Table I. Recovery of Chlorflurenol from Fortified Cucumber Samples

Chlorflurenol added, μg	Chlorflurenol recovered,[a] μg	Recovery,[a] %	Range, %
1	0.90	90	
2	1.57	79	75-91
4	3.51	88	85-101
5	4.45	89	86-93
8	7.15	89	80-105

[a] Average of several determinations.

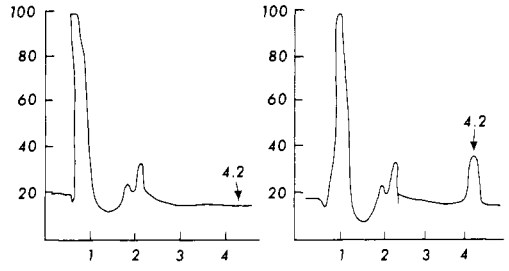

Figure 1. Chromatogram of: (A, left) unfortified cucumber sample; (B, right) cucumber sample fortified with 2 μg of chlorflurenol. The retention time of chlorflurenol is marked at 4.2 min.

column and the concentrate transferred to the column with several small benzene rinses. The column was eluted with 200 ml of 5% acetone in benzene and this fraction was discarded. The column was then eluted with 200 ml of acetone and this fraction was collected. The sample was reduced to 10 ml on a rotavap at 30 °C for GLC analysis.

Gas-Liquid Chromatography. Determinations were performed on a Micro-Tek 220 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was a 1.83 m × 4 mm i.d. glass column containing 6% DC 200 on 80–100 mesh Gas-Chrom Q. Operating parameters were nitrogen carrier at the rate of 80 ml/min; injector 225 °C; column 200 °C; detector 275 °C. Under these conditions chlorflurenol had a retention time of 4.2 min.

Standard Curve. One to four microliters of a 0.1 μg/ml solution of chlorflurenol was injected into the chromatograph. Peak area was plotted vs. concentration or nanograms injected. Normally 4 μl of sample was injected for quantitation.

RESULTS AND DISCUSSION

Blank and samples fortified in the blender with 1, 2, 4, 5, and 8 μg of chlorflurenol were taken through the extraction and cleanup procedures described above. These fortifications correspond to 0.02, 0.04, 0.08, 0.10 and 0.16 ppm residue samples. The average recovery for this series was 87%. The actual recoveries are reported in Table I.

Chlorflurenol was extracted quantitatively from aqueous acetone by benzene in control experiments. In separate control experiments, recovery of chlorflurenol from the Florisil column ranged from 85 to 100%.

Response is linear with both concentration and amount injected. The sensitivity was determined by assuming quantitation of a peak at two times the noise level, and was found to be 0.005 ppm. A typical chromatogram is shown in Figure 1.

ACKNOWLEDGMENT

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Antioxidant Activity of Browning Products Prepared from Low Molecular Carbonyl Compounds and Amino Acids

The antioxidant effect of browning products on safflower oil was investigated. The browning products were obtained by the reaction of low molecular carbonyl compounds with amino acids. Carbonyl compounds tested were methylglyoxal, glyoxal, glyoxylic acid, and dihydroxyacetone. The antioxidant effectiveness changed with the combination of carbonyl compounds and amino acids. Of four carbonyl compounds, methylglyoxal and dihydroxyacetone gave the most potent antioxidative products, followed by glyoxal and then glyoxylic acid. On the other hand, branched chain amino acids such as leucine and valine were most effective as amino acids.

It is well known that a nonenzymatic browning reaction between carbonyl compounds and amino acids functions in the formation of antioxidative substances (Kato, 1973; Maleki, 1973; Yamaguchi and Fujimaki, 1974). Most work was done on the products obtained by the reaction of hexose or pentose with amino acid. In our previous report (Itoh et al., 1975), the antioxidant activity of browning products made from a triose sugar (dihydroxyacetone) and amino acids was compared with the activity of those obtained from glucose or xylose and amino acids. The results indicated that dihydroxyacetone gave the most effective products. El-Zeany et al. (1973) reported the brown pigments produced by condensation of glyoxal with glycine showed excellent antioxidant effect. These facts

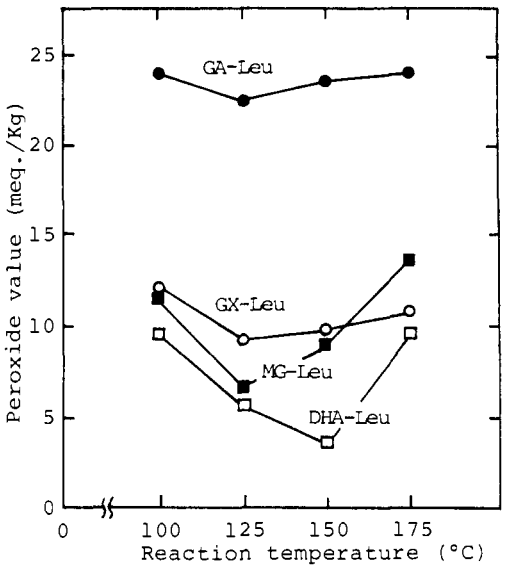

Figure 1. Effect of temperature in the browning reaction on antioxidant activity. The browning oils were prepared by heating low molecular carbonyl compounds and leucine in corn oil. Experimental conditions and abbreviations are described in the text. The peroxide value of blank after oxidation was 30.0 mequiv/kg. The data represent the average of three replications. Variability of the value shown did not exceed ±4%.

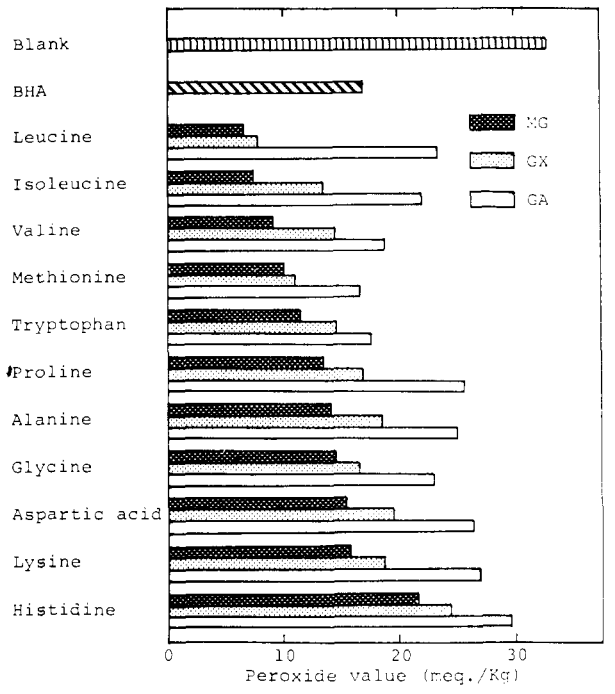

Figure 2. Comparison of antioxidant activity of browning oils prepared from various combinations of amino acids with glyoxal derivatives. Experimental conditions and abbreviations are described in the text. BHA was added in a concentration of 0.02%. The data represent the average of three replications. Variability of the value shown did not exceed ±4%.

stimulated us to study the antioxidant property of products prepared by the browning reaction of amino acid with methylglyoxal which is one of the fission products of dihydroxyacetone. Glyoxal and glyoxylic acid are also considered to be of interest because of their structural relationship to methylglyoxal.

The present report concerns the comparison of the antioxidant activity of browning products from amino acids and four carbonyl compounds described above.

EXPERIMENTAL SECTION

Materials. Methylglyoxal (40% aqueous solution) was obtained from Aldrich Chemical Co., Inc.; glyoxal (40% aqueous solution), glyoxylic acid, and corn oil (peroxide value, 0.1 mequiv/kg) were from Katayama Chemical Co., Ltd., Osaka, Japan; safflower oil (peroxide value, 0.3 mequiv/kg) was from Sigma Chemical Co. All of the amino acids (L form) and dihydroxyacetone are the products of our company, Tanabe Seiyaku Co., Ltd.

Preparation of Browning Oil. One millimole of each carbonyl compound and amino acid were placed in a test tube. Five milliliters of corn oil was added and then the oil was heated at a constant temperature (100–175 °C) for 5 min. The insoluble materials formed were removed by filtration. The filtrate was tentatively named as browning oil, and used as the sample for the antioxidant test. In this paper, the following types of special designations are used to identify various browning oils. Blank is interpreted as heated corn oil without addition of any compound; MG-Leu, GX-Val, GA-Trp, and DHA-Ala are, respectively, interpreted as browning oil prepared by the reaction of methylglyoxal with leucine, glyoxal with valine, glyoxylic acid with tryptophan, and dihydroxyacetone with alanine.

Antioxidant Test. One milliliter of browning oil and 19 ml of safflower oil were placed in a test tube of an apparatus for the active oxygen method (AOM) (Kuramochi Kagaku Co., Ltd., Tokyo, Japan). The test tube was set in the AOM apparatus maintained at a temperature of 97.8 ± 0.1 °C. Air was bubbled into the oil at a constant rate of 2.33 ml/s. After 8 h of oxidation, the oil was titrated according to the KI–$Na_2S_2O_3$ titration procedure of the Association of Official Agricultural Chemists (1975). The peroxide value thus obtained was used for evaluating the antioxidant effectiveness of browning oil.

RESULTS AND DISCUSSION

The effect of temperature in the browning reaction on the antioxidant activity is illustrated in Figure 1. Leucine was chosen as the amino acid since the antioxidant activity of browning oils prepared from this amino acid was relatively higher than those from many other amino acids (Itoh et al., 1975). When dihydroxyacetone was used as the carbonyl compound, the optimal temperature giving the maximal activity was 150 °C.

On the other hand, the antioxidant activity of MG-Leu was highest when the browning oil was produced at 125 °C. Thus, the optimal temperature for producing MG-amino acid seemed somewhat lower than that for DHA-amino acid. Lento et al. (1960) showed that methylglyoxal could be formed from a solution of dihydroxyacetone by heating. So, it might be considered that dihydroxyacetone converted at least partly into methylglyoxal, which reacted with amino acid to yield antioxidative substances.

The optimal temperature for both GX-Leu and GA-Leu was also 125 °C, although the antioxidant effect changed to a lesser extent with varying reaction temperatures.

The comparison of antioxidant activity of the browning oils prepared from varieties of a combination of glyoxal derivatives and amino acids is summarized in Figure 2. These browning oils were prepared at 125 °C. It is evident that almost all of the browning oils have antioxidant activity, more or less. Of the three derivatives tested, methylglyoxal produced the most potent antioxidants independent of the type of amino acid, followed by glyoxal and then glyoxylic acid. Stability of the oil treated with MG-amino acid was much higher than that treated with blank. Furthermore, the former was more stable than the

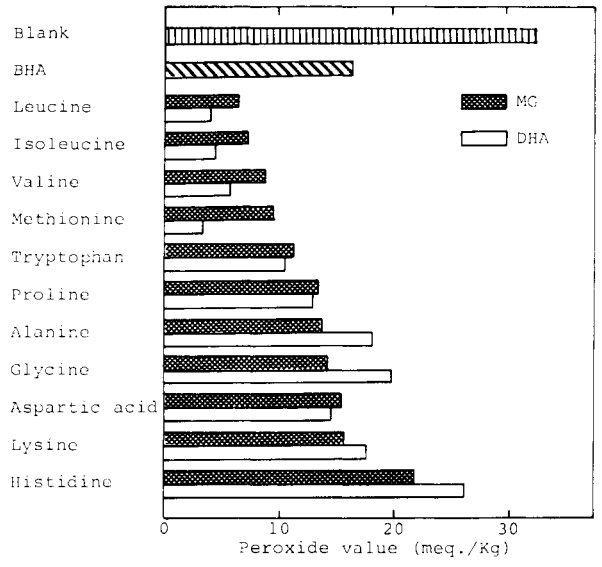

Figure 3. Comparison of antioxidant activity of browning oils prepared from various combinations of amino acids with methylglyoxal or dihydroxyacetone. Experimental conditions and abbreviations are described in the text. BHA was added in a concentration of 0.02%. The data represent the average of three replications. Variability of the value shown did not exceed ±4%.

oil added with 0.02% of butylated hydroxyanisol (BHA) except for MG-His. The highest activity in MG-amino acids was shown by MG-Leu, followed in order by MG-Ile, MG-Val, MG-Met, and MG-Trp.

GX-amino acids also inhibited the oxidation of safflower oil although they were considerably less efficient than MG-amino acids. El-Zeany et al. (1973) wrote that the brown pigments produced by condensation of glyoxal with glycine or ethylamine showed sufficient inhibitory effect to increase the shelf-life of heated fatty foodstuffs. In our study, however, either GX-Leu or GX-Met was much more active than GX-Gly.

The effect of GA-amino acids on oxidation of the oil seems rather weak except for GA-Trp and GA-Met. This is probably because glyoxylic acid was less reactive to amino acid than methylglyoxal or glyoxal.

Figure 3 shows the comparison of MG-amino acids with DHA-amino acid in the antioxidant activity. All of these browning oils had pronounced antioxidant activity. The difference in the activity between both browning oils depended on the type of amino acid used in the browning reaction. In the cases of branched chain amino acids and methionine, dihydroxyacetone was better than methylglyoxal in order to produce browning oils having higher antioxidant activity. On the contrary, methylglyoxal gave better antioxidants when alanine, glycine, or histidine was used as amino acid.

The trend in recent years is the use of more natural compounds as food additives. In this sense, the browning oils seem to be desirable since they can be prepared from natural products. Major problems in the browning oils are the relatively intense flavors and colors accompanying them. The antioxidant mechanism of the browning oils is also not clear. One possible mechanism may be the combined effect with natural tocopherols, which exist in vegetable oils. Considerable work will be needed before using the browning oils for practical application to foodstuffs.

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Natural Occurrence of Alternariols in Discolored Pecans

Two metabolites of *Alternaria* spp., alternariol monomethyl ether (AME) and alternariol (AOH), were extracted from discolored pecans (pickouts) from commercial shellers. AME and AOH isolated from an aqueous acetone extract of pecans were identified by comparison of their IR, UV, and mass spectra with spectra from authentic samples of AME and AOH. Melting points and thin-layer chromatography also confirmed the identifications.

Species of *Alternaria* are common and prevalent in the mycoflora, infesting and often parasitizing the seeds of a wide variety of food crops. Huang and Hanlin (1975) isolated *A. alternata* (Fr.) Keissler from 25% of 36 market samples of pecans. *A. raphani* Groves and Skolko was also isolated from one sample (2.8%). In our laboratory (unpublished data), species of *Alternaria* have been associated with dark discolored pecan kernels.

Because *Alternaria* spp. are commonly found in food and feed products, a number of workers have studied their production of metabolites in culture. Alternariol monomethyl ether (AME) and alternariol (AOH) were first isolated and identified from the mycelium of *A. tenuis* Nees (*A. alternata*) by Raistrick et al. (1953). Freeman (1965) reported the isolation of these compounds from *A. dauci* (Kuhn) Groves and Skolko. Lucas et al. (1971)