong tea; roasted green tea; volatile antioxidants

INTRODUCTION

Tea is one of the most popular beverages in the world. People in China were drinking tea in 59 B.C. (*1*). Black tea is most commonly drunk in the West, whereas green tea is most commonly consumed in Asia. People enjoy tea for its taste and flavor. Therefore, most studies conducted on tea were investigations of taste and flavor. In the 1970s and 1980s, many researchers analyzed the flavor constituents of various tea extracts (*2*−*4*).

Even though some health benefits of tea have been known for many years, scientific studies of biological activities, including antimutagenic (*5*), anticarcinogenic (*6*, *7*), and antioxidative (*8*, *9*), were started only recently. Among these studies, antioxidative chemicals have received much attention by many researchers because ingestion of these chemicals helps to prevent *in vivo* oxidative damage, such as lipid peroxidation, associated with many diseases, including cancer, atherosclerosis, diabetes, aging, arthritis, brain dysfunction, and immune deficiency (*10*).

Most studies of tea associated with human health have focused on the less volatile constituents, such as catechins (*9*, *11*−*13*). Only a few studies exist on the biological activity of volatile chemicals from tea, in contrast to numerous studies on that of less volatile chemicals. In the present study, the antioxidative activity of tea extracts was investigated to assess the health benefit of tea drinking.

MATERIALS AND METHODS

Tea Samples. Green teas and roasted green teas were harvested at Fukuroi-shi, Japan, in the summer of 1999. Oolong teas were obtained from the Li Mountains of Taiwan in the autumn of 1998. Darjeeling black tea and Assam black tea were collected from the State of West Bengal and from the Assam region (far northeast) in India, respectively, in the autumn of 1998. Ceylon black tea was collected from the Dimbula region of Sri Lanka in the autumn of 1998. New leaves were processed at a corresponding local plant within 24 h of being picked from tea trees. For over 100 years, the above countries have used the same tea leaf processing method. Therefore, their products are very consistent. All tea samples were gifts from Nikken Foods Co., Ltd., Fukuroi-shi, Japan, in dry form.

Sample Preparation of Organic Solvent Extracts from Teas. Dry tea leaves (200 g) were placed in a 3-L round-bottom flask with 1.5 L of distilled water. The sample was distilled under reduced pressure

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(95 mmHg) at 55 °C for 3 h. The distillate was extracted with 100 mL of dichloromethane using a liquid−liquid continuous extractor for 3 h. After the volatile extract was dried over anhydrous sodium sulfate, it was condensed to 2 mL in volume using a rotary evaporator. The condensed sample was further condensed with a purified nitrogen stream to 0.6 mL in volume. The experiment was done in triplicate.

Measurement of Antioxidative Activities. Antioxidative activities of the samples were tested using their inhibitory effect toward oxidation of aldehyde to acid (*14*). Various amounts of the extracts (20, 50, 100, and 200 μg/mL) 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. Oxidation of the sample solution was initiated by heating at 60 °C for 10 min in a sealed vial, and the sample was 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 intervals for 40 days. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology. Quantitative analysis of hexanal was conducted according to an internal standard method (*15*). A Hewlett-Packard (HP) model 6890 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 detector temperatures were 300 and 280 °C, respectively. The oven temperature was programmed from 40 to 180 °C at 8 °C/min and held for 10 min.

Antioxidative activities of the samples were also tested using a conjugated diene assay (*16*). Briefly, extracts were tested at various concentrations (20, 50, 100, and 200 μg/mL) by addition to methyl linoleate (1 g) in 15-mL screw-cap amber glass vials (National Scientific, Lawrenceville, GA). Dichloromethane solvent was removed by a purified nitrogen stream. The samples were incubated at 40 °C for 3 days. Sample aliquots (10 mg) were taken at regular intervals and dissolved in 5 mL of isooctane for spectrophotometric measurements (Hewlett-Packard, 8452A diode array UV spectrophotometer) of conjugated diene absorption at 234 nm. Isooctane was used as the blank. All analyses were carried out in triplicate. The antioxidative activity was expressed as the amount of hydroperoxides (micromoles per gram of methyl linoleate) formed in the samples. Natural antioxidant α-tocopherol was used in each experiment as a control antioxidant. Benzyl alcohol (200 μg/mL) was also examined after incubation at 40 °C for 3 days.

Analysis of the Volatile Constituents of Tea Samples. The GC Kovats retention index I (*17*) and the MS fragmentation pattern of each component were compared to those of the authentic compound to identify the volatiles in the samples. An HP MS ChemStation Data System was also used to confirm MS identification of the GC components. 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. Column and GC conditions were as stated above.

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 readily converted 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 BHT, α-tocopherol, and caffeine (*20*) and used successfully to examine antioxidative activities of volatile extracts from various natural plants, including various beans (*21*, *22*), clove bud (*23*), eucalyptus (*24*, *25*), and various herbs and spices (*26*).

The total yields of volatile chemicals were 0.0039% (7.78 μg/200 g) from green tea, 0.007% (15.46 μg/200 g) from oolong tea, 0.0122% (24.44 μg/200 g) from roasted green tea, 0.02% (39.9 μg/200 g) from Darjeeling tea, 0.0065% (13.05 μg/200 g) from Ceylon tea, and 0.0067% (13.4 μg/200 g) from Assam tea.

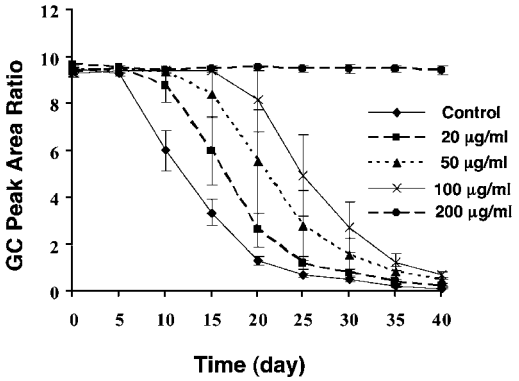

Figure 1. Inhibitory effect of green tea toward hexanal oxidation.

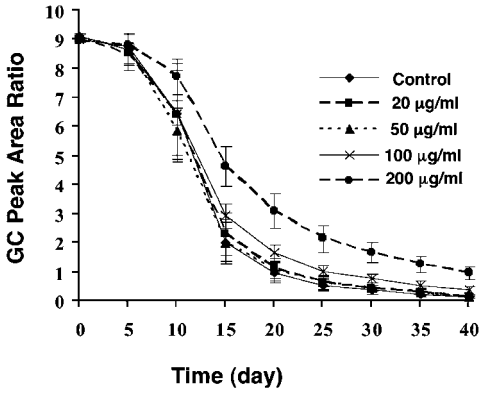

Figure 2. Inhibitory effect of oolong tea toward hexanal oxidation.

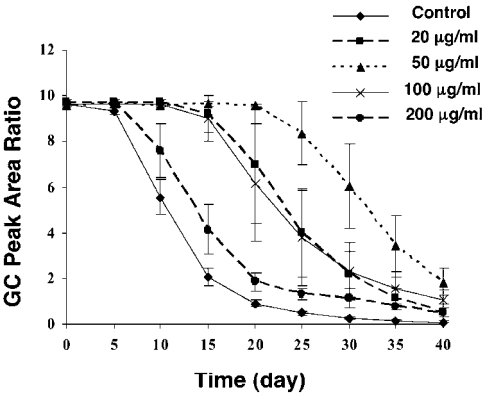

Figure 3. Inhibitory effect of roasted green tea toward hexanal oxidation.

Figures 1−**6** show the results of antioxidative tests on volatile tea extracts. The values are relative amounts of hexanal remaining in samples (GC account of hexanal/GC account of internal standard, undecane). The values are presented as mean ± standard deviation (SD) ($n = 3$). Hexanal in the control samples, which did not contain testing extracts, was oxidized to hexanoic acid at almost 100% over 40 days. All extracts except roasted green tea exhibited dose-dependent inhibitory activity. As presented in **Figure 1**, a green tea extract inhibited hexanal oxidation by almost 100% over 40 days at the level of 200 μg/mL. Also, the same extract inhibited oxidation for 5 days at all levels tested. Oolong tea extract inhibited hexanal oxidation for 5 days at all levels tested (**Figure 2**). This extract inhibited hexanal oxidation by 50% in 15 days.

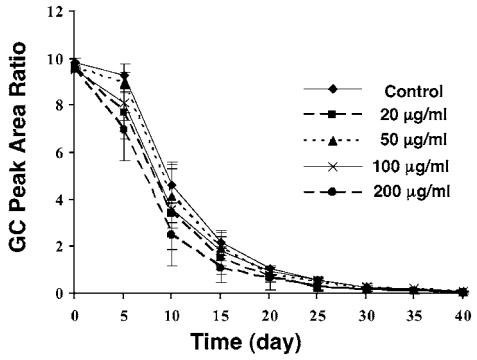

Figure 4. Inhibitory effect of Darjeeling tea toward hexanal oxidation.

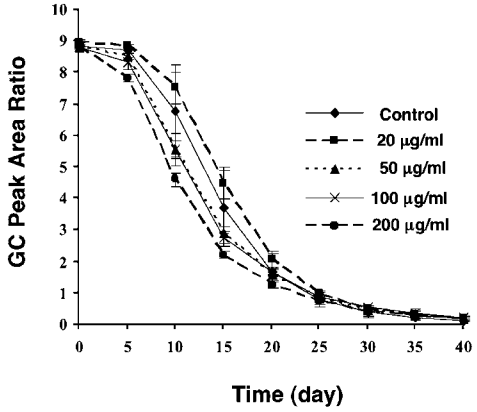

Figure 5. Inhibitory effect of Assam tea toward hexanal oxidation.

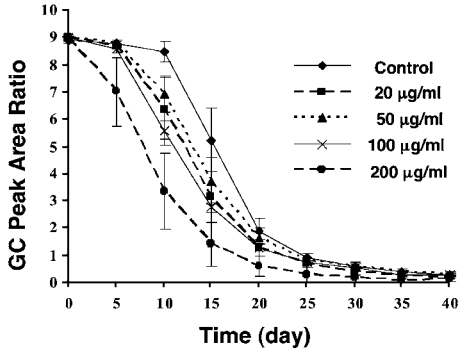

Figure 6. Inhibitory effect of Ceylon tea toward hexanal oxidation.

In the case of roasted green tea extract (**Figure 3**), hexanal oxidation was inhibited over 5 days at all of the levels tested. The extract inhibited hexanal oxidation for 10 days at the levels of 20, 50, and 100 μg/mL. It is interesting that the extract with the highest concentration (200 μg/mL) exhibited the lowest activity. Lower antioxidative activity at the level of 200 μg/mL than at the three other levels suggests that the extract from roasted green tea contained some pro-oxidants. Roasted green tea extracts were the only ones that did not exhibit dose-dependent inhibitory effects.

The extracts from the three black teas showed only slight activities of either anti- or pro-oxidation (**Figures 4**−**6**). In the case of Darjeeling tea (**Figure 4**), volatile extracts showed slight pro-oxidative activity at all levels tested. The volatile extract from Assam tea (**Figure 5**) exhibited slight antioxidative activity at the level of 200 μg/mL. The volatile extract from Ceylon tea exhibited the highest pro-oxidative activities among the tea volatiles tested. At the level of 200 μg/mL, hexanal was oxidized to hexanoic acid by 70% in 10 days, whereas it was oxidized to hexanoic acid by only 5% in a control in the same period of time.

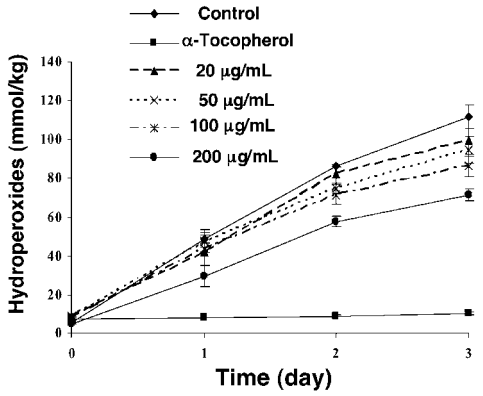

Figure 7. Inhibitory effect of green tea toward methyl linoleate oxidation.

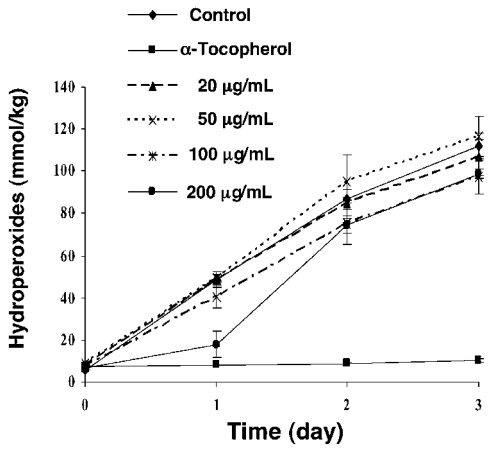

Figure 8. Inhibitory effect of oolong tea toward methyl linoleate oxidation.

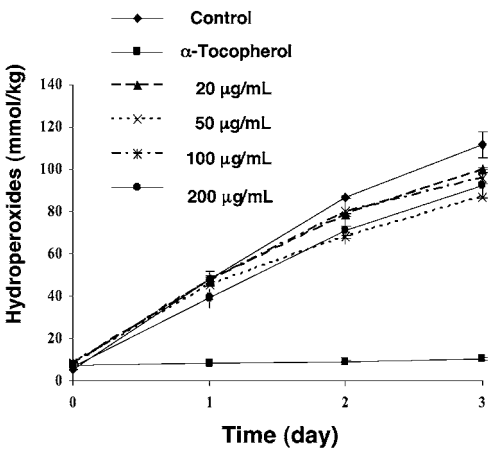

Figure 9. Inhibitory effect of roasted green tea toward methyl linoleate oxidation.

Figures 7−**12** show the inhibitory effects of volatile extracts from tea samples toward oxidation in the conjugated diene assay. The values are mean ± SD (n = 3). The control natural antioxidant, α-tocopherol, inhibited oxidation of methyl linoleate by 100% in 3 days at the level of 10 μg/mL. All samples except the one from green tea did not exhibit clear dose-dependent activity. The volatile extract from green tea exhibited the highest antioxidative activity among the samples tested. The results were consistent with those obtained from the aldehyde/carboxylic acid assay (**Figure 7**). This extract inhibited methyl linoeate oxidation by 36% at the level of 200 μg/mL. In the case of oolong tea

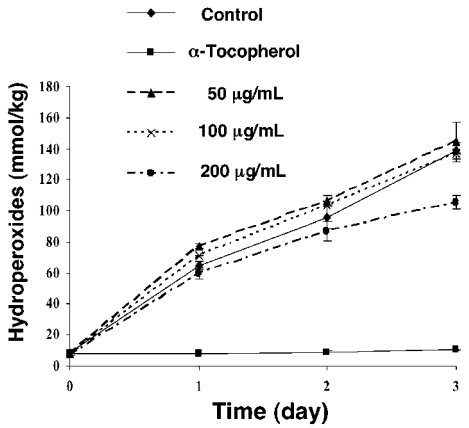

Figure 10. Inhibitory effect of Darjeeling tea toward methyl linoleate oxidation.

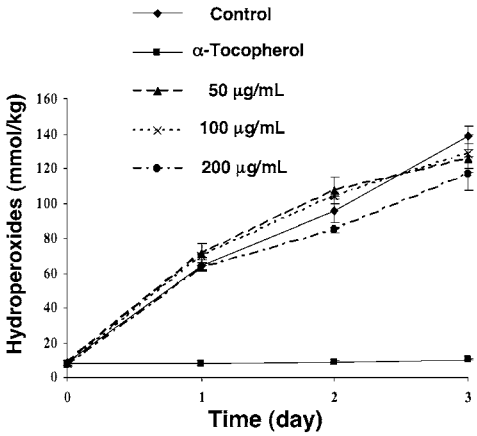

Figure 11. Inhibitory effect of Assam tea toward methyl linoleate oxidation.

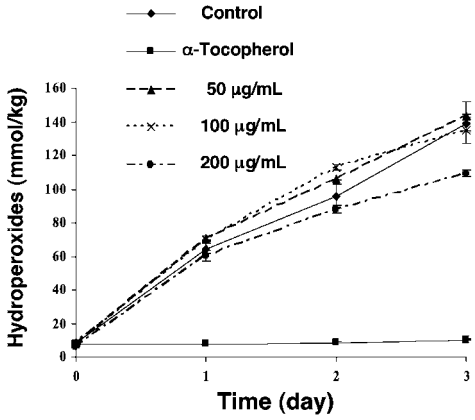

Figure 12. Inhibitory effect of Ceylon tea toward methyl linoleate oxidation.

(**Figure 8**), the volatile extract inhibited oxidation of methyl linoleate by nearly 80% at the level of 200 μg/mL in 1 day. However, this extract exhibited exactly the same activity at the levels of 200 and 100 μg/mL after 2 days. This extract exhibited slight pro-oxidative activity at the level of 20 μg/mL.

Referring to **Figure 9**, the volatile extract from roasted green tea inhibited methyl linoleate oxidation most at the level of 50 μg/mL, which was consistent with the results obtained by the aldehyde/carboxylic acid assay. At this level, the volatile extract inhibited the oxidation by 23%. The other levels (20, 100, and 200 μg/mL) tested showed dose-dependent activities.

Figures 10−12 show the results from black teas. The volatile extract from Darjeeling tea (**Figure 10**) exhibited antioxidative activity only at the level of 200 μg/mL, which inhibited methyl linoleate oxidation by 27% after 3 days. This extract showed slight pro-oxidative activities at the levels of 50 and 100 μg/mL. The volatile extract from Assam tea also exhibited anti- and pro-oxidative activities at the different levels. After 2 days, this extract exhibited antioxidative activity only at the level of 200 μg/μL and pro-oxidative activities at the levels of 50 and 100 μg/mL. However, this extract showed slight antioxidative activities at all levels tested after 3 days. The extract from Ceylon tea also exhibited the most antioxidative activity of the level of 200 μg/mL, which inhibited methyl linoleate oxidation by 27% after 3 days. This extract showed slight pro-oxidative activities at the levels of 50 and 100 μg/mL. Generally, volatile extracts of black teas exhibited appreciable antioxidative activity only at the level of 200 μg/mL. These extracts showed slight pro-oxidative activities at levels lower than 200 μg/mL. The results suggest that oxidants in black tea extracts became predominant at levels >200 μg/mL.

Significant antioxidative activities were obtained only from the volatile extracts of green tea and roasted green tea among the extracts tested. Therefore, the major constituents of these two extracts were identified by GC-MS, and the results are shown in **Table 1**. The concentration of each chemical was calculated using a method previously reported (*23*, *27*). The volatile extracts obtained in the present study did not contain known antioxidants such as α-tocopherol, polyphenols, and flavonoids. Therefore, antioxidative activities of the volatile extracts from green tea and roasted green tea were due to the presence of some antioxidative volatile chemical(s). Antioxidative activities of volatile chemicals have been reported only recently. For example, volatile extracts from various beans (soybeans, mung beans, kidney beans, azuki beans, and coffee beans) exhibited equal antioxidative activities at levels of 200 to 50 μg/mL concentration of α-tocopherol (*22*). Antioxidative activities of coffee volatiles have been reported in several scientific articles (*20*, *28*, *29*). As mentioned above, volatile green tea extract exhibited potent antioxidative activity at the level of 200 μg/mL. Among the major volatiles identified in a volatile green tea extract, benzyl alcohol (4.67 mg/kg) has previously been proved to be an antioxidant. Benzyl alcohol inhibited hexanal oxidation over 30 days at the level of 500 μg/mL (*24*). At a concentration of 400 μg/mL, benzyl alcohol inhibited malonaldehyde formation from blood plasma by 31% (*20*). Benzyl alcohol also inhibited methyl linoleate oxidation by 47 $\pm$ 2.7% (n = 3) at a level of 200 μg/mL in the present study.

Benzaldehyde (2.13 mg/kg) also showed slight antioxidative activity (*24*). The other components found in volatile green tea extracts were not tested for antioxidative activity. However, some alkyl compounds with double bond(s) may have a certain hydroxy radical scavenging activity. For example, 1-octen-3-ol inhibited hexanal oxidation for 40 days by 38% at the level of 500 μg/mL (*21*). A total of 10 compounds with double bond(s) were identified as major components of volatile green tea extracts.

Major constituents of volatile extracts from roasted green teas were heterocyclic compounds, in particular, pyrazines. These heterocyclic compounds have been known to form in heat-treated foods and beverages (*30*). The presence of pyridines and pyrazines has also been identified in some pan-fried green teas (*31*). Alkylpyrazines possess a toasted flavor with a low odor threshold (*32*). Therefore, the pleasant toasted flavor of roasted green teas is due to the presence of alkylpyrazines. However, alkylpyrazines do not possess high antioxidative activities. On the other hand, pyrroles have the greatest antioxidative activities

Table 1. Major Volatile Compounds Identified in Volatile Extracts from Green Tea and Roasted Green Tea

compound	concentration (mg/kg)	
	green tea	roasted green tea
aldehydes and ketones		
hexanal	0.37	0.35
2,3-pentanedione	–[a]	0.47
(E)-2-pentenal	1.06	0.09
heptanal	0.26	–
(E)-2-hexenal	1.47	0.12
(Z)-4-heptenal	0.22	0.25
6-methyl-5-hepten-2-one	0.19	–
3-hydroxy-2-butanone	–	0.62
benzaldehyde	2.13	–
acetophenone	–	1.39
alcohols		
1-penten-3-ol	1.11	1.12
3-methylbutan-1-ol	0.41	–
1-pentanol	0.24	0.10
2-penten-1-ol	1.35	–
1-hexanol	0.40	–
3-hexen-1-ol	6.01	–
3,7-dimethyl-1,6-octadien-3-ol	8.04	–
1-octanol	0.98	–
3,7-dimethyl-2,6-octadien-1-ol	6.10	–
2-phenylpentane-1-ol	1.83	–
benzyl alcohol	4.67	1.35
heterocyclic compounds		
N-ethylpyrrole	–	1.11
trimethyloxazole	–	0.15
pyrazine	–	0.17
methylpyrazine	–	4.80
2,5-dimethylpyrazine	–	3.86
2,6-dimethylpyrazine	–	1.69
ethylpyrazine	–	1.06
2,3-dimethylpyrazine	–	0.30
2-ethyl-6-methylpyrazine	–	0.17
3-ethyl-2,5-dimethylpyrazine	–	7.67
2-ethyl-3,5-dimethylpyrazine	–	1.90
furfural	–	7.67
1-(2-furanyl)ethanone	–	1.10
furfuryl alcohol	–	3.92
N-ethylpyrrole-2-carboxaldehyde	0.96	3.35
indole	0.30	0.30
others		
1-ethylcyclohexene	4.44	–
butyrolactone	–	1.31
heptadecane	0.84	–
methyl salicylate	3.76	–

[a] Not detected.

among heterocyclic compounds, including pyrazines, thiophens, furans, and thiazoles (*29*). An analogue of *N*-ethylpyrrole-2-carboxaldehyde (3.35 mg/kg), *N*-methylpyrrole-2-carboxaldehyde, inhibited hexanal oxidation over 40 days at the level of 10 μg/mL. Also, furfural (7.67 mg/kg) inhibited hexanal oxidation by nearly 50% at the end of a 40-day storage period at the level of 500 μg/mL (*29*). This volatile extract contained only four alkyl compounds with a double bond. Benzyl alcohol (1.35 mg/kg) was also found in this extract.

It is difficult of pinpoint the constituents that give antioxidative activities to volatile extracts from green teas or roasted green teas because there are many volatile antioxidants present in these teas. Although the activities of these constituents are not as strong as the known natural antioxidants such as α-tocopherol, the total activity of these compounds might be comparable to those of known antioxidants.

The aldehyde assay involves abstraction of a hydroxy radical (*19*), whereas the conjugated diene assay involves the scavenging of an alkyl radical and/or a peroxy radical at a propagation phase of lipid autoxidation (*23*). The results of the present study indicate that volatile mixtures from tea samples contained various scavengers of radicals.

The present study suggests that the antioxidative activities of teas are in part due to the contributions of volatile compounds. Drinking a tea may help to prevent *in vivo* oxidative damage as mentioned above because of the presence of various volatile compounds with antioxidative activities.

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