Biological Activities of Low-Molecular Weight Compounds Found in Foods and Plants

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ABSTRACT

Among 27 essential oils tested, 11 oils exhibited strong antioxidant activity (95 - 100%) with dose response, which was comparable to that of α-tocopherol. The essential oils showing such activity were ylang-ylang, rose, parsley seed, jasmine, celery seed, basil, anise star, clove leaf, bergamot, thyme, and cinnamon leaf. Among antioxidant components found in these essential oils, eugenol, benzaldehyde, and thymol exhibited comparable activity to that of known natural antioxidant α-tocopherol. Benzyl alcohol, maltol, γ-butyrolactone, and terpinene-4-ol had moderate antioxidant activity. Low-molecular weight heterocyclic compounds, which are the chemicals responsible for cooked flavor, comprise one fourth of the over 400 volatile compounds identified in cooked foods. Among heterocyclic compounds found in the Maillard reaction products (MRP), furans and pyrroles exhibited relatively potent antioxidative activities. Moreover, these heterocyclic compounds showed significant synergic effects. Although the activity of each low-molecular weight compound is not as strong as the known antioxidant, α-tocopherol or BHT, the total activity of numerous compounds might be comparable to those of known antioxidants because tremendous numbers of these chemicals are present either in essential oils or in MRP. Therefore, constant and consistent consumption of foods and beverages containing these low molecular weight antioxidants may prevent diseases caused by oxidative damage.

Key words: Low-molecular weight compounds, essential oils, antioxidants, Maillard reaction products, heterocyclic compounds

INTRODUCTION

The definition of low-molecular weight compounds varies among scientists. There is no specific molecular weight range to define low-molecular weight compounds. Therefore, many names have been used to define low-molecular weight compounds, including volatile chemicals, low boiling point chemicals, organic solvent extractable chemicals, aroma or fragrance chemicals, and flavor chemicals as well as plant essences. These words have been used in many articles according to the nature of the studies. In this report, volatile and less-volatile chemicals were obtained according to the experimental diagrams shown in Figure 1.

There are two sources of volatile chemicals. One is from natural plants, the volatile chemicals of which are commonly called essential oils. The other is from foods (mainly cooked foods), the volatiles of which are commonly called food flavors.

Essential oils are concentrated hydrophobic liquids obtained from plants by steam distillation (lavender, peppermint, eucalyptus, *etc*.), organic solvent extraction (jasmine), or expression (citrus peel). Essential oils have

been studied mainly from the aspects of flavor and fragrance chemistry until recently. The recent discovery of the antimicrobial and antioxidant potential of these essential oils, however, has extended their use as natural preservatives for prolonging the shelf life of food products[1,2]. For example essential oils isolated from medicinal plants such as chamomile, clove, and eucalyptus have anti-microbial and antioxidant properties[1,3]. Volatile chemicals or aroma chemicals present in essential oils have been widely used in aromatherapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor[4]. Antioxidant activities of aroma chemicals, with potential medical applications, have also been discovered lately. The high antioxidant activity found in essential oils, such as ylang-ylang, rose, and jasmine oils, has been determined[5]. The discovery of the antioxidant activity of essential oils, suggesting that essential oils possess great health benefits[6], has gained considerable attention among researchers[7]. Moreover, due to recent safety concerns over synthetic compounds, there has been increasing interest in the use of natural plant substances, including essential oils, for food and

medicinal therapy.

Low-molecular weight flavor compounds have long been considered to play a secondary role in foods, after nutrition. They are known to play an important role in the palatability of foods. It is also well known that heat treatment produces preferable cooked odors and attractive colors, resulting in increased palatability. Many volatile flavor chemicals have been isolated and identified in cooked foods. Among the many volatile flavor chemicals identified in cooked foods, numerous heterocyclic compounds-which comprise one fourth of the volatile compounds identified in foods[8]-have been reported as the chemicals responsible for characteristic cooked flavors[9,10]. It is also well known that these chemicals are formed by the so-called Maillard browning reaction and many studies have been performed using this system to investigate subjects related to the culinary appeal of cooked foods and beverages (including flavors, tastes, colors and textures), as well as their biological properties (mutagenicity, carcinogenicity, and antioxidant)[11].

Antioxidants have received much attention among food scientists as inhibitors of lipid peroxidation. Lipid peroxidation and DNA damage caused by reactive oxygen species are associated with various diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, malaria, arthritis, and aging[12,13]. Synthetic antioxidants, such as BHA and BHT, have been used to maintain the quality of foods[14]. However, BHA and BHT have been reported to demonstrate carcinogenic effects[15]. Therefore, in addition to natural antioxidants found in plants (such as vitamins, polyphenols, and flavonoids), volatile chemicals including heterocyclic flavor chemicals found in cooked foods and beverages have begun to receive attention as nontoxic and safe antioxidants.
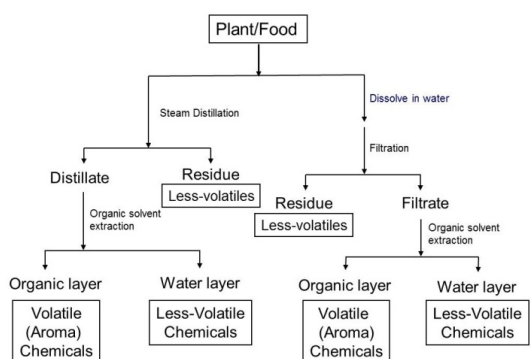

Figure 1. Experimental diagram for preparation of volatile and less-volatile chemicals from plant and food.

MATERIALS AND METHODS

I. *Chemicals and Materials*

Eucalyptol, p-cymene, benzyl alcohol, benzyl aldehyde, terpinene-4-ol, thymol, hexanal, hexanoic acid, undecane, N-methylhydrazine (NMH), 2-methylpyrazine (2-MP), sodium dodecyl sulfate (SDS), 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). Asparagine, cysteine, glycine, histidine, methionine, phenylalanine, threonine, tryptophan, butylated hydroxytoluene (BHT), and hexanal were purchased from Sigma Chemical Co. (St. Louis, MO). Undecane was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Dried clove buds [*Syzygium aromaticum* (L.) Merr. et Perry] were purchased from a local market. Fresh eucalyptus leaves (*Eucalyptus polyanthemos, E. globulus, E. perriniana*) were purchased from Faylor's Eucalyptus Farms (Temecula, CA). All other essential oils were received as a gift from International Flavors and Fragrances Inc. (Union Beach, NJ) and used without any pre-treatment.

II. *Sample Preparations from Plants*

Plant samples (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 (95 mmHg). The distillate (900 mL) was extracted with 100 mL dichloromethane using a liquid-liquid continuous extractor 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 approximately 1 mL, and then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.6 mL.

III. *Sample Preparations from MRP*

D-Glucose (0.05 mol) and 0.05 mol each of the different amino acids were dissolved in 90 mL of deionized water. The pH of the solution was adjusted to 9 with 6 N NaOH. The solution was then brought to a final volume of 100 mL with deionized water. The solution was heated at 100°C for 16, 24, or 40 h and the reaction mixture was cooled to room temperature. The solution was extracted with 80 mL of dichloromethane using a liquid-liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate overnight. After removal of the sodium sulfate, the dichloromethane extract was concentrated to 0.3 mL by a rotary flash evaporator and subsequently by a purified nitrogen stream to 0.1 mL.

IV. *Determination of Volatile Chemicals in the Samples*

Volatile chemicals in samples obtained from plants and a *D*-glucose/amino acid Maillard reaction system were identified by comparison with the Kovats gas chromatographic retention index I and by the MS fragmentation pattern of each component compared with those of authentic chemicals. 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 m) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) was used for a GC. The linear velocity of the helium carrier gas was 30 cm/sec. 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.

V. *Aldehyde/Carboxylic Acid Antioxidant Assays*

The inhibitory effect of samples and components toward oxidation of aldehyde to carboxylic acid was measured[16]. Various amounts of samples and 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 seconds) 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. All tests were performed in triplicate.

The quantitative analysis of hexanal was conducted according to an internal standard method. An HP model 6890 GC equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 m) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and an NPD was used for analysis of 1-MP.

The antioxidant activity was calculated according to the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Amount of hexanal in blank} -}{\text{Amount of hexanal in blank}} \times 100$$

VI. *1,1- Diphenyl-2-picryhydrazyl Radical (DPPH) Antioxidant Assay*

The antioxidant activity of the extracts was measured by a previously reported method, with slight modification[16]. A sample (200 µL) was added to 600 µL of ethanol solution of DPPH (0.3 mM). For a blank, only 200 µL of the extraction solvent was added to the DPPH solution. The absorbance was measured at 517 nm after 30 min of incubation at 37°C using a Hewlett-Packard 8452A diode array spectrophotometer. The antioxidant activity was calculated according to the following equation:

Antioxidant activity (%)=

$$\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

All tests were performed in triplicate.

RESULTS AND DISCUSSION

I. *Antioxidant Activity of Essential Oils and Their Components*

Essential oils were examined for antioxidant activity by aldehyde/carboxylic acid assay. The oils tested are ylang-ylang, rose, parsley seed, jasmine, celery seed, juniper berry, patchouli, angelica seed, lavender, ginger, sandalwood, chamomile, peppermint, basil, anise star, clove leaf, bergamot, thyme, cinnamon leaf, bitter orange, lemon, eucalyptus, rosemary, sage, and aloe vera. The aldehyde/carboxylic acid assay is convenient for evaluating the effects of antioxidants against slow oxidation phenomena occurring over prolonged periods of time, such as the shelf life of foods. Also, the solution system used in this assay is organic. Therefore, organic samples, such as essential oils and most volatile chemicals, can be examined without use of a surfactant[16]. Among 27 essential oils tested, 11 oils exhibited strong antioxidant activity (95 - 100%) at the level of 200 L/mL, which was comparable to that of the well-known natural antioxidant α-tocopherol. The essential oils showing such activity were ylang-ylang, rose, parsley seed, jasmin, celery seed, basil, anise star, clove leaf, bergamot, thyme, and cinnamon leaf. The essential oils of chamomile, rosemary, juniper berry, patchouli, and angelica seed showed moderate (50 - 70%) antioxidant activity. Cinnamon, lavender, ginger, sandalwood, peppermint, bitter orange, lemon, eucalyptus, rosemary, sage and aloe vera had only slight activity (5 - 20%). Figure 2 shows the antioxidant activity of representative oils from each group at four different levels of concentration. Clear dose responses were observed in all oils. Jasmine oil exhibited antioxidant activity by 60% at the level of 100 L/mL, whereas α-tocopherol showed 100% at the same level. However, jasmine oil possessed comparable antioxidant activity to α-tocopherol at the level of 200 L/mL.
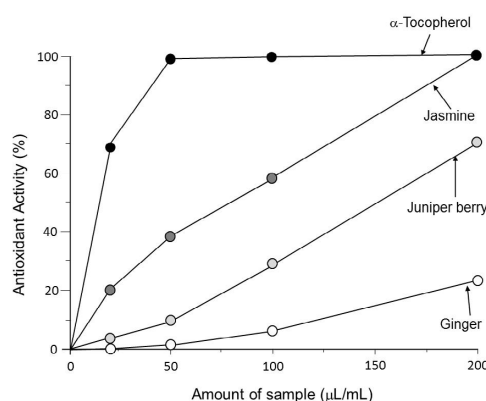

Figure 2. Antioxidant activity of representative essential oils tested by aldehyde/carboxylic acid assay at the level of 200 µL/mL after 30 days.
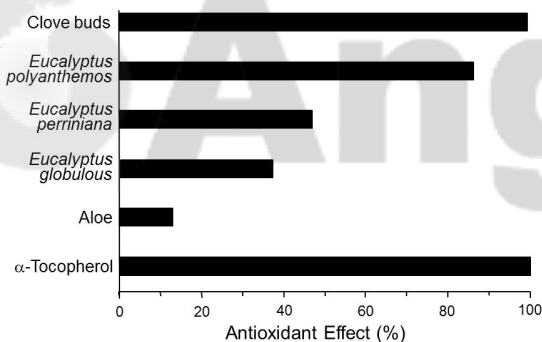
Figure 3. Antioxidant activity of essential oils prepared in our laboratory tested by aldehyde/carboxylic acid assay at the level of 200 μL/mL after 30 days.

Figure 3 shows the antioxidant activity of essential oils prepared in our laboratory tested by aldehyde/carboxylic acid assay at the level of 200 μL/mL after 30 days. Clove bud oil exhibited strong antioxidant activity at the same level as the clove leaf oil mentioned above. Eucalyptus oil did not show appreciable antioxidant activity but *Eucalyptus polyanthemos* leaf oil prepared in our laboratory showed 90% antioxidant activity at the level of 200 L/mL. In addition, two other eucalyptus leaf oils (*perriniana and plobulos*) exhibited moderate antioxidant activity (50 and 40%, respectively). These results indicate that the antioxidant activity of eucalyptus oil varies among species. It is interesting that the oil from the well-known medicinal plant, aloe, exhibited only slight antioxidant activity. On the other hand, aloe oil possessed strong anti-inflammatory activity[17].

Table 1 shows the major components and known antioxidant components in selected essential oils.

Figure 4 shows the antioxidant activity of typical essential oil components tested by aldehyde/carboxylic acid assay. Eugenol, benzaldehyde, and thymol exhibited 100% antioxidant activity at the level of 200 L/mL after 30 days. In particular, eugenol and thymol showed comparable activity to that of known natural antioxidant α-tocopherol at the level of 50 L/mL. Benzyl alcohol, maltol, γ-butyrolactone, and terpinene-4-ol had moderate antioxidant activity.

II. *Antioxidant Activity of Extracts from Maillard Reaction Systems and Their Components*

Figure 5 shows the antioxidant activity of the extracts from MRP tested by aldehyde/carboxylic acid assay at the level of 200 μL/mL for 30 days. The extracts from the reaction mixture of *D*-glucose/tyrosine, methionine, and asparagine exhibited 100% antioxidant activity, which was comparable to that of BHT. The extracts from the reaction mixture of *D*-glucose/glycine, tryptophan, histidine, or phenylalanine showed 90 - 95% antioxidant activity. The extracts from *D*-glucose/cysteine or threonine possessed moderate antioxidant activity, nearly 50%.

Table 1. Major components and known antioxidant compounds in selected essential oils

Essential oil	Major components (GC area%)	Antioxidant (GC area%)
Anise star	anethole (88.00)	terpinene-4-ol (0.14)
Basil	linalool (53.36)	eugenol (13.32)
Bergamot	d-limonene (38.46)	p-cymene (0.49)
Cinnamon leaf	β-trans-caryophyllene (53.22)	p-cymene-8-ol (0.25)
Clove leaf	eugenol (76.51)	eugenol (76.51)
Thyme	p-cymene (44.84)	thymol (22.83)
Ylang-ylang	germacrene (19.10)	cinnamyl acetate (4.81)
Rose	citronelol (34.2)	eugenol (2.20)
Parsley seed	myristicin (44.00)	apiol (12.10)
Jasmine	benzyl acetate (22.90)	benzyl alcohol (6.60)
Juniper berry	α-pinene (33.70)	terpinene-4-ol (1.70)

III. *Antioxidant Activity of Extracts from Maillard Reaction Systems and Their Components*

Figure 5 shows the antioxidant activity of the extracts from MRP tested by aldehyde/carboxylic acid assay at the level of 200 L/mL for 30 days. The extracts from the reaction mixture of *D*-glucose/tyrosine, methionine, and asparagine exhibited 100% antioxidant activity, which was comparable to that of BHT. The extracts from the reaction mixture of *D*-glucose/glycine, tryptophan, histidine, or phenylalanine showed 90 - 95% antioxidant activity. The extracts from *D*-glucose/cysteine or threonine possessed moderate antioxidant activity, nearly 50%.
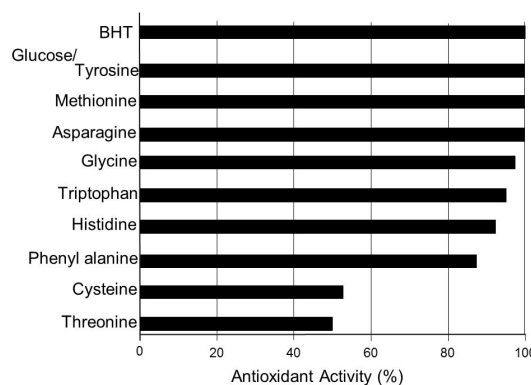

Figure 5. Antioxidant activity of the extracts from MRP tested by aldehyde/carboxylic acid assay at the level of 200 μL/mL for 30 days.

Figure 6 shows the antioxidant activity of the extracts from MRP tested by DPPH assay. In this assay, only the extract from *D*-glucose/histidine showed 100% antioxidant activity, which was comparable to that of BHT, at the level of 200 L/mL. The extracts from *D*-glucose/asparagine or cysteine also exhibited strong antioxidant activity of over 80%. All extracts showed dose response antioxidant activity. The results obtained from the two different assays exhibited some differences. For example, the extract from D-glucose/tyrosine showed

strong antioxidant activity in the aldehyde/carboxylic with the DPPH assay. These results are due to the different nature of the two assays. The aldehyde/carboxylic acid assay determines a level of hydroxyl radical scavenging activity. On the other hand the DPPH assay examines the proton donating activity [16]. However, the majority of the extracts showed over 70% antioxidant activity in both assays, suggesting that the MRP contain some volatile antioxidants.
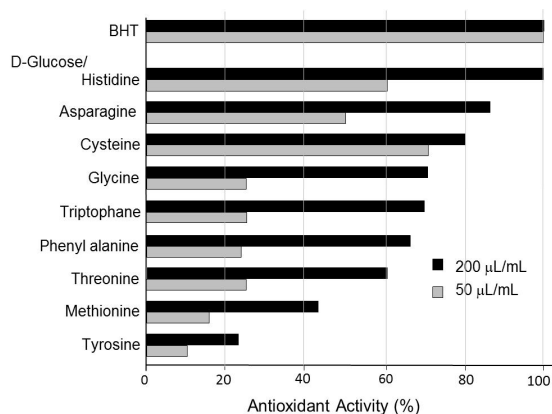

Figure 6. Shows the antioxidant activity of the extracts from MRP tested by DPPH assay.

There have been numerous reports on the biological activities of the MRP, including-mutagenicity, carcinogenicity, and antioxidant effects[11]. For example, one recent study reported that melanoidin isolated from MRP possessed antiproliferative activity[18]. Among biological studies on MRP, antioxidants formed have been studied most intensively. Volatile fractions extracted from Maillard reaction systems consisting of *D*-glucose and different amino acids exhibited potent antioxidant activity[19,20]. Many researchers have been trying to pinpoint the chemicals responsible for the antioxidant activity of MRP. Some MRP, such as such as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, maltol, and 5-hydroxy methylfurfural, were reported to possess appreciable antioxidant activities[21]. As mentioned above, the greatest number of heterocyclic compounds has been identified as chemicals possessing characteristic cooked flavors among volatile compounds found in MRP—In decreasing order, the approximate numbers of derivatives of volatile heterocyclic compounds found in MRP are furans (approximate number, 140) > pyrazines (110) > pyrroles (80) > oxazoles (40) > thiophenes (35) > thiazoles (30) > pyridines (25 > imidazoles [10,20,22]. Later, the antioxidant activities of these volatile heterocyclic compounds were discovered [22-27].

Figure 7 shows the antioxidant activity of representative heterocyclic compounds found in MRP tested by aldehyde/carboxylic acid assay. A radical specie tends to be attracted to an electron rich ring carbon.
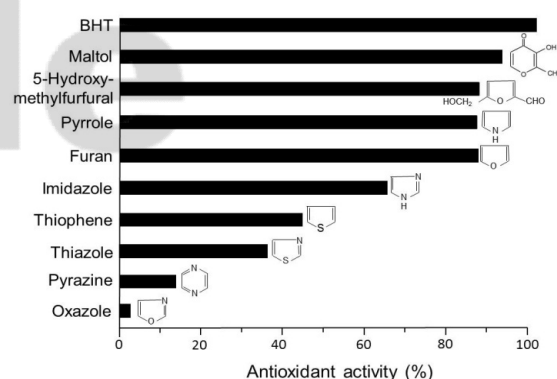

Figure 7. The antioxidant activity of representative heterocyclic compounds found in MRP tested by aldehyde/carboxylic acid assay.

Figure 8 shows the proposed hydroxyl radical scavenging mechanisms by N-methylpyrrole. In fact, when N-methyl pyrrole was treated with Fenton's reagent, hydroxyl radical adducts, 1,5-dihydro-1-methyl-2H-pyrrole-2-one and 1-methyl 2,5 pyrrolidine dione were produced[26], suggesting that pyrroles scavenge a hydroxyl radical. Because the hydroxyl radical scavenging activity is dependent on the electron density of ring carbons, the nature of the substituent changes the scavenging activity. When a substituent was added at the number 2 ring carbon, the antioxidant activities of furan, thiophene, thiazole and pyridine were increased by an electron donating alkyl group, whereas their activity was reduced by an electron withdrawing acetyl group. Both alkyl and acetyl groups increased the antioxidant activity of pyrrole. Both substituents deleted the antioxidant activity of unstubstituted imidazole completely.

The results from the present study indicate that MRP contain many volatile antioxidants, including heterocyclic compounds. Although the activity of each component is not as strong as the known antioxidant, BHT, the total activity of numerous compounds might be comparable to those of known antioxidants. Moreover, these heterocyclic compounds exhibited significant synergic effects. When the antioxidant activity of N-methylpyrrole and a mixture of N-methylpyrrole/pyridine (50/50) were tested at the level of 100 μL/mL by aldehyde/carboxylic acid assay, N-Methylpyrrole exhibited only 10% antioxidant activity after 80 days, whereas a mixture of N-methylpyrrole/pyridine showed 100% antioxidant activity under the same conditions. Figure 9 shows the proposed mechanisms of the synergic effect, suggesting that one compound produces a radical with higher electron density on the other compound, and then a chain reaction like phenomenon occurs to increase the total antioxidant activity.

CONCLUSIONS

Naturally occurring antioxidants that have been reported to date have generally been less volatile chemicals, such as polyphenols, glycosyl flavonoids, and
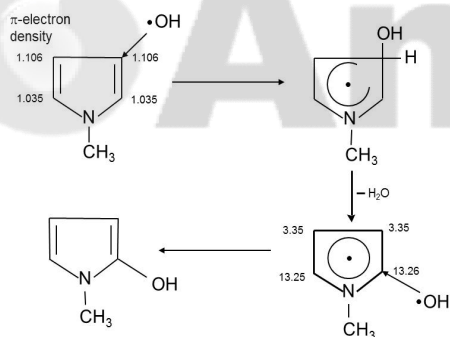
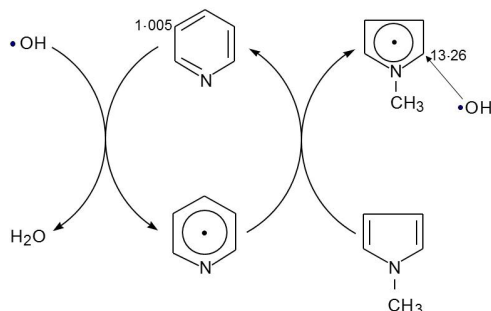**Figure 8.** Proposed hydroxyl radical scavenging mechanisms by N-methylpyrrole.

Figure 9. Proposed mechanisms of the synergic effect between N-methylpyrrole and pyridine.

anthocyanines, as well as melanoidin from the MRP. The present study demonstrates that volatile aroma chemicals in essential oils and volatile flavor chemicals in MRP also possess antioxidant activity. Some essential oils, such as clove bud, thyme, and jasmine, possess potent antioxidant activity, comparable to that of α-tocopherol. Volatile antioxidants found in essential oils were mainly phenols, such as eugenol and thymol, and related compounds such as benzyl alcohol and terpinene-4-ol. Various volatile extracts obtained from the Maillard reaction systems also showed strong antioxidant activity, comparable to that of BHT. Volatile heterocyclic compounds present in the extracts, including pyrroles, furans, and thiophenes, were found to have appreciable antioxidant activity and may be the chemicals responsible for the antioxidant activity of volatile MRP. The antioxidant activity of these volatile chemicals is not as strong as known antioxidants such as a-tocopherol or BHT. However, tremendous numbers of these chemicals are present either in essential oils or in MRP. Therefore, constant and consistent consumption of foods and beverages containing these volatile antioxidants may prevent diseases caused by oxidative damage.

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