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Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties

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Abstract

Aroma compounds in the extracts of basil leaves (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major aroma constituents of basil were 3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-methoxy-4-(2-propenyl) benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g). The major aroma constituents of thyme were 2-isopropyl-5-methylphenol (thymol; 8.55 mg/g), 4-isopropyl-2-methylphenol (carvacrol; 0.681 mg/g), linalool (0.471 mg/g), α-terpineol (0.291 mg/g), and 1,8-cineole (0.245 mg/g). Twelve aroma constituents of basil and thyme were examined for their antioxidant activities using the aldehyde/carboxylic acid assay. Eugenol, thymol, carvacrol, and 4-allylphenol showed stronger antioxidant activities than did the other components tested in the assay. They all inhibited the oxidation of hexanal by almost 100% for a period of 30 days at a concentration of 5 μg/ml. Their antioxidant activities were comparable to those of the known antioxidants, α-tocopherol and butylated hydroxy toluene (BHT).

Keywords: Aroma chemicals; Basil; Thyme; Natural antioxidants

1. Introduction

There have been great efforts to find safe and potent natural antioxidants from various plant sources. As harmless sources of antioxidants, wild herbs, spices, fruits, nuts, and leafy vegetables have been investigated, for their antioxidant properties, for example, rosemary (Trojakova, Reblova, Nguyen, & Pokorny, 2001), sage (Cuvelier, Richard, & Berset, 1996), thyme (Miura & Nakatani, 1989) and summer savory (Bertelsen, Christophersen, Nielsen, Madsen, & Stadel, 1995).

Basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.) are aromatic herbs that are used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics (Simon, Morales, Phippen, Vieira, & Hao, 1999; Javanmardi, Khalighi, Kashi, Bais, & Vivanco, 2002; Senatore, 1996). Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction (Simon et al., 1999). Thyme also possesses various beneficial effects, e.g., antiseptic, carminative, antimicrobial, and antioxidative properties

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(Baranauskiene, Venskutonis, Viskelis, & Dambrauskiene, 2003).

The antioxidant activities of basil and thyme have been investigated using various model systems and assays. The antioxidant activity of ethanol extract of basil (*O. basilicum* L.) was investigated by electrochemical measurements (Madsen, Nielsen, Bertelsen, & Skibsted, 1996). The total antioxidant activities in the acetone extracts of 23 varieties of Iranian basil were determined as trolox equivalent antioxidant capacity (TEAC), and showed a linear positive relationship with the total phenolic contents (Javanmardi, Stushnoff, Locke, & Vivanco, 2003). The non-polar (hexane) fraction of thyme was examined for the occurrence of phenolic compounds and their antioxidant activities. The phenolic compound isolated from hexane extract of thyme, *p*-cymene-2,3-diol (2,3-dihydroxy-4-isopropyl-1-methylbenzene), showed strong antioxidant activity which was greater than those of α-tocophenol and butylated hydroxyanisole (Schwarz, Ernst, & Ternes, 1996). The antioxidant activity of acetone extract of thyme was evaluated in sunflower oil and its 20% oil-in-water emulsion. The thyme extract inhibited generation of hexanal and pentanal in both the oil and in the emulsion (Abdalla & Roozen, 1999). The antioxidant activities of 23 essential oils isolated from various spices and herbs, inhibiting the copper-catalyzed oxidation of human-low-density lipoproteins (LDL) were determined in vitro. Essential oil of thyme (which was abundant in thymol, carvacrol, cuminol, or eugenol) showed moderate inhibition of LDL oxidation (20–27%) (Teissedre & Waterhouse, 2000). Peroxide levels of triacylglycerols of lard and sunflower oil, with the addition of various levels of thymol and carvacrol, major components of thyme essential oil, were measured to assess their antioxidant properties. During autoxidation of lipids at ambient temperature, thymol was more effective in preventing oxidation than carvacrol (Yanishlieva, Marinova, Gordon, & Raneva, 1999). Deodorized aqueous extracts of four herbs, including thyme, showed varying degrees of reductive and radical scavenging capacity, and were capable of a marked prolongation of the lag-time in the LDL oxidation assay (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

Among the many studies to determine the antioxidant activities of basil and thyme, most studies have focussed mainly on the antioxidant activities of crude extracts, using methanol, acetone, or water as a solvent. Although basil and thyme are mainly used for their distinctive aromas, there have been few studies on the identification of aroma components distilled from basil and thyme. In addition, the antioxidant potentials of the aroma components identified in the extracts have not yet been studied.

In the previous study in our laboratory, volatile extracts of thyme and basil showed potent antioxidant activities against hexanal oxidation. Especially noteworthy, the inhibitory effect of thyme at 10 μg/ml and basil at 50 μg/ml were comparable to those of BHT and α-tocophenol at concentrations varying from 10 to 500 μg/ml (Lee & Shibamoto, 2002). Since the volatile extracts of basil and thyme did not contain known antioxidants such as BHT, α-tocopherol, polyphenols, or flavonoids, the antioxidant activities of those extracts were considered to be due to the presence of some antioxidant volatile chemical(s). Thus, the antioxidant potentials of the aroma chemicals found in the volatile extracts of thyme and basil were investigated in this study. First, the aroma compounds in the volatile extracts of basil and thyme were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), and then the selected aroma chemicals identified were examined for their antioxidant activities by the aldehyde/carboxylic acid assay.

2. Materials and methods

2.1. Materials

Dried basil leaves (*O. basilicum* L.) and thyme leaves (*T. vulgaris* L.) were purchased from a local market in northern California (Davis, CA, USA).

2.2. Chemicals

Eugenol, thymol, carvacrol, 4-allylphenol, 1-octen-3-ol, benzyl alcohol, linalool, methyl-salicylate, estragole, 1,8-cineole, 4-terpeneol, benzylaldehyde, hexanal, hexanoic acid, undecane, 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). Dichloromethane was bought from Fisher Scientific Co. Ltd. (Fair Lawn, NJ).

2.3. Isolation of aroma chemicals by steam distillation under reduced pressure

Basil or thyme leaves (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 (DRP) (95 mm Hg). The distillate (900 ml) was extracted with 100 ml dichloromethane using a liquid–liquid continuous extractor (LLE) 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 ≈1 ml, and then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.2 ml and stored at −20 °C for subsequent analyses.

2.4. Identification and quantification of aroma chemicals in the extracts from basil and thyme leaves

Aroma chemicals obtained by DRP and LLE 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.

The mass of each concentrate was determined using an analytical balance. The concentrate was then analyzed by GC, using a flame ionization detector, to determine the percentage total peak areas of aroma chemicals and solvent. The total mass of aroma chemicals was calculated by multiplying the percentage representing the total peak area of components by the total mass of extract. Each injection was triplicated. The detector response to solvent was found to be linear over a range of 0.2–1.0 μl injected, with an R^2 value of 0.99. GC conditions and parameters were the same as for GC–MS analysis. The average concentration of each chemical was calculated using the following equation:

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

2.5. Verification of the antioxidant potential of the major constituents in aroma extracts by aldehyde/carboxylic acid assay

To assess if the antioxidant properties of aroma extracts isolated from basil and thyme can be attributed to their aroma constituents, 12 aroma constituents marked with an asterisk (*) in Table 1 were selected, based on molecular structure and GC peak area. Measuring the antioxidant activities of the constituents was conducted using the aldehyde/carboxylic acid assay (Macku & 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 antioxidant activity using the same methodology.

2.6. Instrumental analysis

The quantitative analysis of hexanal was conducted according to an internal standard method previously reported (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 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 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.

3. Results and discussion

3.1. Volatile composition

The total yields of volatile chemicals from basil and thyme (relative to the amount of dried herbs used) were 1.24 ± 0.14%, and 1.05 ± 0.1%, respectively. Table 1 shows the compounds identified in extracts obtained from basil and thyme, along with their calculated concentrations and Kovats indices on a DB-Wax column. Among 200 GC peaks observed in chromatograms of both basil and thyme extracts, 129 aroma chemicals were identified.

Aroma chemicals identified in the basil extract were 30 monoterpenes, 14 sesquiterpenes, 20 aromatic compounds, 8 alcohols, 4 aldehydes, 7 ketones and esters, and 3 miscellaneous compounds. The class of aromatic compounds displayed the highest amount, followed by monoterpenes, among the above classes. Over 85% of the total volatiles quantified in this study originated from five volatiles: linalool (39.8%); estragole (20.5%); methyl cinnamate (12.9%); eugenol (9.1%); and 1,8-cineole (2.9%). Major volatiles detected in this study were consistent with those of previously published studies in which volatile components were isolated by various organic solvent extractions (Grayer et al., 1996; Hasegawa, Tajima, Toi, & Sugimura, 1997). In the study of essential oils produced from various cultivars of *O. basilicum* L.,

Table 1
Volatile components identified in the extracts of basil and thyme leaves

Compound	I^a	Concentration (mg/g)[b]	
		Basil	Thyme
Monoterpene hydrocarbons			
α-Terpinene	1167	0.002	0.005
γ-Terpinene	1233	0.004	0.007
p-Cymene	1257	0.001	0.013
Oxygenated monoterpenes			
1,8-Cineole	1202	0.288	0.245
Terpinolene	1271	–[d]	0.001
Linalool *cis*-furanoid	1438	0.034	n.d.
Linalool oxide *cis*-furanoid	1439	n.d.	0.018
trans-Sabinene hydrate	1463	0.021	0.030
Linalool *trans*-furanoid	1467	0.037	n.d.
Linalool oxide *trans*-furanoid	1468	n.d.	0.017
Ocimene oxide	1479	0.001	n.d.
Camphor	1505	0.031	0.148
3,7-Dimethyl-1,6-octadien-3-ol (linalool)	1550	3.939	0.479
Linalyl acetate	1556	0.003	n.d.
trans-*p*-menth-2-en-1-ol[c]	1561	0.003	0.001
Bornyl acetate	1574	0.023	0.004
Carvacryl methyl ether	1583	n.d.	0.001
Exo-methylcamphenilol[c]	1592	n.d.	0.002
4-Terpineol	1597	0.003	0.111
cis-Dihydrocarvone	1606	n.d.	0.003
Hotrienol	1607	0.002	n.d.
Terpinen-1-ol[c]	1621	n.d.	0.002
L-menthol	1639	n.d.	0.008
trans-Pinocarveol	1648	n.d.	0.006
δ-Terpineol[c]	1668	n.d.	0.037
Lavandulol	1670	0.020	0.004
trans-Verbenol	1675	n.d.	0.012
p-menth-1,8-dien-4-ol	1679	0.002	0.002
Terpinyl formate	1683	0.002	n.d.
α-Terpineol	1691	0.112	0.291
Borneol	1692	0.026	0.244
Verbenone	1695	0.001	0.091
Exo-2-hydroxycineole acetate	1716	0.003	n.d.
Dihydrocarveol	1720	n.d.	0.007
α-Citral	1721	0.008	n.d.
EXO-2-hydroxycineole[c]	1723	n.d.	0.003
L-carvone	1728	0.001	0.090
Linalool oxide *cis*-pyranoid	1732	0.005	0.005
trans-piperitol	1743	n.d.	0.003
Linalool oxide *trans*-pyranoid	1759	0.008	0.004
Citronellol	1761	n.d.	0.006
Myrtenol	1789	n.d.	0.005
Nerol	1797	0.002	0.005
trans-Carveol	1833	n.d.	0.011
p-Cymen-8-ol	1846	n.d.	0.046
Geraniol	1847	0.016	0.031
Geranyl acetate	1848	0.003	n.d.
Guaiacol	1860	n.d.	0.006
Exo-2-hydroxycineole	1861	0.002	0.001
Piperitenone	1918	n.d.	0.003
L-perillyl alcohol	2003	n.d.	0.002
Cuminyl alcohol	2098	n.d.	0.016
Sesquiterpene hydrocarbons			
β-Cubebene[c]	1662	0.002	n.d.
δ-Cadinene	1745	0.002	n.d.
Valencene	1884	0.001	n.d.
α-Amorphene[c]	1969	0.001	n.d.
δ-Selinene[c]	2231	n.d.	0.010
Dehydroaromadendrene	2287	0.001	n.d.
Oxygenated sesquiterpenes			
Caryophyllene oxide	1974	0.011	0.038
α-Humulene oxide	2038	0.003	0.004
Elemol	2083	n.d.	0.008
Viridiflorol[c]	2092	n.d.	0.001
Spathulenol[c]	2120	0.028	0.037
α-Cadinol	2167	0.182	n.d.
T-murolol[c]	2192	n.d.	0.003
β-Bisabolol[c]	2211	0.002	n.d.
β-Bisabolol isomer[c]	2213	0.002	n.d.
α-Eudesmol	2220	n.d.	0.007
Isospathulenol[c]	2222	0.002	0.001
β-Eudesmol	2225	0.009	0.014
Caryophylla-4(12), 8(13)-dien-5β-ol	2292	n.d.	0.006
Dihydroactinidiolide	2337	0.012	0.005
Caryophylla-3, 8(13)-dien-5α (or β)-ol	2371	n.d.	0.010
Aliphatic alcohols			
1-Penten-3-ol	1155	0.001	0.002
3-Methyl-3-buten-1-ol	1245	n.d.	0.013
(*Z*)-2-pentenol	1318	0.001	n.d.
3-Methyl-2-buten-1-ol	1320	n.d.	0.002
Hexanol	1351	0.003	0.006
(*Z*)-3-hexenol	1381	0.051	0.017
3-Octanol	1393	0.004	0.010
Cyclohexanol	1403	0.001	n.d.
1-Octen-3-ol	1448	0.053	0.054
Octanol	1559	0.011	n.d.
Aliphatic aldehydes			
Hexanal	1073	0.002	n.d.
(*E*)-2-hexenal	1209	0.012	0.003
(*E,Z*)-2,4-heptadienal	1455	0.002	0.004
(*E,E*)-2,4-heptadienal	1483	0.001	0.003
0			
Aliphatic esters			
Methyl 2-methylbutyrate	1005	n.d.	0.001
(*Z*)-3-hexenyl acetate	1309	0.001	n.d.
Aliphatic ketones			
3-Octanone	1247	n.d.	0.006
3-Hydroxy-2-butanone	1270	0.001	n.d.
6-Methyl-5-heptenone	1329	0.002	n.d.
6-Methyl-(*E,E*)-3, 5-heptadien-2-one	1587	0.002	n.d.
β-Ionone	1932	0.005	n.d.
cis-Jasmone	1938	0.002	n.d.
trans-Beta-ionone-5,6-epoxide	1989	0.001	0.003
Methyl jasmone	2332	n.d.	0.012
Aliphatic acids			
Butanoic acid	1616	n.d.	0.002
Octanoic acid	2056	n.d.	0.004
Decanoic acid	2268	n.d.	0.003
Aromatic compounds			
Benzaldehyde	1508	0.018	n.d.
Methyl benzoate	1615	0.005	n.d.
Phenyl acetaldehyde	1638	0.003	n.d.

Table 1 (*continued*)

Compound	*I*[a]	Concentration (mg/g)[b] Basil	Thyme
1-Methoxy-4-(2-propenyl) benzene (estragole)*	1661	2.029	0.011
Methyl salicylate*	1762	0.003	n.d.
p-Methylacetophenone	1763	n.d.	0.005
Cuminaldehyde	1767	0.011	0.011
Anethol	1815	0.006	0.026
Safrole	1863	0.001	n.d.
Benzyl alcohol	1870	0.018	n.d.
Phenethyl alcohol	1905	0.026	0.023
Methyl cinnamate	1946	0.215	n.d.
Methyl eugenol	2007	0.065	0.002
α,α-Dimethylphenylethyl alcohol	2015	n.d.	0.163
Anisaldehyde	2015	0.015	n.d.
trans-Cinnamaldehyde	2032	n.d.	0.019
Methyl cinnamate	2073	1.278	n.d.
p-Cresol	2076	n.d.	0.002
Ethyl cinnnamate	2132	0.003	n.d.
Eugenol*	2161	0.896	0.092
2-Isopropyl-5-methylphenol (thymol)*	2179	0.015	8.554
2-Isopropyl-2-methylphenol (carvacrol)*	2206	0.003	0.681
5-Isopropyl-3-methylphenol	2287	n.d.	0.003
4-Allylphenol*	2329	0.257	n.d.
Dillaiole[c]	2351	n.d.	0.001
p-Methoxycinnamaldehyde	2544	0.003	n.d.
Miscellaneous compounds			
2,6-Dimethylpyrazine	1246	0.001	n.d.
γ-butyrolactone	1626	0.009	n.d.
Myristicin	2257	0.001	n.d.

[a] On DB-Wax column.
[b] Solvent peak excluded and values are on dried weights of basil and thyme in mg/g.
[c] Identified by GC/MS only.
[d] Not detected.
* Tested for antioxidant activity.

linalool (21.1–33.8% of total quantified volatile compounds), estragole (35.9–56.2%), eugenol (1.12–4.36%), and 1,8-cineole (3.40–4.37%) were also determined as major constituents (Hasegawa et al., 1997).

Aroma chemicals identified in the thyme extract were 43 monoterpenes, 16 sesquiterpenes, 14 aromatic compounds, 7 alcohols, 3 aldehydes, 4 ketones and esters, and 3 acids. Phenolic compounds were the aroma principles in this chemo-type of thyme. Quantitatively, the most important compounds were thymol (72%) and isothymol (carvacrol) (5.7%), which constitute almost three-quarters of the quantified total volatiles, followed by monoterpenes, linalool (4.0%), α-terpineol (2.4%), 1,8-cineole (2.1%), and borneol (2.0%). Major volatile constituents, such as thymol and isothymol, were distinctive for *T. vulgaris* species and were also found as major compounds in other published results (Venskutonis, Poll, & Larsen, 1996; Venskutonis, 1997; Baranauskiene et al., 2003).

3.2. Antioxidant activities of aroma chemicals

To measure antioxidant activities of the aroma chemicals in this study, the aldehyde/carboxylic acid conversion assay was used. This is a relatively simple assay that can be used to measure the antioxidant potential of a chemical in an organic, non-aqueous phase (Macku & Shibamoto, 1991). In this assay, volatile chemicals or extracts dissolved in the organic solvent, dichloromethane, can easily be evaluated for their antioxidant potential. This method has been validated to measure the antioxidant activities of natural volatile extracts, various volatile chemicals and known antioxidants, such as BHT and vitamin E (Yanagimoto, Lee, Ouchi, & Shibamoto, 2002; Yanagimoto, Ouchi, Lee, & Shibamoto, 2004; Park, Lee, Shibamoto, Lee, & Takeoka, 2003; Lee & Shibamoto, 2002). Fig. 1 shows the relative amounts of hexanal and hexanoic acid that formed over time in a control sample in which no testing chemicals were added. Hexanal in the control sample was oxidized to hexanoic acid at almost 100% over 30 days. The concentration of hexanoic acid increased as the hexanal decreased.

Table 2 shows the percent of hexanal remaining in each sample containing the different amounts of aroma chemicals, BHT, and α-tocopherol throughout a storage period of 30 days. The values are means ± standard deviation ($n = 3$). All chemicals exhibited dose-dependent inhibitory activity. Among the chemicals identified in the extracts from thyme and basil, thymol, carvacrol (isothymol), 4-allylphenol and eugenol exhibited potent antioxidant activities among the tested materials; those aroma chemicals inhibited hexanal oxidation by 95–99% at 5 μg/ml over 30 days, which is comparable to that of α-tocopherol or BHT. Both α-tocopherol and BHT inhibit hexanal oxidation by 89% and 99% at 5 μg/ml over

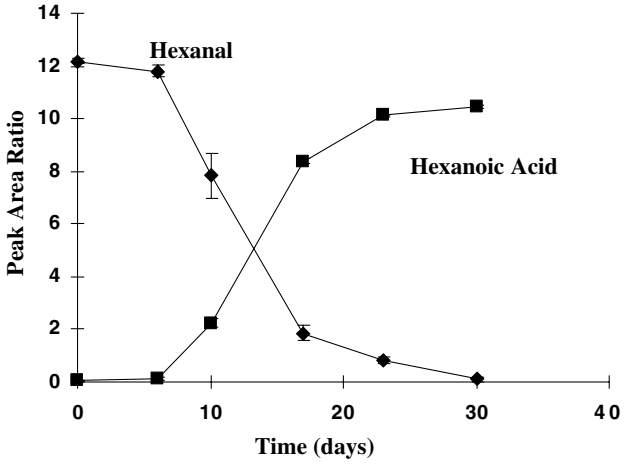

Fig. 1. Relative amounts of hexanal and hexanoic acid over time in a control.

Table 2
Percentage of hexanal remaining in solutions treated with different amounts of volatile components in thyme and basil, α-tocopherol and BHT throughout a storage period of 30 days

Components	Inhibitory effect[a] (%)				
	0 μg/ml	1 μg/ml	5 μg/ml	10 μg/ml	50 μg/ml
Eugenol	6 ± 2.7	32 ± 7.6	99 ± 2.8	99 ± 1.3	100 ± 5.7
Thymol	5 ± 0.4	11 ± 7.1	95 ± 6.2	100 ± 0.5	100 ± 0.7
Carvacrol	6 ± 3.3	15 ± 8.2	100 ± 2.9	100 ± 0.8	100 ± 0.8
4-Allylphenol	2 ± 1.6	14 ± 10	100 ± 1.5	100 ± 0.4	99 ± 4.2
1-Octen-3-ol	2 ± 3.6	4 ± 3.2	9 ± 3.5	11 ± 2.4	19 ± 2.0
Benzyl alcohol	2 ± 1.6	8 ± 5.8	10 ± 4.5	32 ± 5.4	59 ± 10
Linalool	6 ± 1.8	7 ± 4.7	3 ± 3.4	8 ± 3.7	23 ± 4.9
Methyl-salicylate	3 ± 1.6	9 ± 2.7	9 ± 0.5	9 ± 0.3	10 ± 1.4
Estragol	5 ± 0.4	8 ± 2.2	9 ± 0.8	10 ± 0.4	12 ± 2.0
1,8-Cineole	3 ± 0.5	4 ± 2.8	5 ± 4.1	7 ± 3.7	11 ± 0.9
4-Terpeneol	4 ± 0.7	2 ± 2.5	4 ± 2.6	8 ± 2.7	14 ± 0.9
Benzylaldehyde	5 ± 5.2	7 ± 5.8	5 ± 2.7	8 ± 2.5	10 ± 2.1
α-Tocopherol	2 ± 0.2	17 ± 9.9	89 ± 0.6	92 ± 2.2	98 ± 3.0
BHT	5 ± 2.0	100 ± 0.1	99 ± 0.8	99 ± 0.7	100 ± 0.1

[a] The values are means ± SD ($n = 3$).

30 days, respectively. The antioxidant activity of eugenol has been reported several times, tested on various systems (Satoh, Ida, Sakagami, Tanaka, & Fusisawa, 1998; Nagababu & Lakshmaiah, 1992). Thymol and carvacrol, major components of aroma extract of eucalyptus leaves, have also shown strong antioxidant activities in several studies (Aeschbach et al., 1994; Yanishlieva et al., 1999). However, 4-allylphenol, which was quantified in basil (0.257 mg/g), has not been investigated for antioxidant activity to date. The hexanal oxidation was almost completely inhibited by 4-allylphenol at 5 μg/ml over 30 days. At the lowest level of 1 μg/ml, eugenol displayed the highest antioxidant activity among the chemicals tested in this study. Eugenol inhibited hexanal oxidation by 32% at 1 μg/ml over 30 days. Among the other aromatic components tested for their antioxidant activity, only benzyl alcohol showed slight antioxidant activity at the level of 50 μg/ml in the present study. Benzyl alcohol has previously been reported to possess antioxidant activity (Lee & Shibamoto, 2001).

An alkyl compound with a double bond, 1-octen-3-ol, also showed slight antioxidant activity; 1-octen-3-ol inhibited hexanal oxidation by 19% at a level of 50 μg/ml. However, this compound did not show much antioxidant activity at a level of 10 μg/ml or lower. Except for the aroma chemicals mentioned above, the other aroma components tested did not show any appreciable antioxidant activities.

Considering the concentration and the antioxidative performance of each aroma chemical investigated in this study, eugenol (0.896 mg/g) and 4-allylphenol (0.257 mg/g) were considered the main contributors of the antioxidant activity of volatile extract of basil, which showed a potent inhibitory effect against hexanal oxidation at a concentration of 50 μg/ml (Lee & Shibamoto, 2002). Thymol (8.55 mg/g), which constituted 70% of quantified total volatiles, was found to be a main contributor to the antioxidant activity of volatile extract of thyme at 10 μg/ml, comparable to BHT and α-tocophenol at the same concentration as found in a previous study (Lee & Shibamoto, 2002).

4. Conclusion

Major aroma compounds found in volatile extracts of basil and thyme exhibited varying amounts of anti-oxidative activity. In particular, eugenol, thymol, carvacrol and 4-allylphenol, found in basil and thyme, exhibited potent antioxidant activity, comparable to the known antioxidants, BHT and α-tocopherol. Considering the abundance of these aroma chemicals in natural plants, the total activity may be comparable, or more, than 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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