

## Chemical Compositions and Antioxidant/ Anti-inflammatory Activities of Steam Distillate from Freeze-Dried Onion (*Allium cepa* L.) Sprout

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Freeze-dried onion sprout was steam-distilled, and the distillate was extracted with dichloromethane (volatile sample). Water sample I was obtained from the residual aqueous solution in the extractor. The filtrate and the methanol extract of filtrate from the residual aqueous solution in the steam distillation flask were named water sample II and methanol sample, respectively. Among the total of 71 components identified in the volatile sample, 24 were sulfur-containing compounds, which comprised 36.87% of the total volatile chemicals identified. The volatile sample inhibited hexanal oxidation for 40 days by >99% at levels > 100  $\mu\text{g/mL}$ . The volatile sample and water sample II exhibited moderate antioxidant activity in a malonaldehyde/gas chromatography assay and thiobarbituric acid assay, whereas water sample I did not show appreciable activity. The volatile sample, water sample I, and water sample II exhibited anti-inflammatory activity with a dose-related response in the lipoxygenase inhibitor screening assay. However, the methanol sample did not show appreciable activity in either antioxidant or anti-inflammatory tests. The results suggest that onion sprouts can be an excellent food source.

**KEYWORDS:** Anti-inflammatory activity; antioxidant; freeze-dry; onion sprout; sulfur-containing compounds; volatile chemicals

### INTRODUCTION

For thousands of years, the onion (*Allium cepa* L.), a family of *Allium* plants, has been used in spices, foods, flavors, and remedies. In addition to the fresh form, onions have been used in various forms, such as a powdered form as a flavor enhancer and a dried form as a seasoning.

In China and India, onion has been also used medicinally in combination with fresh juice, honey, or ginger. Many studies demonstrate various biological activities of onion extract, including anticancer (1–3), antioxidant (4), antimicrobial (5), antiplatelet (6), antidiabetic (7), anti-inflammatory (8), and antiasthmatic (9) effects. Additionally, the World Health Organization supports the use of the onion bulb for the treatment of age-dependent changes in blood vessels and loss of appetite (10).

There have been some reports on biological studies of bean sprouts. The antioxidant activity of cell wall polysaccharides in mung bean sprout hypocotyls reportedly resulted from decreased esterification in the main chains of pectin molecules (11). Phenolic compounds extracted from mung bean sprouts exhibited moderate antioxidant activity in a  $\beta$ -carotene/linoleic acid model system (12). Flavonoids have been known as principal antioxidants in various plants including onion (13, 14).

However, there are virtually no studies on flavonoids found in bean sprouts. Only daidzein, glycitein, and genistein have been reported in soybean sprouts (15).

Although several health effects and chemical compositional properties of various onion products, including fresh, dried, and powdered, have been studied, the chemical composition and antioxidant activity of onion sprouts in any form have not been reported yet. Therefore, the scope of the present study is to identify the chemical composition and to investigate the antioxidant and anti-inflammatory activities of onion sprouts in the most common powdered form used as a spice and flavor in order to investigate their chemical and biological natures.

### MATERIALS AND METHODS

**Chemicals and Materials.** HPLC grade methanol, acetonitrile, acetic acid, sodium dodecyl sulfate (SDS), hydrogen peroxide, and butylated hydroxytoluene (BHT) were bought from Fisher Scientific Co. (Rochester, NY). Tributylamine was purchased from Fluka Chemie GmbH (Sigma-Aldrich) (Steinheim, Switzerland). Cod liver oil,  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , *N*-methylhydrazine, 1-methylpyrazole, 2-methylpyrazine, tris(hydroxymethyl)aminomethane (Tris), tris(hydroxymethyl)aminomethane hydrochloride, thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP),  $\alpha$ -tocopherol, and malonaldehyde tetrabutylammonium salt were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO). Arachidonic acid, linoleic acid, and nordihydroguaiaretic acid were purchased from Cayman Chemical Co. (Ann Arbor, MI).

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Sprout of onion (*A. cepa* L.) was provided by the Cosmo Salad Co., Ltd. (Dixon, CA) as a gift. The sprouts were collected 6 days after germination.

A Lipoyxygenase Inhibitor Screening Assay (LISA) Kit was purchased from Cayman Chemical Co.

**Sample Preparations.** The freeze-dried onion sprout (30 g) was mixed with 1.5 L of deionized water in a 3 L two-neck round-bottom flask. The water in the flask was boiled using a mantle heater, and 500 mL of water distillate was collected in a beaker. The distillate was extracted with 150 mL of dichloromethane for 6 h using a liquid–liquid continuous extractor. The extract was dried over anhydrous sodium sulfate for 12 h. After removal of the sodium sulfate, the extract was condensed to approximately 3 mL in volume using a rotary evaporator. The extract was further condensed to exactly 0.4 mL under a purified nitrogen stream (volatile sample) and then stored at  $-20\text{ }^{\circ}\text{C}$  until used for experiments. Residual aqueous solution in the extractor was transferred into a flask, and the volume was reduced to 5 mL (water sample I) and then stored at  $5\text{ }^{\circ}\text{C}$  until used for further experiments.

The residual aqueous solution remaining in the distillation flask after water distillation was filtered, and the filtrate was reduced to 5 mL in volume (water sample II). A series of dilution samples (100, 80, 40, 20, 10, 5, 2.5, and 1.75%) was prepared with deionized water and then stored at  $5\text{ }^{\circ}\text{C}$  until used for inflammatory experiments. The residual onion sprout trapped in a filter paper (filtrand) was placed in a 100 mL beaker, and 50 mL of methanol was added. The methanol solution was shaken vigorously and allowed to stand for 1 h. After the residual onion sprout was filtered off, the filtrate was reduced in volume to 5 mL (methanol sample) and then stored at  $5\text{ }^{\circ}\text{C}$  until used for further experiments.

**Identification of Compounds in a Volatile Sample.** Chemicals in the volatile sample were identified by comparison with the Kovats gas chromatographic retention index I (16) and by the mass spectral fragmentation pattern of each component compared with those of authentic compounds. The identification of the gas chromatographic components was also conducted with the NIST AMDIS version 2.1 software.

An Agilent model 6890 gas chromatograph interfaced to an Agilent 5971A mass selective detector was used for mass spectral identification of the gas chromatographic components at a mass spectral ionization voltage of 70 eV. A 30 m  $\times$  0.25 mm i.d. ( $d_f = 0.25\text{ }\mu\text{m}$ ) DB-Wax bonded phase fused silica capillary column (J&W Scientific, Folsom, CA) was used for a gas chromatograph. The linear velocity of the helium carrier gas was 30 cm/s. The injector and detector temperatures were  $250\text{ }^{\circ}\text{C}$ . The oven temperature was programmed from 50 to  $180\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$  and held for 40 min.

The calculated concentration of each chemical was obtained using the following equation (23):

$$\text{concn } (\mu\text{g/g}) = \frac{\text{wt of extract (without solvent)} \times \text{GC peak area } \%/100 (\mu\text{g})}{\text{wt of freeze-dried onion sprout (30 g)}} \quad (1)$$

**Antioxidant Test of Volatile Sample by Aldehyde/Carboxylic Acid Assay.** The antioxidant activity of the volatile sample, water sample II, and the methanol sample was tested using its inhibitory effect on the oxidation of hexanal to hexanoic acid (17). Various amounts of volatile sample were added to a 2 mL dichloromethane solution of hexanal (3 mg/mL) containing 0.2 mg/mL of undecane as a gas chromatographic internal standard. The oxidation of the sample solution was initiated by heating at  $60\text{ }^{\circ}\text{C}$  for 10 min in a sealed vial and then storing at room temperature. A blank sample was prepared following the same procedure without a test sample. BHT was used as a positive control and to validate the assay. The decrease in hexanal was monitored at 5 day intervals for 40 days. Measurement was done in triplicate.  $\alpha$ -Tocopherol was used as a positive control of the assay.

**Antioxidant Test of Samples by Malonaldehyde/Gas Chromatography (MA/GC) Assay.** The antioxidant activity of samples was also tested by a previously reported MA/GC assay (18). Various concentrations of the samples were added to an aqueous solution (5 mL) containing 10  $\mu\text{L}$  of cod liver oil, 0.05 M Tris buffer (pH 7.4),

0.5  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , 1.0  $\mu\text{M}$   $\text{FeCl}_2$ , 0.75 mM KCl, and 0.2% of SDS. The sample solution was put in a 20 mL test tube, vortex mixed for 10 s, and incubated at  $37\text{ }^{\circ}\text{C}$  for 18 h in a water bath with shaking. The sample test tube was covered with aluminum foil during incubation to avoid photo-oxidation. BHT was used as a positive control of the assay. A control sample was prepared following the same procedure without a test sample. After incubation, a 4% ethanol solution of BHT (50  $\mu\text{L}$ ) was added to stop the oxidation. Malonaldehyde formed in the sample was derivatized to 1-methylpyrazole by adding 10  $\mu\text{L}$  of *N*-methylhydrazine. Fifty microliters of a 2-methylpyrazine stock solution (4 mg/mL) was added as a gas chromatographic internal standard. Malonaldehyde formed was quantified as 1-methylpyrazole by a gas chromatogram with a nitrogen–phosphorus detector. Then 10  $\mu\text{L}/\text{mL}$  each of three dilutions (100, 50, and 25%) of water sample II and the methanol sample were also tested using the method described above. Measurement was done in triplicate. BHT was used as a positive control of the assay.

An Agilent Technologies model 6890N gas chromatograph equipped with a 30 m  $\times$  0.25 mm i.d. ( $d_f = 25\text{ }\mu\text{m}$ ) ZB-Wax fused-silica capillary column (Phenomenex, Torrance, CA) and a nitrogen–phosphorus detector was used. The gas chromatographic oven temperature was held at  $60\text{ }^{\circ}\text{C}$  for 1 min and then programmed to  $180\text{ }^{\circ}\text{C}$  at  $12\text{ }^{\circ}\text{C}/\text{min}$  and held for 1 min. The injector and detector temperatures were 200 and  $300\text{ }^{\circ}\text{C}$ , respectively. The helium carrier gas flow rate was 1 mL/min.

**Antioxidant Test of Samples by Thiobarbituric Acid (TBA) Assay.** The TBA assay was performed using a previously reported method (19). Three concentrations of the volatile sample (200, 100, 50  $\mu\text{g}/\text{mL}$ ) were added to an aqueous solution (2 mL) containing 200  $\mu\text{L}$  of Tris buffer (pH 7.4), 300  $\mu\text{L}$  of 1 M KCl, 400  $\mu\text{L}$  of 1% SDS, 10  $\mu\text{L}$  of cod liver oil, 40  $\mu\text{L}$  of 1.0  $\mu\text{M}$   $\text{FeCl}_2$ , and 20  $\mu\text{L}$  of 0.5  $\mu\text{M}$   $\text{H}_2\text{O}_2$  in a brown nontransparent vial. The sample vial was then incubated for 18 h at  $37\text{ }^{\circ}\text{C}$  with shaking. After the incubation, oxidation was terminated by adding 50  $\mu\text{L}$  of 4% BHT in ethanol solution, and 2 mL of the TBA reagent (0.67% TBA, TCA, 1% SDS, 5 N HCl) was added to the sample. The sample was heated at  $80\text{ }^{\circ}\text{C}$  for 1 h and then cooled in an ice bath for 10 min. A blank sample was prepared following the same procedure without a test sample. The TBA–malonaldehyde adduct formed was measured using a Hewlett-Packard 8452A diode array spectrophotometer at 532 nm ( $A_{532}$ ). Three 10  $\mu\text{L}/\text{mL}$  dilutions (100, 50, and 25%) of water sample II and the methanol sample were also tested according to the method described above. Measurement was done in triplicate. BHT was used as a positive control of the assay.

**Anti-inflammatory Test of Samples.** Anti-inflammatory tests were conducted using the LISA Kit. Several published methods were also referred to in this study of lipoyxygenase activity (20–22)

The solutions provided commercially in the LISA Kit were 0.1 M Tris-HCl assay buffer (pH 7.4), developing agents 1 and 2 (chromogen), soybean–enzyme 15–lipoyxygenase standard, arachidonic acid, and KOH. The assay buffer was diluted 10-fold with HPLC-grade water before use. Chromogen, which was used within 1 h, was prepared by mixing equal amounts of developing agents 1 and 2. A blank well was prepared by adding an assay buffer solution (100  $\mu\text{L}$ ) to a well plate supplied from the LISA Kit. A positive control well was made by mixing 10  $\mu\text{L}$  of a 15-lipoyxygenase solution and 990  $\mu\text{L}$  of the assay buffer. A substrate solution was prepared by mixing 25  $\mu\text{L}$  of arachidonic acid and 25  $\mu\text{L}$  of KOH in ethanol. After the substrate solution had been vortexed, it was diluted with 950  $\mu\text{L}$  of HPLC-grade water. The substrate solution was used within 30 min to prevent degradation. The solution prepared was stored at  $0\text{ }^{\circ}\text{C}$  until used.

A 15-lipoyxygenase solution (10  $\mu\text{L}$ ), various concentrations of the samples, and assay buffer (980  $\mu\text{L}$ ) were placed in the testing well. The reaction was initiated by adding 10  $\mu\text{L}$  of substrate solution to a positive control well and a testing sample well. All testing wells were covered and placed on a shaker (Bellco Biotechnology, Vineland, NJ) for 5 min. Chromogen (100  $\mu\text{L}$ ) was added to the reaction wells to stop the enzyme catalysis and prevent further development of the reaction. The level of hydroperoxide in the samples was measured using a microplate reader (Molecular Devices Corp., Sunnyvale, CA). The entire assay was performed in duplicate.

Ten microliters each of eight concentrations (200, 100, 50, 25, 20, 10, 5, and 0  $\mu\text{g/mL}$ ) of a standard anti-inflammatory chemical, nordihydroguaiaretic acid, and the volatile sample and 10  $\mu\text{L}$  each of nine concentrations (100, 80, 40, 20, 10, 5, 2.5, 1.75, and 0%) of water samples I and II and the methanol sample were tested. The volatile sample and nordihydroguaiaretic acid were diluted with DMSO. The water samples and the methanol sample were diluted with HPLC-grade water.

## RESULTS AND DISCUSSION

**Chemicals Identified in the Volatile Sample.** The total yield of chemicals identified (relative to the freeze-dried onion sprout) was 0.0003% (w/w). The freeze-dried form was used for the present study because it is a commonly used method to prepare dried onions.

**Table 1** shows the compounds identified in the volatile sample, along with their calculated concentrations and Kovats indices on a DB-Wax column. A total of 71 components were identified in a volatile sample of onion sprout in the present study. Among them, 24 were sulfur-containing compounds (heterocyclic compounds are not included), which comprised 36.87% of the total volatile chemicals identified. The major constituents of sulfur-containing compounds in the volatile sample were methyl propyl disulfide (7.56 ppm)—which is present in the greatest amount in the volatile sample—dipropyl trisulfide (3.48 ppm), dipropyl disulfide (2.49 ppm), dimethyl disulfide (2.18 ppm), and methyl propyl trisulfide (1.76 ppm). Sulfide compounds are also the major constituents of volatiles found in onion. In a previous study, they comprised 39.2% of total volatiles recovered from onion (24). Many alkyl mono-, di-, and trisulfides, which are known to yield the typical flavor and aroma of onions (25), have been reported in onion (26–28). These volatile sulfides with the characteristic onion flavor have been known to form from less volatile sulfur-containing precursors such as *S*-alk(en)ylcysteinesulfoxides by the action of the enzymes such as lyase and allinase (28). Also, *Allium* vegetable components, such as *S*-propylcysteine, *S*-propylcysteine sulfoxide, *S*-methylcysteine, and its sulfoxide, were known to produce various volatile aroma compounds including alkyl sulfides and heterocyclic compounds upon thermal degradation (29, 30). Therefore, it can be hypothesized that these precursors and enzymes are also present in the onion sprout, and consequently these sulfides were found in the onion sprout in a large number and amount in the present study. On the other hand, although the thiosulfates occur in mature onions (12), they were not found in onion sprouts in the present study.

Carbonyl compounds (aldehydes and ketones) were the second major components of the volatile sample of onion sprout. Among 13 carbonyl compounds identified—which comprised 20.24% of the total chemicals identified—2-methyl-(*E*)-2-pentenal (4.17 ppm) is present in the greatest amount in the sample, followed by hexanal (3.38 ppm) and propanal (2.65 ppm). Among 15 heterocyclic compounds identified in the volatile sample, 7 compounds were sulfur-containing compounds including thiophene, thiazoline, and thiazole. These compounds have been known as chemicals contributing the roasted or toasted flavor in cooked foods (31). However, the amounts of these found in onion sprout in the present study were relatively low (total = 3.12 ppm) compared with sulfides. There is virtually no work reported on these sulfur-containing heterocyclic compounds in fresh onion.

The other compounds identified in the volatile sample were eight alcohols (total = 3.93 ppm)—2-methyl-2-butanol (1.43 ppm) was present in the greatest amount—five alkanes and alkenes, and six miscellaneous compounds. Some high molec-

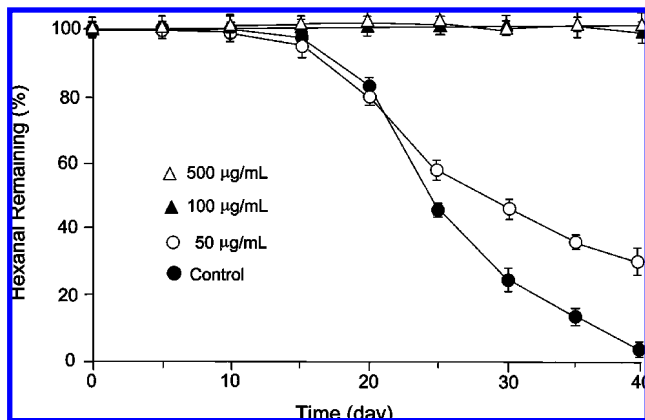
**Table 1.** Compounds Identified in a Volatile Sample from Dried Onion Sprout

compound	Kovats index <sup>a</sup>	concn (ppm)
<b>alkanes and alkenes</b>		
3-methyl-1-butene	597	0.17
tricosane	2300	1.00
tetracosane	2400	0.15
pentacosane	2500	1.87
heptacosane	2700	0.45
<b>alcohols</b>		
ethanol	927	0.84
2-methyl-2-butanol	1016	1.43
3-methyl-3-buten-2-ol	1172	0.16
hexanol	1357	0.49
2-butoxyethanol	1399	0.07
2-ethylhexanol	1494	0.25
benzyl alcohol	1875	0.46
phenylethyl alcohol	1907	0.23
<b>aldehydes and ketones</b>		
propanal	792	2.65
acetone	820	0.26
2,3-pentanedione	973	0.33
valeraldehyde	975	0.24
hexanal	1078	3.38
( <i>E</i> )-2-methyl-2-butenal	1089	0.34
( <i>E</i> )-2-methyl-2-pentenal	1153	4.17
2-heptanone	1179	0.05
acetoin	1280	0.24
hydroxyacetone	1295	0.36
2,3-octanedione	1323	0.18
3-hydroxypentan-2-one	1338	0.28
phenyl acetaldehyde	1632	0.15
<b>sulfur-containing compounds</b>		
allyl mercaptan	829	0.18
propanethiol	836	0.32
allyl methyl sulfide	949	0.30
dimethyl disulfide	1066	2.18
allyl propyl sulfide	1104	0.16
methyl propyl disulfide	1224	7.56
(methylthio)acetaldehyde	1253	0.38
methyl ( <i>Z</i> )-1-propenyl disulfide	1255	0.11
allyl methyl disulfide	1272	0.56
methyl ( <i>E</i> )-1-propenyl disulfide	1281	0.69
1-mercaptopropanon	1359	0.04
dipropyl disulfide	1370	2.49
( <i>Z</i> )-1-propenyl propyl disulfide	1404	0.53
allyl propyl disulfide	1421	0.60
( <i>E</i> )-1-propenyl propyl disulfide	1428	0.55
3-(methylthio)propanal	1445	0.13
methyl propyl trisulfide	1516	1.76
dimethyl sulfoxide	1578	0.08
allyl methyl trisulfide	1584	0.12
2-ethyl-5-methyl-1,3-dithiolane	1509	0.32
3,5-dimethyl 1,2,4-trithiolane	1588	0.30
dipropyl trisulfide	1658	3.48
3-mercapto-2-methylpentanol	1812	0.09
2-decen-5-olide	2201	0.08
<b>heterocyclic compounds</b>		
pyridine	1181	0.06
2,4-dimethylthiophene	1183	0.77
2-methylpyrazine	1263	0.12
2,3-dimethylpyrazine	1361	0.04
2-methyl-2-thiazoline	1366	0.06
2-ethyl-4,5-dimethyl-3-thiazoline isomer	1440	0.01
4-methyl-3-thiazoline	1442	trace
furfural	1458	0.38
2-ethyl-4,5-dimethyl-3-thiazoline isomer	1465	0.03
2-ethyl-4-methyl-3-thiazoline	1468	0.34
2-acetylfuran	1499	0.09
1-(2-furyl)-1-propanone	1569	0.42
2-acetylthiazole	1640	0.48
benzothiazole	1942	0.14
2,4-dimethylquinoline	2132	0.18
<b>miscellaneous compounds</b>		
ethyl acetate	908	0.25
octanoic acid	2060	0.27
4-vinylguaicol	2182	0.59
diethyl phthalate	2378	0.99
dibutyl phthalate	2695	1.00
valeric acid	2813	4.94

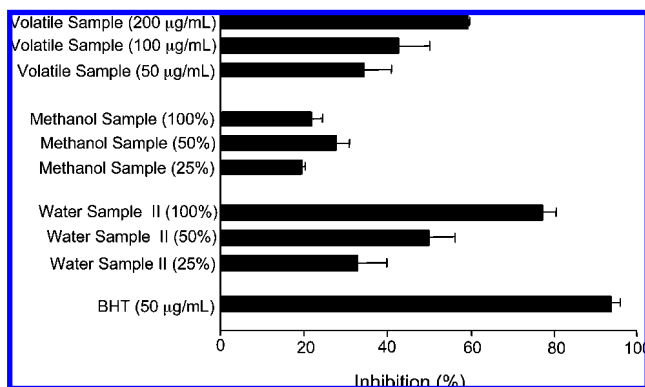
<sup>a</sup> Kovats index on DB-WAX column.

ular weight *n*-hydrocarbons were identified. Pentacosane was found in relatively high levels (1.87 ppm). These *n*-hydrocarbons may be degradation products of wax in the onion sprout skin.





**Figure 1.** Results of antioxidant tests on the volatile samples tested by aldehyde/carboxylic acid assay.

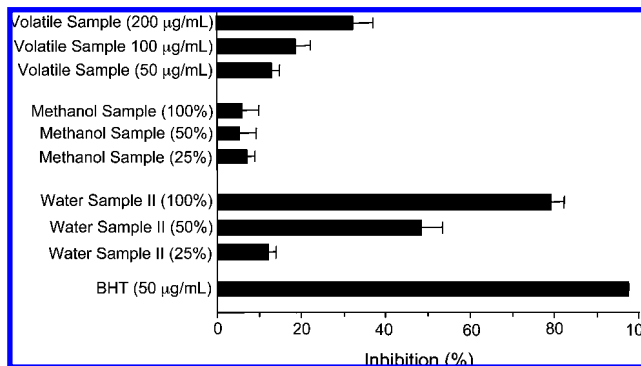


**Figure 2.** Results of antioxidant test on the samples of volatile, methanol, and water sample II tested by MA/GC assay.

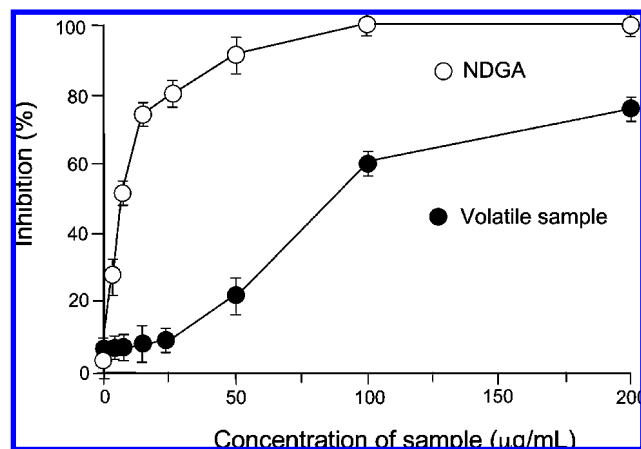
Valeric acid was recovered in significantly high levels (4.94 ppm), the second highest level after methyl propyl sulfide in the present study. Some unusual compounds, such as pyridine, 2-methylpyrazine, 2,3-dimethylpyrazine, 2,4-dimethylquinoline, 4-vinylguaiacol, diethyl phthalate, and dibutyl phthalate, were found in relatively low levels. These compounds may be formed or contaminated during experimental processes.

**Results of Antioxidant Activity Tests.** Figure 1 shows the results of antioxidant tests on the volatile samples using an aldehyde/carboxylic acid assay. The autoxidation of hexanal to hexanoic acid was monitored for 40 days. It is not shown in Figure 1, but  $\alpha$ -tocopherol, a standard antioxidant, inhibited hexanal oxidation by 100% throughout the 40-day period at the level of 50  $\mu\text{g/mL}$ . Volatile samples inhibited hexanal oxidation by >99% for 40 days at levels of 100 and 500  $\mu\text{g/mL}$ . The 50  $\mu\text{g/mL}$  volatile sample inhibited hexanal oxidation by approximately 50% over 31 days. Dose-related antioxidant activity was observed after 15 days.

Figures 2 and 3 show the results of antioxidant tests on the samples of volatile, methanol, and water sample II by MA/GC assay and TBA assay, respectively. BHT, a standard antioxidant, inhibited malonaldehyde formation by >95% in both assays. The methanol sample showed slight antioxidant activity without a dose-response in both assays. The volatile sample exhibited moderate antioxidant activity with a dose-response in both assays. The water sample without dilution (100%) inhibited malonaldehyde formation by nearly 80%. However, the calculated concentration of this water sample was 10 mg/mL, which is a rather high concentration compared with that of the volatile sample. Therefore, the volatile sample may have the most potent antioxidant activity among these three samples.



**Figure 3.** Results of antioxidant test on the samples of volatile, methanol, and water sample II tested by TBA assay.



**Figure 4.** Results of anti-inflammatory tests of the volatile sample and nordihydroguaiaretic acid.

Antioxidant activities of onion associated with various constituents, including phenols (32, 33), methanol extract (34), flavonols (35), and flavonoids (36), have been reported. However, the antioxidant activity of volatile samples from onion has not received much attention. Moreover, there are no reports of antioxidant activity of samples prepared from onion sprout prior to the present study.

**Results of Anti-inflammatory Activity Test.** Previous *in vivo* studies have demonstrated that overexpression of 15-lipoxygenase, found to be present within atherosclerotic plaques of humans and rabbits, was associated with increased occurrence of oxidized LDL (37). Also, overexpression of the arachidonate 15-lipoxygenase type I in transgenic rabbits gave, upon challenge, a phenotype that leads to enhanced endogenous anti-inflammation (38). Therefore, lipoxygenase is an enzyme that plays a vital role in the synthesis of inflammation. The present study utilized 15-lipoxygenase (provided in the LISA Kit) to examine the inhibitory activity of the samples obtained from onion sprout. In this assay, the hydroperoxides produced by 15-lipoxygenase from arachidonic acid were measured at a UV absorbance of 490 nm.

Figure 4 shows the results of anti-inflammatory tests of the volatile sample and nordihydroguaiaretic acid. A known anti-inflammatory compound, nordihydroguaiaretic acid, was tested for its activity to validate the LISA. The dose-response-related anti-inflammatory activity of nordihydroguaiaretic acid reached 100% at 100  $\mu\text{g/mL}$ . The anti-inflammatory activity of the volatile sample was 74.9% at 200  $\mu\text{g/mL}$ , 60.0% at 100  $\mu\text{g/mL}$ , and 21.2% at 50  $\mu\text{g/mL}$ , demonstrating a dose-related

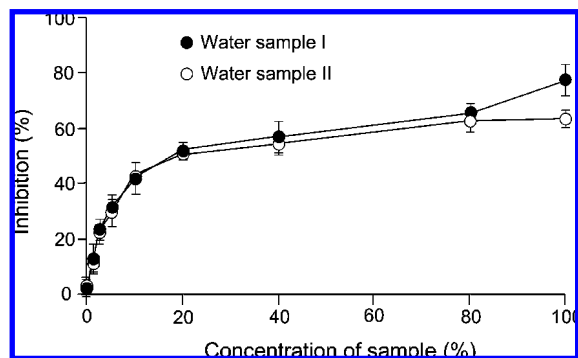


Figure 5. Results of anti-inflammatory tests of water samples I and II.

response. The results indicate that volatile chemicals possess potent anti-inflammatory activity as well as strong antioxidant activity.

Figure 5 shows the results of the anti-inflammatory tests of water samples I and II. Water sample I showed strong dose-related anti-inflammatory activity. It exhibited 77.9% inhibition at no dilution. Water samples I and II exhibited similar dose-related inhibition up to 80% concentration. Both samples inhibited hydroperoxide production by 60 and 50% at 80 and 20% concentrations, respectively. The methanol sample did not show any appreciable anti-inflammatory activity.

Results of the antioxidant tests and the anti-inflammatory test were quite consistent among the samples. This is reasonable because LISA measures the inhibitory activity of hydroperoxides induced by lipoxygenase. The strong relationship between antioxidant and anti-inflammatory activities has been reported previously (39, 40). For example, the crude extract of oregano herb (*Origanum vulgare* L.) exhibited strong activity in both antioxidant and anti-inflammatory tests (41).

Due to their characteristic odor and taste, onions (*A. cepa* L.) are considered to be an important flavor ingredient and have been used in a wide variety of dishes all over the world (42). In addition, onions have also received a great deal of attention due to their medicinal value (43). Diallyl disulfide found in onion (5) has been shown to inhibit the formation of carcinogenic heterocyclic aromatic amines found in boiled pork broth (2).

Similarly, medicinal and chemical compositional properties found in onions have been also found in onion sprouts. Many sulfur-containing compounds reported in onions and onion sprouts, such as allyl methyl sulfide and allyl propyl sulfide, exhibited strong antimicrobial activity (5). Antioxidant and anti-inflammatory activities of samples prepared from onion sprout also exhibited consistent results with those of onions. The flavor and taste of onion sprouts are quite similar to those of onion. The chemical compositions of onion sprout and onion are also similar. These results suggest that onion sprouts can be consumed in the same way as onion. Moreover, it is quite easy to prepare edible onion sprouts. It requires only UV light, water, and less than 1 week between germination and harvest, suggesting that onion sprouts can be an excellent food source for people living in sterile areas in the world.

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