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Formation of *N*-alkylpyrroles in corn oil/amino acid model systems

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Corn oil was heated with alanine, cysteine, glycine, phenylalanine, ammonia, methylamine, or ethylamine at 180°C for 4 h. The volatiles formed in the headspace were collected using a simultaneous purging and solvent extraction apparatus and analyzed by gas chromatography and gas chromatography/mass spectrometry. Fourteen mono- and di-alkylpyrroles were identified in the headspace extracts of the corn oil/amino acid mixtures and the corn oil/amine mixtures. It is proposed that α,β-unsaturated fatty aldehydes reacted with the alkylamine produced from the corresponding pyrolyzed amino acid to form 1-alkylpyrroles.

INTRODUCTION

Pyrroles are heterocyclic compounds generated from the thermal interactions among food constituents. The compounds have been found in several processed foodstuffs, and in general they are thought to contribute to the sweet, burned, and earthy aroma noted (Maga, 1981; Vernin & Vernin, 1982). Pyrroles have not been found in fresh, unprocessed foods (Maga, 1981).

Alkylpyrroles are found in roasted nuts and beans such as roasted peanuts (Ho *et al.*, 1981), roasted filberts (Kinlin *et al.*, 1972), roasted cocoa beans (Gill *et al.*, 1984), and particularly in roasted coffee beans, in which more than 15 alkylated pyrroles were found (Tressl *et al.*, 1981). 1-Methylpyrrole and 1-propylpyrrole have been identified in bread (Folkes & Gramshaw, 1981). Nine 1-alkylpyrroles have also been found in tobacco and tobacco smoke (Schmeltz & Hoffmann, 1977). *N*-Alkylpyrroles have been reported to be present in cooked chicken (Noleau & Toulemonde, 1986) and in processed seafood such as in cooked shrimp (Kubota *et al.*, 1989) and dried squid (Kawai *et al.*, 1991).

Compared to other well studied flavor compounds such as pyrazines and thiazoles, little is known about the mechanism of formation of pyrroles, and particularly about that of alkylpyrroles. Dialkylated pyrroles can be formed through several pathways involving several food constituents. The thermal interaction between proteins and either carbohydrates or lipids is likely to produce these nitrogen-containing compounds. The objective of the present study was to determine the contribution of fatty acids and free amino acids to the formation of 1,2-dialkylated pyrroles in lipid-rich processed foodstuffs.

MATERIALS AND METHODS

Materials

Corn oil (Mazola brand, Englewood Cliffs, NJ) was purchased from a local market. Alanine (Ala), cysteine (Cys), glycine (Gly), phenylalanine (Phe), and serine (Ser) were brought from Sigma Chemical Co. (St Louis, MO). Methylamine (40 wt % solution in water), ethylamine (70 wt % solution in water), and 2,5-dimethylhexane were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ammonium hydroxide (38 wt % solution in water) was purchased from Mallinckrodt (Paris, KY).

Sample preparations

Corn oil/amino acid

Corn oil (100 g) was mixed with Ala, Cys, Gly, Phe, or Ser (2·0 g) and placed in a 500 ml two-neck, round-bottom flask (RBF), which was interfaced to a simultaneous purging and solvent extraction apparatus (SPE) devised by Umano and Shibamoto (1987). The

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mixture was stirred and kept at 180°C for 4 h. The headspace was purged into the SPE with a purified air stream (flow rate, 10 ml min^{-1}). The dichloromethane extract was concentrated to 2 ml by fractional distillation. 2,5-Dimethylhexane (final concentration = 100 μg ml^{-1}) was added to the sample as a GC internal standard.

Corn oil/amine

A 500 ml two-neck RBF, which contained 200 ml of aqueous solution of ammonium hydroxide, methylamine, or ethylamine, was purged with an air stream (flow rate, 10 ml min^{-1}). The gaseous amine was introduced into a second 500 ml two-neck RBF, which contained 100 g of corn oil and was interfaced to a SPE. The oil was stirred and heated at 180°C for 4 h. The rest of the procedure was the same as that for the corn oil/amino acid mixtures.

Instruments

A Hewlett-Packard (HP) 5790 gas chromatograph equipped with a 60 m × 0·25 mm i.d. DB-5 bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used to analyze the headspace extracts. The GC peak areas were integrated with a HP 5880A Series terminal. The injector temperature was 250°C, and the detector temperature was 300°C. The oven temperature was held at 40°C for 5 min and then programmed to 180°C at 2°C min^{-1}. The linear helium carrier gas flow rate was 30 cm s^{-1}. The samples were co-injected with a series of hydrocarbons to calculate the GC retention indexes of the peaks of interest (Kovats, 1965).

An HP 5890 gas chromatograph interfaced to a VG Trio II mass spectrometer with a VG 11-250 computer data system was used for MS identification of the GC components. The column and oven conditions for GC/MS were as described for the HP 5790 gas chromatograph.

RESULTS AND DISCUSSION

Table 1 shows the pyrroles detected in the headspace of all corn oil/amino acid and corn oil/amine mixtures. The corn oil/glycine mixture produced a homologous series of 1-methyl 2-alkylpyrroles, which was also produced from the corn oil/methylamine mixture. The amounts of these 1-methylpyrroles were found to be significantly higher when corn oil was heated with methylamine than with glycine. The corn oil/alanine mixture produced a homologous series of 1-ethyl 2-alkylpyrroles, which was also produced from the corn oil/ethylamine mixture. These 1-ethylpyrroles were produced in significantly higher amounts from corn oil/ethylamine than from corn oil/alanine. The corn oil/cysteine and corn oil/serine mixtures produced appreciable amounts of 1-ethylpyrrole, but in smaller quantities than did the corn oil/alanine mixture. The corn oil/cysteine mixture produced trace amounts of 1-ethyl 2-propylpyrrole and 1-ethyl 2-butylpyrrole. The corn oil/phenylalanine mixture produced only trace amounts of 1-phenethylpyrrole, which was detected by GC/M due to the presence of its molecular ion (m/z = 171), as reported by Thomas (1973). The corn oil/ammonia mixture produced only the unalkylated

Table 1. Headspace pyrroles found in corn oil/glycine, corn oil/alanine, corn oil/cysteine, corn oil/serine, corn oil/phenylalanine, corn oil/ammonia, corn oil/methylamine and corn oil/ethylamine mixtures. Amounts expressed as relative peak areas (GC peak area of compound divided by GC peak area of 2,5-dimethylhexane)

Compound	$I_{\mathrm{DB\text{-}5}}$[a]	Gly	Ala	Cys	Ser	Phe	NH_3	CH_3NH_2	$C_2H_5NH_2$
Pyrrole	762	+	+	+	+	+	+	–	–
1-Methylpyrrole	750	0·45	–	–	–	–	–	+	–
1,2-Dimethylpyrrole	878	0·15	–	–	–	–	–	0·17	–
1-Methyl 2-ethylpyrrole	967	+	–	–	–	–	–	+	–
1-Methyl 2-propylpyrrole	1056	0·18	–	–	–	–	–	0·42	–
1-Methyl 2-butylpyrrole	1154	0·036	–	–	–	–	–	0·41	–
1-Methyl 2-pentylpyrrole	1252	0·14	–	–	–	–	–	1·2	–
1-Ethylpyrrole	820	–	3·8	1·0	0·18	–	–	–	4·9
1-Ethyl 2-methylpyrrole	932	–	+	–	–	–	–	–	+
1,2-Diethylpyrrole	1018	–	0·034	–	–	–	–	–	0·031
1-Ethyl 2-propylpyrrole	1100	–	+	–	tr	–	–	–	+
1-Ethyl 2-butylpyrrole	1198	–	+	–	tr	–	–	–	+
1-Ethyl 2-pentylpyrrole	1296	–	0·038	–	–	–	–	–	0·34
1-Phenethylpyrrole	>1400	–	–	–	–	tr	–	–	–

[a] Kovats Index on a DB-5 capillary column.

+, Indicates presence of the compound found by GC/MS. Quantitation not possible by GC/FID due to overlapping with neighbouring peak.

–, Indicates compound not found by GC/MS or GC/FID (Kovats index).

tr, Indicates traces. Presence of compound found by GC/MS but not detected by GC/FID.

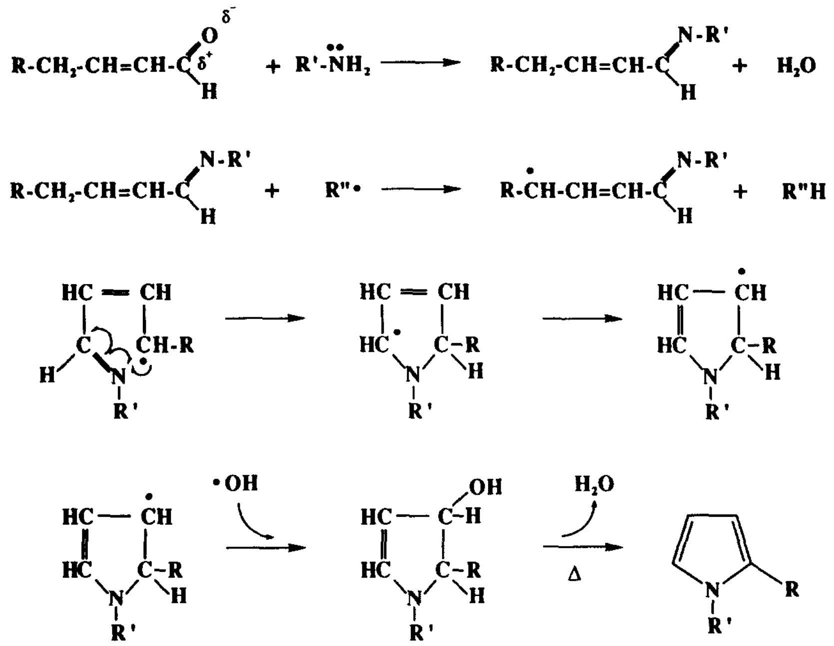

Fig. 1. Proposed mechanism for the thermally formed 1,2-dialkylated pyrroles found in the headspace of corn oil/amino acid mixtures.

pyrrole, which was found in all corn oil/amino acid mixtures. Pyrrole has been proposed to be formed from the interaction between 2-butanol and ammonia gas (Henderson & Nawar, 1981).

During headspace extraction, corn oil undergoes peroxidation (Macku & Shibamoto, 1991*a*), and the free amino acids undergo pyrolysis (Simmonds *et al.*, 1972). The peroxidation of corn oil at high temperatures yields a homologous series of α,β-unsaturated aldehydes. The amounts of these carbonyl compounds have been found to decrease dramatically when the oil is heated with increasing amounts of glycine (Macku & Shibamoto, 1991*a*). Also, methylamine is a pyrolysis product of glycine (Simmonds *et al.*, 1972), and ethylamine is a pyrolysis product of alanine (Simmonds *et al.*, 1972), cysteine (Fujimaki *et al.*, 1969) and serine (Kato *et al.*, 1970). 2-Phenylethylamine is a pyrolysis product of phenylalanine (Shulman & Simmonds, 1968). Table 1 shows a relation between the pyrolysis product of each amino acid and the *N*-alkyl group of the pyrroles found in each corn oil/amino acid mixture.

A proposed formation mechanism for the 1,2-dialkylated pyrroles is shown in Fig. 1. A nucleophilic attack by the amine on the α,β-unsaturated fatty aldehyde replaces the oxygen atom with a nitrogen atom. The resulting α,β-conjugated imine radical, formed by hydrogen abstraction at the γ-carbon, rearranges its electrons and reacts with a surrounding hydroxyl radical to undergo dehydration and pyrrole-ring formation. A similar reaction could occur between carbohydrates and amino acids at high temperatures. Olsson and coworkers (1977, 1978) found that 2-formyl 5-hydroxymethyl 1-methylpyrrole was formed by heating from either a mixture of D-glucose and glycine or a mixture of D-glucose and methylamine. 2-Acetyl 1-ethylpyrrole was also formed by heating a hexose with either alanine or ethylamine. (Kato *et al.*, 1972; Shigematsu *et al.*, 1972).

Some pyrroles reportedly contribute to the off-flavors in cooked foods (Peterson *et al.*, 1975). 1-Alkylpyrroles play an important role in the stability of cooked foods with respect to oxidation. The headspace volatiles produced from corn oil/alanine, /cysteine, /phenylalanine, and /serine mixtures were found to have antioxidative properties similar to the ones produced from the corn oil/glycine mixture (Macku & Shibamoto, 1991*b*). Further work will be required to better understand the antioxidative effects of these 1-alkylated pyrroles.

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