Volatile Antioxidants Produced from Heated Corn Oil/Glycine Model System

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The headspace volatiles collected from a mixture consisting of corn oil and glycine heated at 180 °C were found to inhibit the aldehyde/carboxylic acid turnover using a newly developed antioxidation test. Among six column chromatographic fractions of the headspace extract, the fraction that contained 1-methylpyrrole and several of its 2-alkyl homologues was found to show a significant antioxidative effect. Authentic 1-methylpyrrole also exhibited antioxidative activity that increased with the presence of pyridine. The antioxidative strength (weight basis) of 1-methylpyrrole was comparable to that exhibited by α-tocopherol.

INTRODUCTION

It has been known for many years that heating improves food stability. For example, in the 1940s, the stabilities of dairy products were improved by heat treatment (El-Rafey et al., 1944; Josephson and Dahle, 1945; Lea et al., 1945; Findlay et al., 1946). Later, meat products, such as ground beef (Zipser and Watts, 1961; Huang and Greene, 1978) and turkey (Einerson and Reineccius, 1977, 1978), showed increased stability following heat treatment. Oils and fats are less susceptible to oxidation when they are mixed with whey powder, wheat flour, casein, starch, or amino acids and then heated at temperatures ranging from 100 to 300 °C (Lips, 1951; Janicek and Pokorny, 1961; Itoh et al., 1975; Kawashima et al., 1977; Dworschák and Szabó, 1986). These findings suggest that some active substances, such as antioxidants, are produced from food when heated.

Even though synthetic antioxidants are widely used as food additives, there is concern about their possible chronic and side effects in humans (Branen, 1975). This is why new antioxidants are being isolated from natural sources (Pratt and Hudson, 1990). In the present study, the antioxidative activity of a headspace extract prepared by heating a mixture of corn oil and glycine was investigated in a search for new natural antioxidants.

EXPERIMENTAL PROCEDURES

Materials. Corn oil was purchased from a local market. Glycine and butylated hydroxytoluene (BHT) were bought from Sigma Chemical Co. (St. Louis, MO). Reagent grade 1-pentanal, 1-hexanal, 1-pentanoic acid, 1-hexanoic acid, α-tocopherol, 1-methylpyrrole (MP), pyridine, nonadecane (ND), and 2,5-dimethylhexane (DMH) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All authentic samples were obtained from reliable commercial sources.

Headspace Sample. Corn oil (100 g) and 5 g of glycine were placed in a 500-mL two-neck round-bottom flask. The flask was connected to a simultaneous purging and solvent extraction apparatus (SPE) devised by Umano and Shibamoto (1987). The mixture was heated at 180 °C for 4 h while stirring with a magnetic stirrer. The headspace was purged with a purified air stream (flow rate 10 mL/min) into 250 mL of deionized water and simultaneously extracted with 50 mL of dichloromethane. The extract (organic phase) was concentrated to 2 mL by fractional distillation.

Column Chromatography (CC). The headspace sample was transferred to a 15 cm × 1 cm i.d. glass column packed with silica gel (Kieselgel 60, E. Merck, Darmstadt, Germany). The extract was developed with hexane and ethyl acetate (30-mL aliquots) into six fractions. Each fraction was concentrated to a final volume (2 mL) by fractional distillation and stored at −5 °C for subsequent experiments.

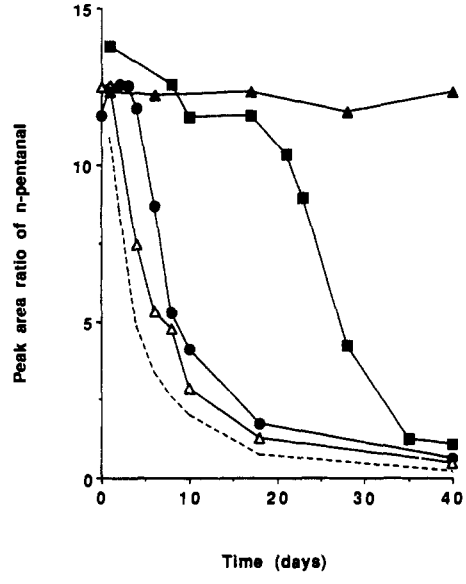

Figure 1. Antioxidative effect of various concentrations (μg/mL) of α-tocopherol on 1-pentanal: control (- - -); 5 (△); 10 (●); 50 (■); 100 (▲). Peak area ratio equals GC peak area of 1-pentanal divided by GC peak area of 2,5-dimethylhexane.

Antioxidation Test. A newly developed aldehyde/carboxylic acid test was used to assess the antioxidative activity of standard antioxidants such as α-tocopherol (5, 10, 50, and 100 μg/mL) and BHT (5, 10, 50, and 100 μg/mL); several amounts of the corn oil/glycine headspace extract (20, 100, and 500 μL); 500 μL of each CC fraction; and pure MP (50, 100, and 500 μg/mL) with or without 100 μg/mL of pyridine. These various extracts and chemicals were individually combined with a mixture of pentyl aldehyde and hexyl aldehyde (1000 and 3000 μg/mL, respectively) and placed in 5-mL vials. DMH and ND were added as gas chromatographic internal standards (100 μg/mL each). The mixtures were diluted with dichloromethane to total volumes of 2 mL and stored at room temperature. The headspace of each vial was purged with air every 2 weeks. These experiments were simultaneously performed with controls that contained the C_5 and C_6 aldehydes, ND, and DMH. The vials were periodically analyzed by gas chromatography (GC) for different periods of time.

A Hewlett-Packard (HP) Model 5790 GC equipped with a 60 m × 0.25 mm i.d. DB-5 bonded-phase fused-silica capillary column

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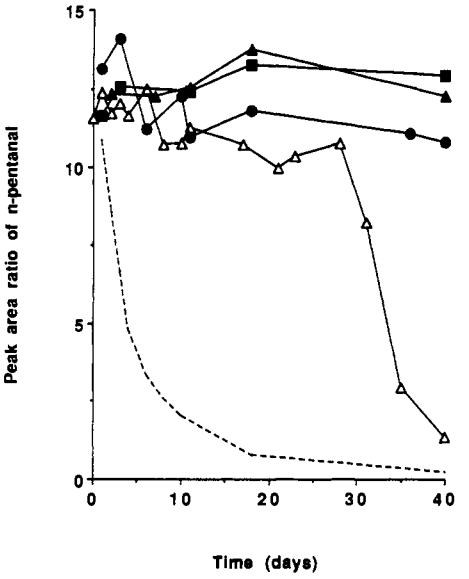

Figure 2. Antioxidative effect of various concentrations (μg/mL) of BHT on 1-pentanal: control (- - -); 5 (△); 10 (●); 50 (■); 100 (▲). Peak area ratio of 1-pentanal is as defined in Figure 1.

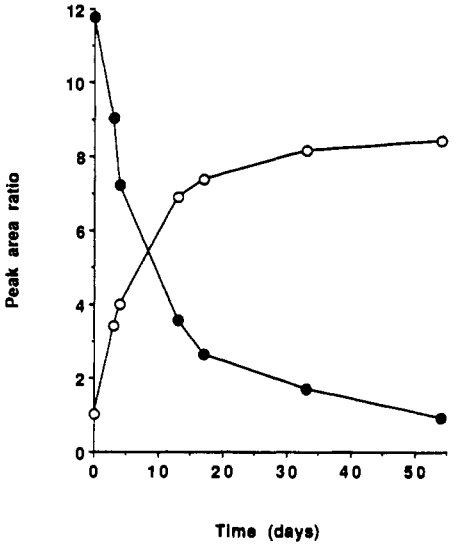

Figure 3. Relative amounts of 1-pentanal (●) and 1-pentanoic acid (○) over time in a control vial. Peak area ratio of 1-pentanal is as defined in Figure 1. Peak area ratio of 1-pentanoic acid equals GC peak area of 1-pentanoic acid divided by GC peak area of nonadecane.

(J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used to monitor the relative amounts of pentyl aldehyde and hexyl aldehyde present in the vials. The GC peak areas were integrated with an HP 5880A series GC terminal. The injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature was programmed from 50 to 200 °C at 6 °C/min. The GC peak areas of pentyl aldehyde and hexyl aldehyde were divided by the GC peak area of DMH to calculate the relative peak areas (RPA) of the C_5 and C_6 aldehydes, respectively.

An HP Model 5890 GC equipped with a 30 m × 0.25 mm i.d. DB-Wax bonded-phase fused-silica capillary column (J&W Scientific) and a FID was used to monitor the relative amounts of pentanoic acid and hexanoic acid present in the vials. The GC peak areas were integrated with a SP4400 (Spectra-Physics, San Jose, CA). The injector and detector temperatures were 200 and 250 °C, respectively. The oven temperature was held at 70 °C for 5 min and then programmed to 180 °C at 2 °C/min. The GC peak areas of pentanoic acid and hexanoic acid were divided by the GC peak area of ND to calculate the RPA of the C_5 and C_6 carboxylic acids, respectively.

Qualitative Analysis of the Headspace Volatiles. CC

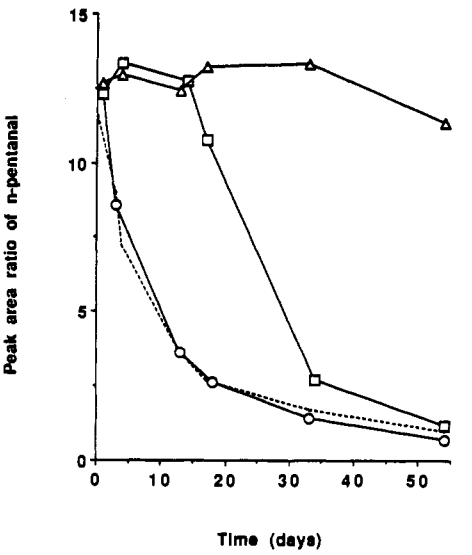

Figure 4. Antioxidative effect of various amounts (μL) of corn oil/glycine headspace extract on 1-pentanal: control (- - -); 20 (○); 100 (□); 500 (△). Peak area ratio of 1-pentanal is as defined in Figure 1.

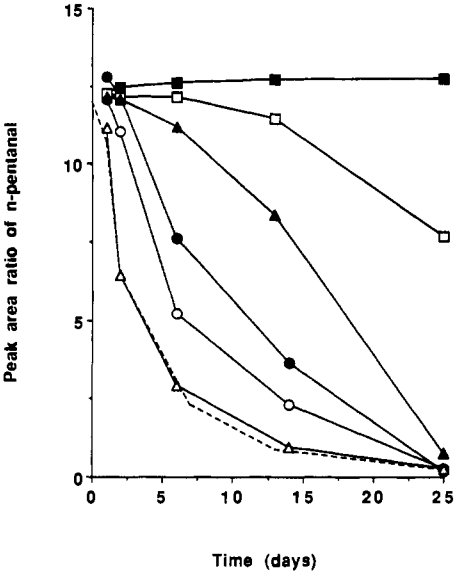

Figure 5. Antioxidative effect of column chromatographic fractions (500 μL) on 1-pentanal: control (- - -); fraction I (○); fraction II (●); fraction III (■); fraction IV (□); fraction V (▲); fraction VI (△). Peak area ratio of 1-pentanal is as defined in Figure 1.

fraction III, which exhibited the highest antioxidative activity, was analyzed by GC, gas chromatography/mass spectrometry (GC/MS), and gas chromatography/infrared spectrometry (GC/IR). The gas chromatographic retention index (Kovats, 1965), IR spectrum, and MS fragmentation pattern of each component were compared with those of the authentic compounds.

An HP Model 5880 GC equipped with the same DB-5 capillary column used for assessment of antioxidative activity and a FID was used for qualitative analysis. The GC peak areas were integrated with an HP 5880A series GC terminal. The injection and detector temperatures were held at 250 and 300 °C, respectively. The oven temperature was held at 40 °C for 5 min and then programmed to 160 °C at 2 °C/min.

An HP Model 5890 GC interfaced to a VG Trio II mass spectrometer with VG 11-250 computer data system was used for MS identification of the GC components at MS ionization voltage of 70 eV. Another HP Model 5890 GC interfaced to an HP Model 5965A detector was also used for IR identification of the GC components. Column and oven conditions for GC/MS and GC/IR were as described for the HP Model 5880 GC.

Table I. *N*-Methylpyrroles Present in Column Chromatography Fraction III

component	I_{DB-5}[a]	MS data,[b] *m/e*	IR data, cm^{-1}
1-methylpyrrole	750	81, 80, 39, 41, 53, 55	3117, 2947, 1516, 1413, 1089
1,2-dimethylpyrrole	878	94, **95**, 42, 53, 39, 65	NA[c]
1-methyl-2-ethylpyrrole	967	94, **109**, 42, 39, 83, 53	NA
1-methyl-2-propylpyrrole	1056	94, **123**, 42, 39, 41, 53	3114, 2950, 1488, 1300, 1091
1-methyl-2-butylpyrrole	1154	94, **137**, 95, 42, 39, 41	3114, 2945, 1488, 1300, 1092
1-methyl-2-pentylpyrrole	1252	94, **151**, 95, 42, 39, 53	3115, 2941, 1488, 1300, 1092

[a] Kovats index values based on DB-5 capillary column. [b] The base ion is the underlined number, and the molecular ion in bold print. [c] NA, not available.

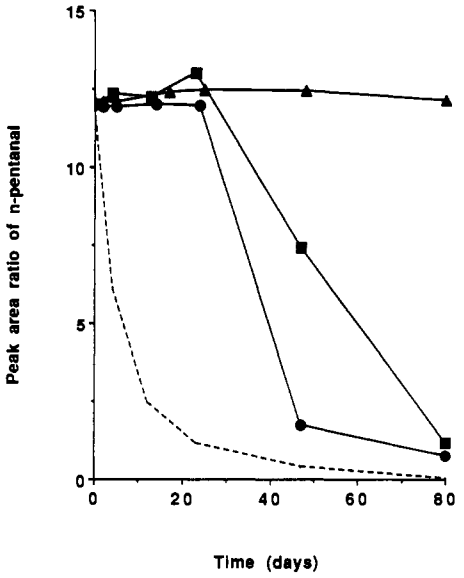

Figure 6. Antioxidative effect of various concentrations (μg/mL) of 1-methylpyrrole on 1-pentanal: control (- - -); 50 (●); 100 (■); 500 (▲). Peak area ratio of 1-pentanal is as defined in Figure 1.

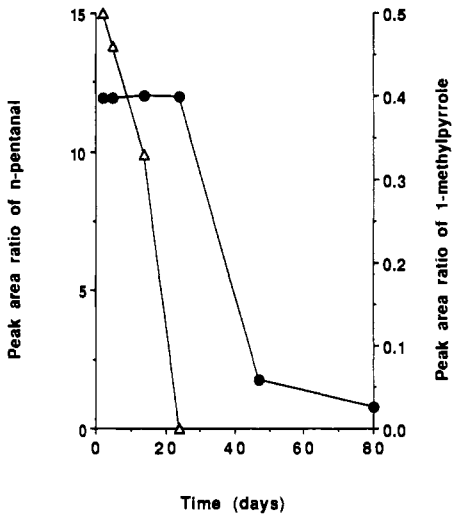

Figure 7. Relative amounts of 1-pentanal (●) and 1-methylpyrrole (△) over time in a vial containing 1-methylpyrrole at 50 μg/mL. Peak area ratio of 1-pentanal is as defined in Figure 1. Peak area ratio of 1-methylpyrrole equals GC peak area of 1-methylpyrrole divided by GC peak area of 2,5-dimethylhexane.

RESULTS AND DISCUSSION

The headspace volatiles isolated from mixtures of corn oil and glycine heated at 180 °C were studied by Macku and Shibamoto (1991). These volatiles, which are dissolved in an organic solvent (dichloromethane), were studied with a newly developed method to assess their antioxidative effect. Simple and affordable materials such as straight-chain aldehydes (pentyl and hexyl aldehydes) and hydrocarbons (DMH and ND, inert compounds used as GC internal standards) are mixed with the headspace sample. If there were no antioxidative volatiles present in solution, the aldehydes would readily oxidize to their corresponding carboxylic acids in the dichloromethane solution through a radical reaction (Nonhebel et al., 1979). Well-known antioxidants, such as α-tocopherol and BHT, were tested to illustrate the use of this assay. Figures 1 and 2 show the effects of α-tocopherol and BHT, respectively, on the relative amounts of 1-pentanal present in the test vials. Increasing amounts of α-tocopherol inhibited the aldehyde/carboxylic acid turnover for increasing periods of time. Within a testing period of 40 days, only the vial with BHT at the smallest concentration (5 μg/mL) showed an aldehyde/carboxylic acid turnover (Figure 2).

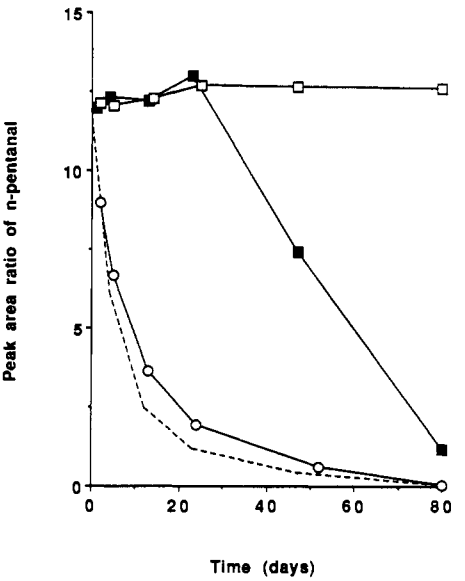

Figure 8. Antioxidative effect of 1-methylpyrrole and pyridine on 1-pentanal: control (- - -); 100 μg/mL 1-methylpyrrole with no pyridine (■); 100 μg/mL of pyridine alone (○); 100 μg/mL 1-methylpyrrole with 100 μg/mL pyridine (□). Peak area ratio of 1-pentanal is as defined in Figure 1.

Figure 3 shows the relative amounts of 1-pentanal and 1-pentanoic acid in a control sample over a period of 55 days. Appearance of 1-pentanoic acid corresponds to the disappearance of 1-pentanal, confirming that the aldehydes are oxidized to their corresponding acids over time. A similar trend was observed for the 1-hexanal/1-hexanoic acid turnover.

The antioxidative activity of the headspace extract was analyzed using the aldehyde/carboxylic acid assay. Figure 4 shows the effect of increasing amounts of added extract on the amount of 1-pentanal present in the vials over a period of 55 days. Increasing amounts of the headspace extract inhibited the aldehyde/carboxylic acid turnover for increasing periods of time.

To isolate the antioxidant(s) formed in the corn oil/glycine headspace extract, each CC fraction was tested for antioxidative activity. Figure 5 shows the individual

effects of these fractions on the amounts of 1-pentanal present in the vials. Fraction III (95% hexane and 5% ethyl acetate), which contained MP and several of its 2-alkyl homologues, exhibited the highest antioxidative activity among the six fractions. The MS and IR spectral data of these heterocyclic compounds (Table I) are comparable with those reported by Budzikiewicz et al. (1964) and Sotoyama et al. (1979). The presence of MP in fraction III is interesting because its antioxidative effect was reported by Gritter and Chriss (1964).

Figure 6 shows the results of antioxidative testing conducted with different amounts of MP. Increasing amounts of this compound inhibited the aldehyde/carboxylic acid turnover for increasing periods of time. Figure 7 shows the relationship between MP (50 μg/mL) and 1-pentanal in the testing solution. After 22 days, MP disappeared completely and the onset of 1-pentanal turnover was observed, suggesting that oxidation of the aldehydes was inhibited in the presence of MP. α-Tocopherol (50 μg/mL) inhibited oxidation for approximately 20 days (Figure 1), suggesting that MP and α-tocopherol have similar antioxidative strengths.

Many alkylpyridines were identified in fractions IV and V, which also showed antioxidative activities (Figure 5). Figure 8 shows the effect of pyridine on the antioxidative activity of MP. Pyridine (100 μg/mL) alone did not inhibit oxidation of the aldehydes. However, 100 μg/mL MP with 100 μg/mL pyridine inhibited oxidation of 1-pentanal for more than 80 days, whereas 100 μg/mL MP alone inhibited it only up to approximately 25 days. These results suggest a synergistic effect of pyridine with MP.

Heterocyclic compounds, including pyrroles and pyridines, are major volatile chemicals produced by the Maillard reaction. 1-Alkylpyrroles and their 2-alkyl homologues have been found in many foodstuffs, such as cooked meats (chicken, shrimp, and squid), roasted beans and nuts (coffee, cocoa, peanuts, and filberts), fermented products (beer, sherry, and tea), and baked goods such as bread (van Straten and Maarse, 1983). In the past, pyrroles have received attention as compounds that contribute to the off-flavors in cooked foods (Peterson et al., 1975). The present study suggests that pyrroles play an important role in the antioxidative activity of Maillard reaction products.

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