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Substitution of Pyrazines by Aldehydes in Model Systems

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The effect of long-chain aldehydes on the formation of long-chain alkyl-substituted pyrazines was investigated in model systems of acetol and ammonium acetate with and without the addition of pentanal or hexanal. When the systems were reacted at 100 °C for 4 h, 2,5-dimethyl-3-pentylpyrazine and 2,6-dimethyl-3-pentylpyrazine were formed in the model system with added pentanal, and the corresponding hexylpyrazines were formed in the hexanal system. Other pyrazines, including 2,3,5-trimethylpyrazine, 2-methyl-5(or 6)-ethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,6-dimethyl-3-ethylpyrazine, 2,5-dimethyl-3-allylpyrazine, 2,5-dimethyl-3-propenylpyrazine, 2,3,5-trimethyl-6- butylpyrazine, 2,3,5-trimethyl-3-pentylpyrazine, 2,3,5-trimethyl-3-hexylpyrazine, 2,5-dimethyl-3-propylpyrazine, and 2,6-dimethyl-3-propylpyrazine, were also obtained from the model systems. Formation pathways are proposed for some of these pyrazines.

The occurrence and formation of pyrazines in foods have been studied extensively in the last 25 years. A comprehensive review of these studies has been prepared by Maga (1982). Many of these studies have focused on the investigation of pyrazine precursors, especially those carbohydrate and amino acid sources most active in the Maillard reaction.

Interest in the influence of lipids on pyrazine formation has recently been generated by the identification of long-chain alkyl-substituted heterocyclic compounds in foods and in model systems. Various nitrogen- and sulfur-containing heterocyclic products with long-chain alkyl substituents have been detected in model systems (Boelens et al., 1974; Henderson and Nawar, 1981), in fried chicken extracts (Tang et al., 1983; Hartman et al., 1984), and in french-fried potato extracts (Carlin et al., 1986;

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Table I. Pyrazines Identified from the Reaction of Acetol and Ammonium Acetate with Pentanal and Hexanal

pyrazine	I_k (HP-1)	mg/g of acetol[a]			
		A_1	A_2	B	C
2,5-dimethyl	889	23.09	7.88	7.81	13.85
2,6-dimethyl	895	0.01	0.01	0.04	0.01
2-methyl-5(or 6-)-ethyl	975	0.14	0.14	0.08	0.14
2,3,5-trimethyl	979	1.45	0.48	0.36	0.51
2,5-dimethyl-3-ethyl	1059	0.89	0.66	0.40	0.70
2,6-dimethyl-3-ethyl	1064	0.62	0.38	0.22	0.31
2,3-diethyl-3-methyl	1136	0.05	0.04	0.01	0.02
2,5-dimethyl-3-propyl	1142	0.38	0.24	0.05	0.12
2,6-dimethyl-3-propyl	1151	0.26	0.18	0.04	0.09
6,7-dihydro-2,5-dimethyl-5*H*-cyclopenta	1202	0.07	0.04	0.02	0.03
2,5-dimethyl-3-allyl	1212	1.36	0.78	0.18	0.43
2,5-dimethyl-*cis*-3-propenyl	1214	0.72	0.53	0.25	0.43
2,5-dimethyl-*trans*-3-propenyl	1218	0.21	0.12	0.15	0.08
2,3,5-trimethyl-6-butyl	1317	–[b]	–	0.07	0.04
2,5-dimethyl-3-pentyl	1335	–	–	0.02	0.01
2,6-dimethyl-3-pentyl	1348	–	–	0.49	–
2,3,5-trimethyl-6-pentyl	1412	–	–	0.05	–
2,5-dimethyl-3-hexyl	1435	–	–	–	0.39
2,6-dimethyl-3-hexyl	1449	–	–	–	0.22
2,3,5-trimethyl-6-hexyl	1510	–	–	–	0.02

[a] Average of two experiments. Key: A_1, acetol/ammonium acetate (open system); A_2, acetol/ammonium acetate; B, acetol/ammonium acetate/pentanal; C, acetol/ammonium acetate/hexanal. [b] Not detected.

Hwang et al., 1986). Pyrazines in this category include 2-heptylpyrazine, isolated from french-fried potatoes (Carlin et al., 1986; Hwang et al., 1986), and 2-methyl-3(or 6)-pentylpyrazine and 2,5-dimethyl-3-pentylpyrazine, isolated from baked and extruded corn-based systems (Huang et al., 1987; Bruechert et al., 1988).

The formation of long-chain alkyl-substituted pyrazines by the thermal interaction of acetol and ammonium acetate with either hexanal or pentanal was investigated in the present study. Acetol, a hydroxy ketone compound, is a possible hydrolysis product of amino ketones during the Strecker degradation, and is an active precursor of pyrazines in the presence of ammonia in weakly acidic aqueous solutions (Rizzi, 1988). Yields of pyrazines from acetol increased with increasing temperatures up to 120 °C. Hexanal and pentanal are well-known oxidative degradation products of lipids. Several mechanisms for the formation of substituted pyrazines based on the condensation of carbonyl compounds with 2,3-dihydropyrazines have been discussed by Flament (1981). Although 2,5-dihydropyrazines have been identified as intermediate structures in the autocondensation of α-amino carbonyl compounds, mechanisms for the condensation of 2,5-dihydropyrazines with carbonyl compounds have not been established.

EXPERIMENTAL SECTION

Materials. Acetol, ammonium acetate, hexanal, pentanal, and sodium sulfate were obtained commercially.

Sample Preparation. The model systems used in this study were acetol/ammonium acetate (A_1 and A_2 in Table I), acetol/ammonium acetate/pentanal (B in Table I), and acetol/ammonium acetate/hexanal (C in Table I). For A_2, B, and C systems, 0.12 mol of each component was dissolved in 100 mL of distilled water and the resultant solution sealed in a reaction vessel. For the A_1 system, 0.12 mol of acetol and ammonium acetate were placed in a round-bottom flask equipped with a refluxed condenser. The reaction vessels and the flask were then heated in an oil bath at 100 °C for 4 h.

Extraction of Volatile Compounds. The heated mixtures were adjusted to pH 12 and extracted with 50 mL of redistilled ethyl ether three times. The ether extract was washed with 50 mL of 10% HCl solution. The acidic aqueous portion was collected and adjusted to pH 7. Volatile components were extracted for 2 h at boiling temperature in a Nickerson–Likens steam distillation/solvent extraction apparatus (Kontes, Vineland, NJ). The extract was then dried with anhydrous sodium sulfate and concentrated on a macro Kuderna–Danish concentrator to a final volume of 3 mL. 2-Methylpyrazine was added as the internal standard. The sample was then ready for GC analysis.

Gas Chromatography. The gas chromatograph was a Varian Model 3400. A nonpolar fused silica capillary column (50 m × 0.32 mm (i.d.); 1.05-μm film thickness; HP-1, Hewlett-Packard) was used for this analysis. The column temperature was increased from 40 to 220 °C at a rate of 2 °C/min and was held for 30 min at the final temperature. The injector temperature was 270 °C, and the detector temperature was 290 °C. The flow rate was approximately 1 mL/min, the split ratio was 58:1, and the injected sample volume was 0.5 μL.

Quantitative determination was accomplished by internal standard previously mentioned. The quantity of each components was finally converted into milligrams of the volatiles generated by 1 g of acetol. Linear retention indeces for the volatile compounds were calculated versus *n*-paraffin standards (C_6–C_{22}; Alltech Associates) as references (Majlat et al., 1974).

Gas Chromatography/Mass Spectrometry. The high-resolution GC/MS consists of a Varian gas chromatograph, Model 3400, a Finnigan MAT mass spectrometer, Model 8230, and a Finnigan SS 300 data system. The column and GC program were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA.

RESULTS AND DISCUSSION

The compounds identified in the three model systems are listed in Table I with their I_k's, and the mass spectral data of several new pyrazines generated from these model systems are listed in Table II.

Possible formation pathways for 2,5-dimethylpyrazine and 2,5-dimethyl-3-pentylpyrazine in the acetol/ammonium acetate/pentanal system are shown in Figure 1. 2,5-Dimethylpyrazine and 2,6-dimethylpyrazine were the major products in the acetol/ammonium acetate reaction. Rizzi (1988) recently reported that the reaction of acetol with ammonia from ammonium acetate yields α-amino ketone precursors of pyrazines. Autocondensation of these amino ketone precursors and dehydrogenation of the resulting dihydropyrazine intermediate explain the formation of 2,5- or 2,6-dimethylpyrazine (Figure 1). The formation of pyrazines by the reaction of acetol and ammonium acetate was investigated both in a closed pressure system and in an open system. Only quantitative differences of various pyrazines formed were observed, and there were no qualitative differences. In the acetol/ammonium acetate/pentanal system, 2,5-dimethyl-3-pentylpyrazine and 2,6-dimethyl-3-pentylpyrazine were formed, and in the acetol/ammonium acetate/hexanal system, 2,5-dimethyl-3-hexylpyrazine and 2,6-dimethyl-3-hexylpyrazine were formed. Flament (1981) has reported high yields of substituted pyrazines from condensation of 2,3-dimethyl-5,6-dihydropyrazines with aldehydes and ketones. In Figure 1, 2,5-dimethyl-3,6-dihydropyrazine is shown as the reactive intermediate. Competition between dehydrogenation of the intermediate and condensation of the intermediate with pentanal or hexanal might account for the decreased amounts of 2,5- and 2,6-dimethylpyrazine observed in the acetol/ammonium acetate systems in the presence of these aldehydes.

The formation of several of the other alkylpyrazines identified depends on the production of intermediates from acetol. Keto–enol tautomerisms, aldol condensations, and retro-aldol condensations of acetol lead to the

Table II. Mass Spectral Data of Newly Identified Pyrazines Generated from the Reactions of Acetol and Ammonium Acetate with Pentanal and Hexanal

compound	mass spectral data, m/z (rel intens)
2,3,5-trimethyl-6-butylpyrazine	177 (2), 149 (13), 136 (26), 108 (9), 56 (9), 42 (13), 41 (18), 39 (11)
2,5-dimethyl-3-pentylpyrazine	178 (2), 149 (13), 135 (21), 122 (100), 121 (17), 80 (98), 53 (12), 42 (21), 41 (17), 39 (23)
2,6-dimethyl-3-pentylpyrazine	178 (2), 149 (13), 135 (21), 122 (100), 121 (17), 80 (10), 53 (12), 42 (17), 41 (7), 39 (23)
2,3,5-trimethyl-6-pentylpyrazine	192 (5), 163 (16), 149 (10), 136 (100), 135 (27), 56 (14), 55 (18), 43 (20), 41 (24)
2,5-dimethyl-3-hexylpyrazine	192 (4), 149 (13), 135 (7), 123 (13), 122 (100), 121 (9), 53 (7), 42 (13), 39 (12)
2,6-dimethyl-3-hexylpyrazine	192 (4), 149 (13), 135 (17), 123 (13), 122 (100), 121 (9), 59 (9), 42 (13), 39 (14)
2,3,5-trimethyl-6-hexylpyridine	206 (2), 163 (7), 149 (14), 137 (11), 136 (100), 137 (7), 53 (14), 42 (10), 41 (7)

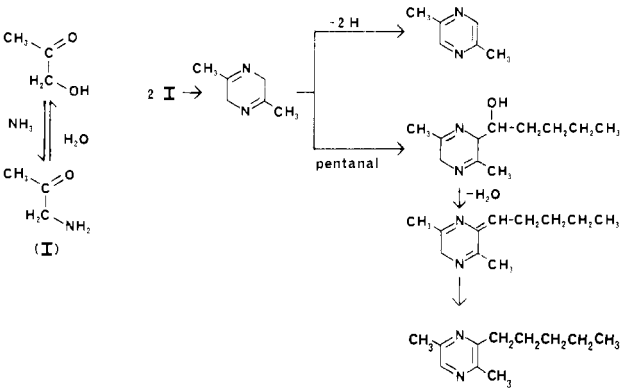

Figure 1. Proposed formation of 2,5-dimethylpyrazine and 2,5-dimethyl-3-pentylpyrazine from acetol, ammonium acetate, and pentanal.

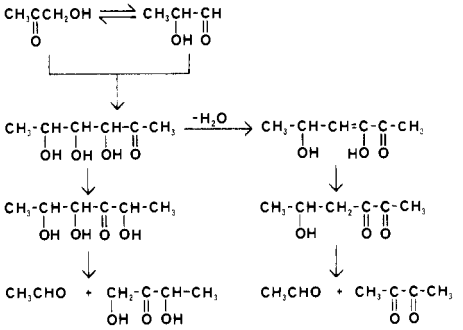

Figure 2. Formation of pyrazine precursors from acetol.

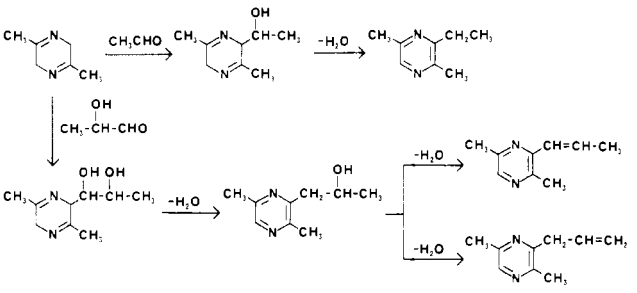

Figure 3. Formation of ethyl-, allyl-, and propenyl-substituted pyrazines from 2,5-dimethyl-3,6-dihydropyrazine.

formation of acetaldehyde, 2,3-butanedione, and 1,3-dihydroxy-2-butanone as shown in Figure 2.

Additional pyrazine formation pathways based on 2,5-dimethyl-3,6-dihydropyrazine are shown in Figure 3. Condensation of the dihydropyrazine intermediate with acetaldehyde, followed by dehydration, yields the 2,5-dimethyl-3-ethylpyrazine identified in the acetol/ammonium acetate system. If the tautomer of acetol condenses with the dihydropyrazine intermediate, the pyrazine generated after the loss of two molecules of water is either 2,5-dimethyl-3-allylpyrazine or 2,5-dimethyl-3-propenylpyrazine.

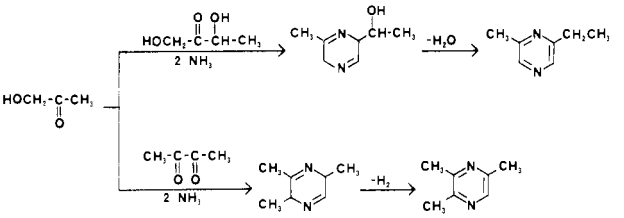

Figure 4. Formation of 2-methyl-6-ethylpyrazine and 2,3,5-trimethylpyrazine from acetol and acetol derivatives.

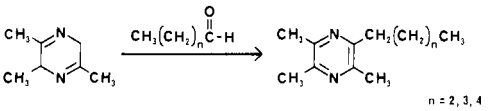

Figure 5. Formation of alkyl-substituted pyrazines from 2,3,5-trimethyl-3,6-dihydropyrazine and aldehydes.

Figure 4 illustrates the condensation of 1,3-dihydroxy-2-butanone or 2,3-butanedione with acetol in the presence of ammonia. Formation of 2-methyl-6-ethylpyrazine by condensation of α-amino carbonyl fragments has been discussed by Shibamoto and Bernhard (1977). Alternatively, condensation of 2,3-butandione with acetol in the presence of ammonia yields the reactive 2,3,5-trimethyl-3,6-dihydropyrazine intermediate, which can be dehydrogenated directly to 2,3,5-trimethylpyrazine. Condensation of a long-chain aldehyde with 2,3,5-trimethyl-3,6-dihydropyrazine can explain the formation of the trimethylpyrazines with butyl, pentyl, and hexyl substitutions (Figure 5). The origin of butanal and pentanal in the acetol/ammonium acetate/hexanal system is unclear.

Small amounts of 2,5-dimethyl-3-propylpyrazine, 2,6-dimethyl-3-propylpyrazine, and 2,3,5-trimethyl-6-butylpyrazine were also identified, but we have no simple explanation for the formation of these compounds at this time.

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Effect of Succinylation on Some Physicochemical and Functional Properties of the 12S Storage Protein from Rapeseed (*Brassica napus* L.)

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The physicochemical and functional properties of the native and succinylated 12S storage protein from rapeseed have been studied and found to depend on the degree of modification. Ultracentrifugation and isoelectric focusing were used for the characterization of the protein samples. The functionality was studied through adsorption kinetics at air/water interfaces and foaming and emulsifying properties. Succinylation results in a significant change of the interface adsorption kinetics of the native rapeseed globulin and in an improvement of foam capacity and stability as well as emulsion stability. These effects, greatly influenced by the level of succinylation, are discussed as being related to the dissociation of the oligomeric 12S globulin into subunits, to the unfolding of these subunits, and to the charge variations, depending on the degree of modification.

The 12S globulin represents a main storage protein in the seeds of *Brassica* species (Bhatty et al., 1968; Goding et al., 1970). It shows the typical properties of the oligomeric 11-12S plant storage proteins: a molecular weight around 300 000 (Schwenke et al., 1980), a quaternary structure composed of six subunits (Plietz et al., 1983; Schwenke et al., 1983a), a low content of α-helix and a high content of β-sheet (Schwenke et al., 1975; Zirwer et al., 1985), and two types of polypeptide chains with molecular weights around 30 000 and 20 000 forming the subunits of MW 50 000 (Schwenke et al., 1983a; Dalgalarrondo et al., 1986).

It has been shown by ultracentrifugation, viscometry, and CD spectroscopy that the spatial structure of the protein can be broken step-by-step by succinylation (Schwenke et al., 1986). This kind of modification, mainly performed for improving the functional properties of food proteins (Kinsella, 1976), has been applied to protein isolates from soybean (Franzen and Kinsella, 1976), peanut (Beuchat, 1977), sunflower seed (Canella et al., 1979; Schwenke and Rauschal, 1983), cottonseed (Choi et al., 1981), faba bean (Rauschal et al., 1981; Schwenke et al., 1983b; Prahl and Schwenke, 1986), and pea (Jonhson and Brekke, 1983).

A first investigation of functional properties of the succinylated rapeseed globulin was described by Nitecka and Schwenke (1986). A certain dependence of functional properties on the kind and level of modifications was found, but the structure-function relationships were not very often studied. It was the aim of the present work to investigate this relationship, expecting that the modification of size and charge induced by succinylation would influence the surface behaviors of the rapeseed globulin. It has been shown indeed by Graham and Phillips (Gra-

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