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# Determination of Antioxidant Potential of Volatile Extracts Isolated from Various Herbs and Spices

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Antioxidant activities of volatile extracts isolated from thyme, basil, rosemary, chamomile, lavender, and cinnamon were evaluated by two independent assays: the aldehyde/carboxylic acid assay and the conjugated diene assay. The volatile extracts were prepared from dried herbs and spices using liquid−liquid continuous extraction following steam distillation under reduced pressure (55 °C and 95 mmHg). The antioxidant activities of the extracts decreased in the following order in both of the lipophilic assay systems: thyme > basil > rosemary > chamomile > lavender and cinnamon. Thyme and basil extracts inhibited the oxidation of hexanal for 40 days at the levels of 10 $\mu$g/mL and 50 $\mu$g/mL, respectively. The extracts of thyme and basil were effective in retarding methyl linoleate deterioration at 40 °C, with activity increasing with concentration in the range 10−200 $\mu$g/mL. At a concentration of 50 $\mu$g/mL, thyme extract was similar in antioxidant activity to BHT and $\alpha$-tocopherol in the conjugated diene assay. The antioxidant potentials of the volatile extracts used in this study were accurately measured by the lipophilic systems, such as the aldehyde/carboxylic acid assay and the conjugated diene assay.

**KEYWORDS: Volatile chemicals; thyme; basil; natural antioxidants**

## INTRODUCTION

The oxidative deterioration of lipids is a great concern in the shelf life of foods. Lipid oxidation leads to development of undesirable off-flavors and decreases the acceptability of foods (*1*). In addition, lipid oxidation decreases food safety and nutritional quality by formation of potentially toxic products and secondary reaction products during cooking or processing (*2, 3*). To prevent and retard lipid oxidation, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary-butylhydroquinone, and propyl gallate have been added to lipid-containing foods (*4*). However, potential health hazards of synthetic antioxidants in foods, including possible carcinogens, have been reported several times (*5, 6*). Since then, the search for antioxidants naturally occurring in plants as alternatives to synthetic antioxidants is of great interest to researchers (*7*). Natural antioxidants of plant origin are generally classified as vitamins, phenolic compounds including flavonoids and phenolic acids, and volatile compounds in herbs and spices (*1*). These natural antioxidants are becoming increasingly important, not only in food but also in preventive medicine. In medicine, natural antioxidants are one of the important sources in curing diseases associated with oxidative damage (*8*).

Spices and herbs provide foods with flavors and food-preserving power, including antiseptic and antioxidant activity. The antioxidant activities of herbs and spices extracted with solvents, such as methanol or acetone, have been evaluated in various test systems, including hydrophilic and lipophilic systems. So far, many investigations on the antioxidant activities of methanol extracts of rosemary (*9*−*13*), sage (*9, 10, 14*), and thyme (*15*−*18*) have been carried out in hydrophilic and lipophilic test systems. However, only a few volatile extracts of herbs and spices obtained by distillation and liquid−liquid continuous extraction were assessed for their antioxidant activities (*19, 20*). Typical assays for antioxidant activities of plant extracts and their components are performed in hydrophilic systems such as water or alcohols. However, volatile components in plant extracts are dissolved in less polar or nonpolar solvents such as dichloromethane and hexane. For this reason, it is less accurate and not robust to measure the antioxidant activity of volatile extracts using the typical hydrophilic assays. The aldehyde/carboxylic acid assay and the conjugated diene assay used in this study were carried out to test the antioxidant potential of volatile extracts in lipophilic environments such as dichloromethane and methyl linoleate.

In this study, the volatile extracts of herbs and spices (thyme, basil, rosemary, chamomile, lavender, and cinnamon) were prepared using liquid−liquid continuous extraction following steam distillation under reduced pressure. The antioxidant properties of the volatile extracts were assessed by modification of established assays, such as the aldehyde/carboxylic acid assay and the conjugated diene assay.

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## MATERIALS AND METHODS

**Materials and Reagents.** Dried thyme leaves (*Thymus vulgaris* L.), basil leaves (*Ocimum basilicum* L.), rosemary leaves (*Rosemarynus officinalis* L.), chamomile flowerheads (*Chamaemelum nobile* L.), lavender flowerheads (*Lavandula angustifolia* P. Miller), and cinnamon bark (*Cinnamomum cassia* Blume) were purchased from a local market. Methyl linoleate was obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Hexanal, hexanoic acid, undecane, 2,2,4-trimethylpentane (isooctane), and α-tocopherol (vitamin E) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Butylated hydroxytoluene (BHT) was purchased from Sigma Chemical Co. (St. Louis, MO), and dichloromethane was from Fisher Scientific Co., Ltd. (Fair Lawn, NJ).

**Isolation of Volatile Extracts by Steam Distillation under Reduced Pressure (DRP) and Liquid−Liquid Continuous Extraction.** Dried thyme, basil, rosemary, chamomile, lavender, or cinnamon (20 g) was placed in a 3-L round-bottom flask with 1 L of deionized water. The solution was steam-distilled at 55 °C for 3 h under reduced pressure (95 mmHg). The distillate (900 mL) was extracted with 100 mL of dichloromethane using a liquid−liquid continuous extractor for 6 h. After the extract had been dried over anhydrous sodium sulfate, the solvent was removed in a rotary flash evaporator (Yamato Sci, Tokyo, Japan). The distillation was stopped when the volume of extract was reduced to approximately 1 mL. Then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.6 mL.

**Determination of Total Volatile Components in Extract.** The mass of each concentrate was determined according to the method reported by Lee and Shibamoto (*21, 22*). Each concentrate was analyzed by gas chromatography (GC), using a flame ionization detector (FID), to determine the percentage of total peak areas of volatile components and solvent. The total mass of volatile components was calculated by multiplying the percentage representing the total peak area of components by the total mass of extract. Each experiment was repeated three times. The detector response to solvent was found to be linear over a range of 0.2 μL to 1.0 μL injected, with a $R^2$ value of 0.99. A Hewlett-Packard (HP; Palo Alto, CA) model 6890 GC equipped with a 30 m × 0.25 mm i.d. ($d_f$ = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a FID was used for analysis of total volatile components in each extract. 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 250 °C. The oven temperature was programmed from 50 to 180 °C at 3 °C/min and held for 20 min.

**Aldehyde/Carboxylic Acid Assay.** The inhibitory effect of each volatile extract on the oxidation of aldehyde to carboxylic acid was determined according to previously published reports (*21−24*). Various amounts of volatile extract and components were added to 2 mL of a 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 then the vial 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. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology. The quantitative analysis of hexanal was conducted according to an internal standard method (*25*). All analyses were carried out in triplicate. A 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) 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 4 °C/min and held for 10 min.

**Conjugated Diene Assay.** Measurements of conjugated diene hydroperoxides in methyl linoleate were carried out according to the procedures previously described (*26*). Various concentrations of each volatile extract (10, 20, 50, 100, and 200 μg/mL) dissolved in dichloromethane (volume of extract with dichloromethane was 284 μL) were added to methyl linoleate (1 g), and dichloromethane was removed under pure nitrogen purging (20 s). Oxidation of methyl linoleate in screw-capped amber vials (15 mL) was carried out at 40 °C in a shaker water bath (Dubnoff model, Precision Scientific, Chicago, IL). An aliquot of sample (10 mg) was taken at 24-hour intervals and dissolved in 5 mL of 2,2,4-trimethylpentane (isooctane) for spectrometric measurement (HP 8452A diode array UV spectrophotometer) of conjugated diene absorption at 234 nm. The spectrophotometer was set to zero with 2,2,4-trimethylpentane. All analyses were carried out in triplicate. Results were calculated as mmol MeLo hydroperoxides per kg of methyl linoleate with a molar absorptivity of 26,000 (*27*).

## RESULTS AND DISCUSSION

**Measurement of Total Volatile Components in Extracts.** The yields of total volatile components (relative to the dried herbs and spices) for thyme, basil, rosemary, chamomile, lavender, and cinnamon were 1.24 ± 0.14, 1.05 ± 0.1, 0.087 ± 0.038, 0.45 ± 0.16, 1.06 ± 0.09, and 1.08 ± 0.09% (w/w), respectively. The mass of total volatile components from thyme, basil, rosemary, chamomile, lavender, and cinnamon were 246 ± 27, 209 ± 20, 17 ± 7.6, 93 ± 32, 212 ± 17, and 215 ± 17 mg, respectively. The values are mean ± standard deviation ($n$ = 4).

**Antioxidant Activities of Volatile Extracts Isolated from Thyme, Basil, Rosemary, Chamomile, Lavender and Cinnamon in the Aldehyde/Carboxylic Acid Assay.** The aldehyde/carboxylic acid assay is a relatively simple assay compared to typical assays for antioxidant activity of an individual chemical or a group of chemicals. Volatile chemicals dissolved in organic solvent (non- or less-polar phase) can be easily measured for their antioxidant potential by this assay. In addition, this assay is appropriate to assess long-term antioxidant potential of natural antioxidants because aldehyde oxidation is monitored over 40 days. This assay has been used to examine antioxidant activity of natural aroma extracts isolated from plant sources (*21−24*, *28*).

**Figure 1** shows the amounts of hexanal remaining in samples over a period of 40 days. GC peak area ratios were calculated by dividing the GC peak area of hexanal by the GC peak area of the internal standard, undecane. Antioxidant activities of the known antioxidants BHT and α-tocopherol were used to confirm the testing system. All extracts exhibited dose-dependent inhibitory activity between concentration (10−500 μg/mL) and antioxidant activity. After 30 days, control samples exhibited over 95% oxidation of hexanal to hexanoic acid. In control samples, the rate of hexanoic acid formation was slow during the first 10 days but increased rapidly after 10 days. Hexanal was completely oxidized to hexanoic acid on and after 40 days. At all concentrations of thyme, almost 100% hexanal remained in samples, which meant the thyme extract possesses potent antioxidant activity against hexanal oxidation (**Figure 1a**). The percentage of hexanal remaining in solutions treated with different amounts of volatile extracts, α-tocopherol, and BHT is shown in **Table 1**. The inhibitory effect of the extracts on formation of hexanoic acid was in the following descending order: thyme > basil> rosemary > chamomile > lavender and cinnamon. At a concentration of 500 μg/mL, thyme, basil, rosemary, chamomile, lavender, and cinnamon extracts inhibited hexanal oxidation by 100, 100, 59, 50, 6, and 5% respectively, for 40 days. For the volatile extract of thyme, the percentages of hexanal remaining were 98−100% at concentrations from 10 to 500 μg/mL. The volatile extract of basil demonstrated 8, 24, 96, 99, 100, and 100% at concentrations of 10, 20, 50, 100, 200, and 500 μg/mL, respectively. The inhibitory effect of thyme

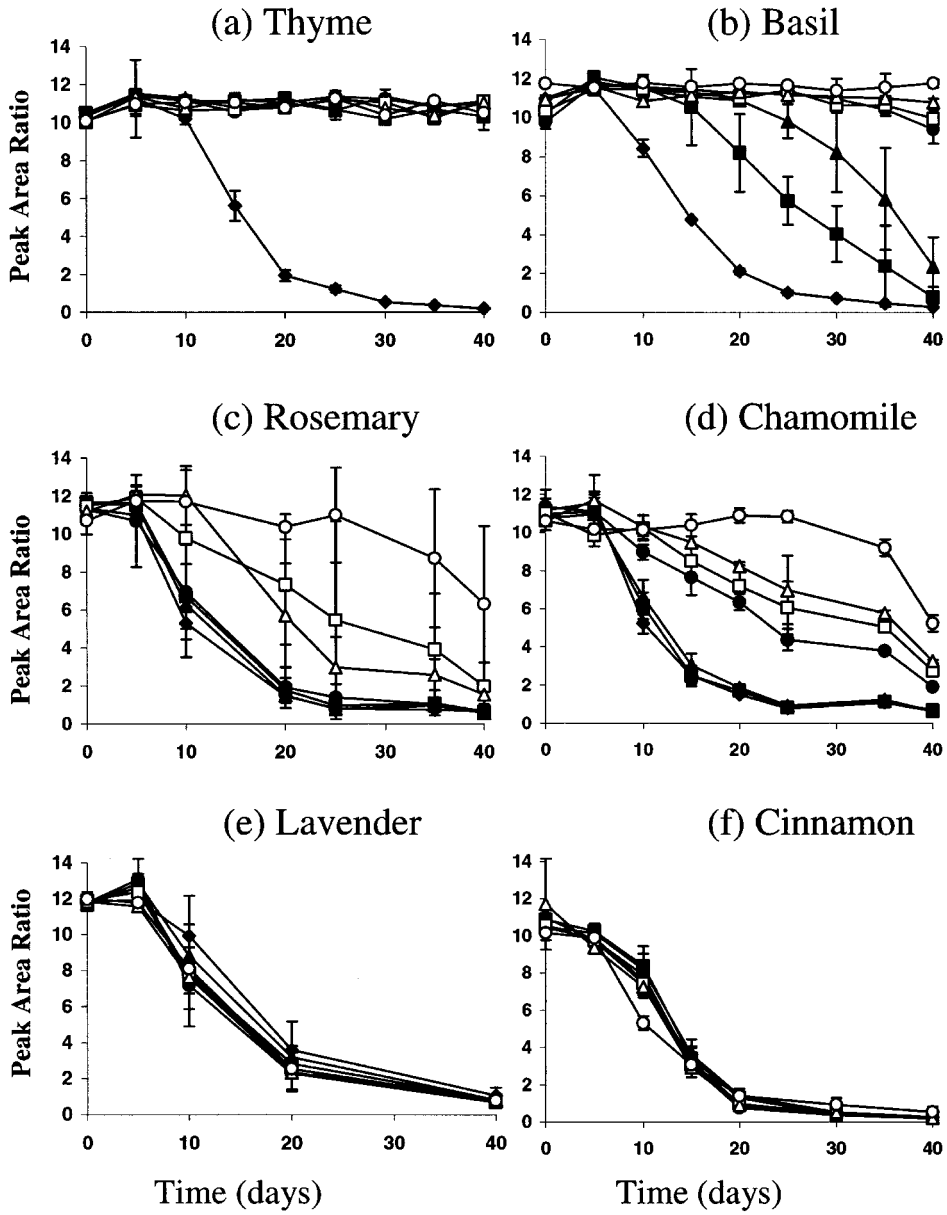


**Figure 1.** Relative amounts of hexanal remaining in solutions treated with different amounts of total volatile extracts isolated from thyme (a), basil (b), rosemary (c), chamomile (d), lavender (e), and cinnamon (f) at concentrations indicated: -◆- control; -■- 10 μg/mL; -▲- 20 μg/mL; -●- 50 μg/mL; -□- 100 μg/mL; -△- 200 μg/mL; and -○- 500 μg/mL.

**Table 1.** Percentage of Hexanal Remaining in Solutions Treated with Different Amounts of Volatile Extracts, α-Tocopherol, and BHT throughout a Storage Period of 40 Days

| conc. (μg/mL) | inhibitory effect[a] (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | thyme | basil | rosemary | chamomile | lavender | cinnamon | α-tocopherol | BHT |
| 0 | 2 ± 0.2 | 2 ± 1.6 | 6 ± 3.3 | 5 ± 0.4 | 6 ± 3.4 | 3 ± 0.4 | 2 ± 0.2 | 5 ± 2.0 |
| 10 | 98 ± 7.3 | 8 ± 5.2 | 5 ± 0.8 | 6 ± 0.3 | 7 ± 0.7 | 2 ± 0.2 | 89 ± 0.6 | 99 ± 0.8 |
| 20 | 99 ± 4.1 | 24 ± 14.9 | 5 ± 0.4 | 6 ± 0.6 | 7 ± 1.2 | 2 ± 0.6 | 92 ± 2.2 | 99 ± 0.7 |
| 50 | 99 ± 0.5 | 96 ± 10.6 | 5 ± 0.6 | 17 ± 1.3 | 6 ± 1.5 | 2 ± 0.5 | 97 ± 2.3 | 100 ± 0.6 |
| 100 | 100 ± 1.3 | 99 ± 1.0 | 17 ± 10.6 | 25 ± 1.0 | 6 ± 2.1 | 2 ± 0.6 | 98 ± 0.2 | 100 ± 0.1 |
| 200 | 100 ± 5.7 | 100 ± 1.0 | 14 ± 2.2 | 31 ± 0.3 | 6 ± 0.8 | 2 ± 0.3 | 99 ± 1.3 | 100 ± 0.1 |
| 500 | 100 ± 4.8 | 100 ± 3.2 | 59 ± 19.2 | 50 ± 4.9 | 6 ± 0.7 | 5 ± 2.8 | 100 ± 1.5 | 100 ± 0.1 |

[a] The values are mean ± standard deviation ($n = 3$).

at 10 μg/mL and basil at 50 μg/mL were comparable to that of BHT, which showed over 99% at concentrations varying from 10 to 500 μg/mL. At concentrations of 10, 20, and 50 μg/mL, the antioxidant activity of thyme was higher than that of α-tocopherol, which inhibited hexanal oxidation by 89, 92, and 97%, respectively.

**Antioxidant Activities of Volatile Extracts Isolated from Thyme, Basil, Rosemary, Chamomile, Lavender, and Cinnamon in the Conjugated Diene Assay.** In the conjugated diene assay, the amount of total conjugated diene hydroperoxides produced from polyunsaturated lipids such as methyl linoleate upon oxidation was determined quantitatively by the absorption

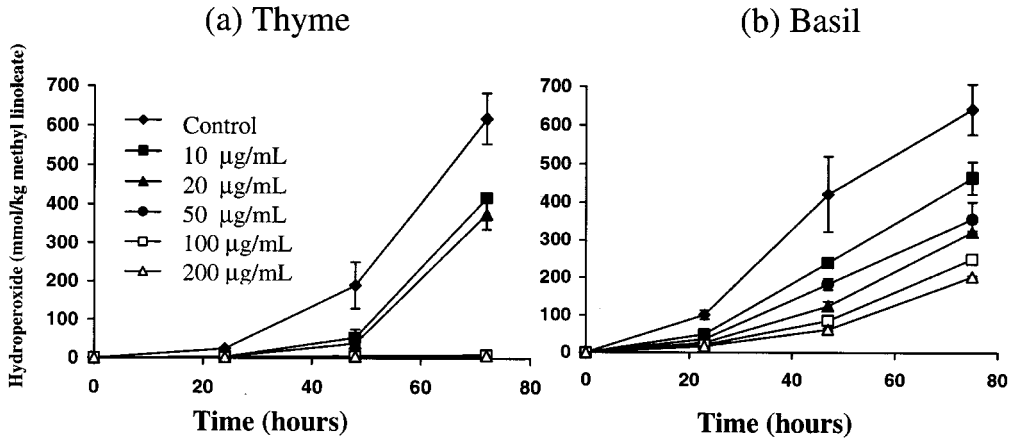


**Figure 2.** Effect of volatile extracts isolated from thyme (a) and basil (b) on the oxidation of methyl linoleate.

**Table 2.** Inhibitory Effects (%) of Various Concentrations of Volatile Extracts, α-Tocopherol, and BHT toward Conjugated Diene Formation from Methyl Linoleanate

| conc. | inhibitory effect[a] (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (μg/mL) | thyme | basil | rosemary | chamomile | lavender | cinnamon | α-tocopherol | BHT |
| 10 | 33 ± 1.0 | 28 ± 6.6 | 0 ± 0.4 | 10 ± 6.1 | 0 ± 0.7 | 0 ± 0.2 | 99 ± 0.4 | 100 ± 0.0 |
| 20 | 39 ± 6.3 | 50 ± 0.5 | 12 ± 3.6 | 25 ± 6.8 | 3 ± 1.2 | 0 ± 0.6 | 99 ± 0.1 | 100 ± 0.0 |
| 50 | 99 ± 0.6 | 45 ± 7.1 | 23 ± 0.6 | 24 ± 2.7 | 0 ± 1.5 | 1 ± 0.5 | 98 ± 0.3 | 100 ± 0.1 |
| 100 | 99 ± 0.1 | 61 ± 1.6 | 28 ± 5.0 | 28 ± 1.3 | 2 ± 2.1 | 2 ± 0.6 | 98 ± 0.1 | 100 ± 0.1 |
| 200 | 99 ± 0.2 | 68 ± 0.4 | 38 ± 2.0 | 31 ± 14 | 5 ± 0.8 | 2 ± 0.3 | 96 ± 0.9 | 100 ± 0.1 |

[a] The values are mean ± standard deviation ($n = 3$).

maximum at 234 nm (*29*). This method has been applied to measure antioxidant activity of plant and wine extracts (*26*, *30*, *31*), flavonoids (*32*), and tocopherols (*12*). In this assay, no initiators or metal-catalysts such as ferrous chloride or hydrogen peroxides were added for initiating and accelerating autoxidation, because the main oxidation phase is not an initiation phase but a propagation phase (*32*). The inhibitory effect in this assay is due to added volatile components because there is no antioxidant (such as tocopherols or carotenoids) in methyl linoleate itself. **Figure 2** illustrates the effect of volatile extracts isolated from thyme (a) and basil (b) on the oxidation of methyl linoleate. The values are mean ± standard deviation ($n = 3$). The formation of conjugated diene hydroperoxides in oxidizing methyl linoleate was the most effectively inhibited by thyme. The volatile extracts of thyme and basil inhibited hydroperoxide formation in methyl linoleate dose-dependently, at concentrations from 10 to 200 μg/mL. Inhibitory effects (%) of various concentrations of volatile extracts, α-tocopherol, and BHT on the formation of conjugated diene hydroperoxides from MeLo are shown in **Table 2**. The effectiveness of the various volatile extracts on MeLo oxidation was in the following descending order: thyme > basil > rosemary and chamomile > lavender and cinnamon. The trend of inhibition of MeLo hydroperoxides was quite similar to that of hexanal oxidation. The inhibitory effects of thyme, basil, rosemary, chamomile, lavender, and cinnamon were 99, 68, 38, 31, 5, and 2%, respectively, at a concentration of 200 μg/mL. The inhibitory effect of thyme was similar to that of α-tocopherol or BHT at concentrations from 50 to 200 μg/mL. At concentrations from 10 to 200 μg/mL, BHT and α-tocopherol inhibited MeLo oxidation by 100% and 96−99%, respectively. The inhibitory effect of thyme was weaker than that of α-tocopherol at lower doses (10 and 20 μg/mL), but it was more effective at higher doses (50, 100, and 200 μg/mL). It is hard to rationalize the decrease in inhibitory effect of α-tocopherol at higher doses in this assay. A similar phenomenon was observed when α-tocopherol was added to squalene (*33*) and to lecithin (*34*) upon UV radiation. The data suggests that α-tocopherol might be degraded into many products (including malonaldehyde and glyoxal) at higher concentrations. However, further investigation is necessary to clarify this phenomenon. In this assay, volatile extracts of lavender and cinnamon did not display any antioxidant activity similar to their results in the aldehyde/carboxylic assay.

The strong antioxidant activity of the volatile extract of thyme is mostly due to its main volatile components, thymol and carvacrol (*35*). The antioxidant activities of thymol and carvacrol have been reported using various testing systems (*16*, *18*, *22*, *36*). In the case of the volatile extract of basil, eugenol, reported as a major volatile component, probably contributes significantly to the strong antioxidant activity of the extract (*35*). Eugenol showed high antioxidant activity in the aldehyde/carboxylic acid assay (*21*). The antioxidant activities of the volatile extracts of thyme and basil in the conjugated diene assay were lower than those in the aldehyde/carboxylic acid assay at the same doses. This can be explained with different amounts of the substrates hexanal and methyl linoleate that were used in the assays. Because concentration of methyl linoleate (2.4 mmol/mL) in the conjugated diene assay is quite high compared with that of hexanal (30 μmol/mL) in the aldehyde/carboxylic acid assay, inhibition for substrate oxidation requires a higher dose of antioxidants.

The potent antioxidant activity of rosemary has been thoroughly investigated (*12*, *16*, *19*, *37*). However, the antioxidant activity of the volatile extract of rosemary was much lower than that of thyme or basil in the present study. Extracts of spices and herbs are traditionally obtained by steam distillation (the extract is called an "essential oil") or by extraction with solvents (such as alcohols, hexane, and acetone) and consequent solvent removal by evaporation (*15*). The major components in the volatile extract of rosemary—1,8-cineole and borneol—are different from those in the solvent extract—carnosol and carnosic acid (*19*, *38*). Though carnosol and carnosic acid in rosemary

extract are potent antioxidants, 1,8-cineole and borneol have not shown high antioxidant activity (*12*).

In conclusion, volatile extracts of thyme and basil exhibited antioxidant activities in lipophilic systems, such as in the aldehyde/carboxylic acid assay and in the conjugated diene assay. The antioxidant activities of these volatile extracts, as well as BHT and α-tocopherol, were consistent in the two assays. The results indicate that the antioxidant activities of thyme and basil extracts are as potent as those of the known antioxidants BHT and α-tocopherol. It is very important to develop and select a reliable and robust assay for accurately measuring antioxidant activity of natural antioxidants. Antioxidant behavior is usually more complicated when evaluated in hydrophilic systems than in lipophilic systems (*39*). Therefore, the volatile antioxidants that are non- or less-polar compounds might be more accurately assessed for their antioxidant activity by lipophilic systems than by hydrophilic systems. Herbs and spices possessing high antioxidant activity, such as thyme and basil, would not only be very useful to maintain food freshness, flavor, taste, and color, but also to alleviate diseases by preventing oxidative deterioration.

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