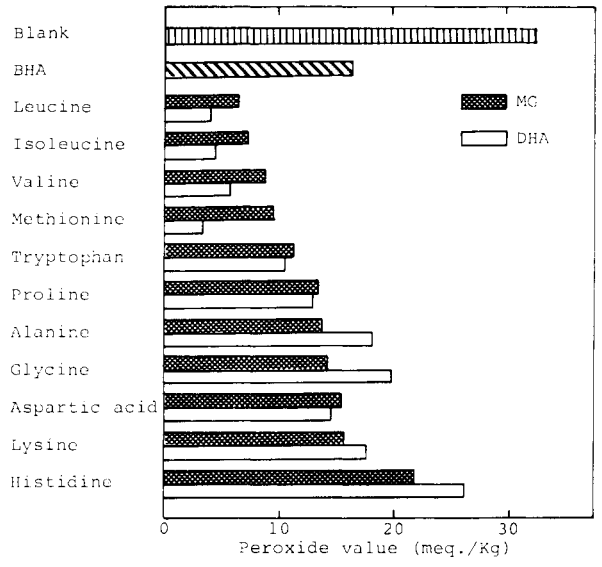

Figure 3. Comparison of antioxidant activity of browning oils prepared from various combinations of amino acids with methylglyoxal or dihydroxyacetone. Experimental conditions and abbreviations are described in the text. BHA was added in a concentration of 0.02%. The data represent the average of three 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)

isolated both compounds from tobacco. Seitz et al. (1975) reported the natural occurrence of the toxins in weathered grain sorghum.

During aflatoxin investigations on a lot of discolored pecan kernels (pickouts) obtained from a commercial pecan sheller, we determined significant amounts of two compounds of suspected fungal origin. After further investigation, they were identified as AME and AOH.

EXPERIMENTAL SECTION

A lot of about 10 kg of damaged, discolored pecan kernels was obtained from a commercial pecan sheller. Forty 50-g samples were selected at random. These samples were individually extracted and assayed for aflatoxins by the method of Pons and Goldblatt (1965). After the assay for aflatoxin the extracts were bulked with the extracts of an additional 4.8 kg of the pickout pecans. The latter were extracted in units of 300 g. Increments of the bulked extracts were applied to silica gel columns and the major components were separated with the aid of a fraction collector. Fractions were monitored by TLC (thin-layer chromatography) and those containing like compounds were bulked and concentrated for further purification.

The crude extract was first separated on 25 mm × 200 mm columns containing Baker 3405 silica gel. These columns were packed with 3% acetone in chloroform and developed with the same solvent system. An easily visualized component fluorescing blue under long-wavelength UV was recognized during the monitoring of fractions of the first separations of the crude extract. A compound with similar fluorescent characteristics but with a lower R_f was also observed, but appeared to be present in smaller amounts. These two compounds were purified by a consecutive series of column separations on 20- and 15-mm diameter columns containing E. M. Laboratories silica gel 60 (particle size less than 0.063 mm) developed with 1.75% methanol in benzene. The extracts were concentrated and yielded high purity crystals, about 10 mg of the one component and 2 mg of the other. TLC analysis suggested that the compounds were AME and AOH, respectively.

TLC analyses were conducted by use of 20 × 20 cm glass plates coated with silica gel GH-R (5:4:1, v/v/v) and developed with chloroform–acetone (73:7, v/v). The compounds were visualized under long-wavelength UV light before and after spraying with 50% ethanolic H_2SO_4 followed by heating for 5 min at 100 °C.

Uncorrected melting points were determined on a Kofler micro-melting point apparatus. Ultraviolet spectra (UV), in methanol, were taken with a Beckman Model DB-G recording spectrophotometer calibrated with a holmium oxide standard. Infrared spectra (IR) were taken with a Perkin-Elmer Model 257 IR spectrophotometer equipped with a 4X beam condenser. Samples were analyzed as a thin film coated onto KBr windows.

High-resolution mass spectral (HRP) and low-resolution mass spectral (LRP) analyses were made with an A.E.I. MS-9 mass spectrometer. Samples were introduced into the ion source by the direct-probe method and ionization was effected by electron impact at 70 eV. The ion-source temperature was programmed to 200 °C, and high-resolution measurements were made by peak matching with perfluorokerosene as the internal standard.

RESULTS AND DISCUSSION

AME. The major component of the extracts was AME, with a melting point of 266–270 °C as compared to the reported melting points of 268–271 °C (Rosett et al., 1957) and 266–267 °C (Freeman, 1965).

The TLC analyses of AME from pecans and of the authentic standard obtained from Mr. Don Harvan were identical and showed a blue fluorescent spot under long- and short-wavelength UV light at R_f 0.51 (chloroform–acetone, 93:7, v/v) and R_f 0.75 (toluene–ethyl acetate–formic acid, 5:4:1, v/v/v). The spots remained blue fluorescent after spraying with 50% ethanolic sulfuric acid, but shifted to a greenish fluorescence after spraying and heating for 5 min at 100 °C.

The mass spectral data showed a molecular ion peak at nominal mass m/e 272 (LRP) and HRP spectrum showed a measured mass of 272.0699 with a computer calculated formula of $C_{15}H_{12}O_5$. The relative intensity of this molecular ion peak was 100% and, therefore, also represented the base peak in the mass spectrum. The calculated mass for a molecular formula of $C_{15}H_{12}O_5$ is 272.0684 which agreed with our measured mass. The fragmentation pattern agreed with that reported by A. E. Pohland and J. S. Sphon at the Mycotoxin Mass Spectral Data Bank, U.S. Food and Drug Administration, Washington, D.C.

The IR spectra of standard AME and AME from pecans are essentially identical. Similarly, the UV spectra of the authentic standards and those isolated from pecan are also identical.

AOH. The TLC characteristics of AOH from pecans were identical with those of the authentic AOH standard. The R_f of the compound from both sources was 0.76 in the toluene–ethyl acetate–formic acid solvent system and 0.21 in chloroform–acetone. Under UV light, AOH from both sources fluoresced blue before and after spraying with H_2SO_4 and continued to fluoresce blue after the sprayed plate was heated.

The LRP spectrum of the AOH from pecan showed a molecular ion peak at nominal mass m/e 258, which is identical with the molecular weight of alternariol. The UV analysis showed λ_{max}^{MeOH} 330–340 (broad band), 201, 290, 256, and 230 nm and these absorptions and their relative intensities matched those of the authentic AOH standard. The IR spectrum of the pecan compound was also identical with the spectrum supplied by Mr. Harvan and with the spectrum we obtained from standard AOH. Major IR absorptions corresponding to the major functional groups were 3460 (OH), 3100 (intramolecular hydrogen-bonded OH), 1672 (lactone shifted downfield due to H bonding with OH), 1590, 1430 (phenyl nucleus), 1365 (CH_3), 1200 (phenolic OH), and 850 cm^{-1} (isolated H on aromatic ring).

The natural occurrence of the *Alternaria* compounds AME and AOH has been established in tobacco (Lucas et al., 1971) and in grain sorghum (Seitz et al., 1975). This report of their occurrence in pecans suggests that they could be present in many other seed crops. The ubiquitous association of *Alternaria* species with agricultural seed crops suggests that contamination of food and feed by these compounds may be common. Current reports suggest that only a few commodities have been analyzed for the compounds. *Alternaria* spp. other than *A. tenuis* and *A. dauci* may also produce AME and AOH.

Although pecans can be contaminated with AME and AOH, our data suggest they are not a probable source leading to consumption of significant amounts by humans. We have only detected the compounds in discolored pecan kernels which are removed from shelled pecans during processing and probably would be rejected by home consumers of in-shell pecans. Also, per capita consumption of pecans is very low and further reduces the chance of significant intake of AME and AOH.

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Formation of Sulfur- and Nitrogen-Containing Compounds from the Reaction of Furfural with Hydrogen Sulfide and Ammonia

Seventeen compounds produced from the reaction of furfural, hydrogen sulfide, and ammonia in aqueous solution under cooking conditions were identified by a GC–MS technique. Major reaction products were cyclic methylene polysulfides and furan derivatives. The formation of oxazole and pyrazine derivatives indicated that furfural produced a smaller number of carbon units. The formation of thiophene and pyrrole derivatives indicated that the oxygen atom in the furan ring exchanged with the sulfur and nitrogen atoms. In addition to those compounds, two self-condensation products of furfural, difurylethylene and furil, were obtained in large amounts from this reaction.

The author has recently studied volatile chemicals generated in a D-glucose–hydrogen sulfide–ammonia heating system (Shibamoto and Russell, 1976), and identified many compounds which are associated with cooked meat flavors; they were thiols, sulfides, thiophenes, thiazoles, and furans. Most compounds identified in this system have also been found in cooked foods (Stoll et al., 1967; Kinlin et al., 1972). Quantitative analysis of these D-glucose–hydrogen sulfide–ammonia reaction products showed that this system produced a large amount of furfural (GC peak area % = 5.64, the third largest constituent following 2-methylthiophene and 2-acetylfuran). Furfural has been well known as a product from sugar caramelization (Hodge, 1967). Also, May (1960) obtained products which possess meat-like flavor from the reaction of cysteine or cystine with furan compounds including furfural.

It was noticed that there were some compounds which could be obtained by the reaction of furfural and hydrogen sulfide in this D-glucose–hydrogen sulfide–ammonia reaction mixture: methyl thiofuroate, 2-furylmethanethiol, and methyl furfuryl sulfide. Bruins (1929) obtained difurfurylethylene from the reaction of furfural and hydrogen sulfide in an aqueous solution with 18% yield. van den Ouweland and Peer (1975) reported ten thiophene derivatives produced from the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone, which had been identified in beef broth, and hydrogen sulfide. They suggested that an oxygen atom in a furanone ring was exchanged with a sulfur atom of hydrogen sulfide to produce thiophene derivatives. Rizzi (1974) reported the formation of *N*-alkyl-2-acylpyrroles from the reaction of furfural and acetylfuran with α-amino acids. He proposed the exchange of an oxygen atom in the furan ring of acetylfurans and a nitrogen atom of amino acid to yield acylalkylpyrroles. Following from their studies, furfural was reacted with hydrogen sulfide and ammonia to investigate the formation of sulfur- and nitrogen-containing compounds under the same conditions used for the D-glucose–hydrogen sulfide–ammonia heating system. If this system creates any of the same compounds which have been found in the sugar–hydrogen sulfide–ammonia model system or in some other food systems, furfural could be a flavor precursor as well as a sugar caramelization product or a flavor ingredient.

EXPERIMENTAL SECTION

Materials. Commercially obtained furfural was distilled at 95 °C (65 mmHg) before the reaction. All other chemicals and authentic samples were obtained commercially or were gifts from Ogawa & Co., Ltd., Tokyo, Japan, and were used without further treatment.

Sample Preparation. Furfural (9.6 g; 0.10 mol) was mixed with 100 ml of deionized water and hydrogen sulfide gas was bubbled through this solution at 0 °C for 10 min in a Kjeldahl flask (~0.02 mol of H_2S). Ammonium hydroxide solution (0.50 mol as NH_3) was then added to the above solution. The neck of the flask was flame-sealed and the flask placed in an oven at 100 °C for 2 h. Approximately 5 g of brown polymeric material was obtained from the reaction mixture which possessed sweet roast beef-like odor. After this polymeric material was removed by filtration, the reaction products were extracted from the filtrate with 200 ml of methylene chloride using a liquid–liquid continuous extractor for 16 h. The methylene chloride solution was dried over anhydrous $MgSO_4$, and concentrated to yield 0.5 g of a brown liquid. The identification of products was conducted following the GC–MS technique described previously (Shibamoto and Russell, 1976).

RESULTS AND DISCUSSION

The compounds identified from the reaction of furfural with hydrogen sulfide and ammonia are listed in Table I and their gas chromatogram is shown in Figure 1. The