Effect of High Hydrostatic Pressure on the Volatile Components of a Glucose–Lysine Model System

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An aqueous glucose–lysine model system (initial pH 10.1) was incubated at 60 °C and atmospheric pressure (system A) or 600 MPa (system B) to the same absorbance value at 420 nm. Volatile reaction products were isolated by solvent extraction and analyzed by gas chromatography/mass spectrometry. Thirty-two compounds were identified; most contained nitrogen, and pyrazines predominated. Yields of all compounds were suppressed at 600 MPa. Further incubation, at either atmospheric pressure (system C) or 600 MPa (system D), of system A, resulted in lower yields of many compounds at 600 MPa, compared to prolonged incubation at atmospheric pressure. Many of the compounds reported may be formed by, or subsequently react via, aldol condensation. The observed differences among the systems in the profiles and yields of volatile compounds suggest that aldol condensations increase in rate in the systems under pressure.

Keywords: *High hydrostatic pressure; Maillard reaction; volatiles; glucose; lysine*

INTRODUCTION

Over the past 10 years, increasing attention has been given to the study of novel food-processing techniques, including high-pressure processing, which render food microbiologically safe while providing alternative functional properties and/or conservation of freshness (Ledward et al., 1995; Hayashi and Balny, 1996). High-pressure processing, involving pressures in the range 100–1000 MPa, is now commerically viable for selected foods. For this reason, interest has grown in the chemical and biochemical changes occurring in food subjected to high pressure, but only a few such studies have been reported concerning the Maillard reaction. Tamoaka et al. (1991) demonstrated the suppression of browning in a system comprising xylose and lysine dissolved in pH 8.2 sodium hydrogen carbonate solution [initial pH shown to be 6.3 by Hill et al. (1996)] incubated at 200–400 MPa, compared to the same system incubated at atmospheric pressure. Hill et al. (1996) showed that the rate of browning in a glucose–lysine system, incubated at 50 °C, was enhanced at 600 MPa, compared to atmospheric pressure, when the initial pH of the system was above ~7, but was retarded by elevated pressure at lower pH. Incubation of a pH 7 xylose–tryptophan system at 80 °C over the pressure range 50–800 MPa resulted in a progressive decrease in absorbance with increasing pressure, giving a volume of activation ($\Delta V^{\#}$) of 23 cm³/mol (Bristow, 1998). It was suggested that this effect could be due to the proven retardation rate of degradation of the Amadori rearrangement product, formed from trytophan and either xylose or glucose, both of which have a $\Delta V^{\#}$ of 17 cm³/mol (Isaacs and Coulson, 1996). Hill et al. (1998) demonstrated differences in the

profile of reaction products for a glucose–lysine system (initial pH 10.1), incubated at 60 °C at either 600 MPa or atmospheric pressure to the same absorbance at 420 nm. Analysis by capillary electrophoresis showed that the two systems were similar qualitatively, but several compounds occurred at significantly different levels in the two systems. Huang et al. (1996) have shown that the formation of tetramethylpyrazine was enhanced by pressure in a weakly acidic 3-hydroxy-2-butanone/ ammonium acetate system incubated at 25 °C and pressures between 1 and 50 MPa.

The aim of the study reported here was to identify and quantify the volatile reaction products formed in a glucose–lysine model system, incubated at either atmospheric or high pressure.

EXPERIMENTAL PROCEDURES

Materials. α-D-(+)-Glucose (ACS reagent), L-lysine (97%), and 1,2-dichlorobenzene (99%, spectrophotometric grade) were from Aldrich (Gillingham, U.K.). Diethyl ether (peroxide-free) was from Rhône Poulenc (Manchester, U.K.). *n*-Pentane (HPLC grade) was from Rathburn Chemicals Ltd. (Walkerburn, U.K.). *n*-Alkanes (C_6–C_{22}) and anhydrous sodium sulfate (AnalaR) were from BDH (Poole, U.K.). Standard chemicals used to determine linear retention indices were principally from Aldrich (Gillingham, U.K.) and Lancaster Synthesis (Morecambe, U.K.), and the highest purity grade available was used.

Preparation of Model Systems. Aqueous 1 molal glucose– lysine solutions (initial pH 10.1) were prepared, and 100 mL aliquots were incubated at 60 ± 0.1 °C in sealed polyethylene bags, either at atmospheric pressure or at 600 MPa, in a prototype Stansted Food Lab 900 high-pressure rig (Stansted Fluid Power Ltd., Stansted, U.K.). Samples were incubated at high pressure and/or atmospheric pressure for the times given in Table 1. Model systems were cooled on ice immediately after the incubation, and the pH and absorbance at 420 nm were measured. Between three and eight replicates of each model system were prepared. Blanks were prepared using pH 9.2 water.

Isolation of Volatile Reaction Products. Model system (100 mL) containing 1,2-dichlorobenzene internal standard (0.1

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Table 1. Incubations Applied to Glucose−Lysine Model Systems and Absorbance Data

code	incubation conditions	A^a
A	8 h at atmospheric pressure	0.44−0.48
B	5 h at 600 MPa	0.44−0.48
C	8 h at atmospheric pressure and then 3 h at atmospheric pressure	nd^b
D	8 h at atmospheric pressure and then 3 h at 600 MPa	nd

^{a} Absorbance of a 250-fold dilution of the incubated model system, measured at 420 nm. ^{b} nd, not determined.

mL of a 1.306 mg/mL solution in diethyl ether) was extracted with diethyl ether/*n*-pentane (80:20 v/v; 3 × 50 mL). The combined extracts were washed with distilled water (2 × 50 mL), dried over anhydrous sodium sulfate, and concentrated to 0.5 mL at 45 °C over 15 min using a Vigreux column. Reduction to a final volume of 0.1 mL was achieved using a stream of oxygen-free nitrogen. All extracts were stored at −18 °C and were analyzed by gas chromatography/mass spectrometry (GC/MS) within 24 h of preparation.

GC/MS. GC/MS was performed using a Hewlett-Packard (Bracknell, U.K.) HP 5890 series II gas chromatograph connected to a Hewlett-Packard 5972 series mass selective detector. The fused-silica capillary column (50 m × 0.325 mm i.d.) was coated with BPX5 (0.5 μm film) (SGE, Milton Keynes, U.K.).

Solvent extracts (1 μL) were injected using a Hewlett-Packard 7673 automatic injection system operated in the split/splitless mode, that is, operating in splitless mode during injection and then switching to split after 1 min (split flow = 20 mL/min). The injector temperature was 200 °C. The helium carrier gas was set at a pressure of 55 kPa at 50 °C (flow rate = 1.9 mL/min). The temperature program was 20 °C for 2 min, followed by a ramp rate of 4 °C/min to 250 °C, and held at 250 °C for 10 min.

Data acquisition and analysis were performed using the HP Chemstation G1034C version C.01.05 data system (Hewlett-Packard). Mass spectra were recorded in the electron-impact (EI) mode, with an ionization voltage of 70 eV and an ionization current of 50 μA. A source temperature of 175 °C and a scan range of 29−400 *m*/*z*, with a scan time of 0.69 s, were used.

Mass spectra were interpreted by spectral matching, using either the mass spectrometer data system library (NBS/NIH/EPA) or other collections of reference spectra. Linear retention indices (LRI values) of compounds were obtained by reference to a series of standard *n*-alkanes, run under the same conditions. Compound identifications were confirmed, when possible, by comparing experimental LRI values with those of authentic compounds.

Quantitative Analysis. Components were quantified by reference to the standard (1,2-dichlorobenzene). Student's *t* test (two-tailed *t* test of two groups of data with equal variance; type 2) (Microsoft Excel, version 5.0) was used to calculate *p* values and levels of significant differences between amounts of compounds subjected to different treatments.

RESULTS AND DISCUSSION

A pressure of 600 MPa was chosen because it represents a realistic pressure for the commercial pressurization of food systems. It symbolizes a balance between cost (which increases with increased pressure) and effectiveness. Only one high-pressure level was chosen for this study, the aim of which was to identify and quantify the volatile reaction products formed when the glucose−lysine system was incubated at either atmospheric or high pressure. Because the Maillard reaction is such a complex network of reactions, no additional information regarding the elucidation of mechanisms of formation of the compounds identified would have been

obtained by the use of additional levels of pressure. In a related study of a xylose−tryptophan system (Bristow, 1998), using additional pressure levels (over the range 50−800 MPa), the only additional information obtained was that levels of volatile compounds were proportional to the pressure applied.

Model systems prior to incubation were very pale yellow and virtually odorless. After incubation, all systems were dark brown with a sweet, "digestive biscuit" aroma. The pH of all systems had dropped from an initial value of 10.1 to pH 8.9 ± 0.5 after incubation. Model systems A and B were incubated at 60 °C, at either atmospheric pressure or 600 MPa, to give samples with the same level of absorbance at 420 nm, that is, 0.44−0.48 absorbance unit for a 250-fold dilution in water. This corresponded to incubation times of 8 and 5 h for the atmospheric and high-pressure systems, respectively. Thus, similar absorbance values were used to indicate similarity between systems A and B, in terms of color development. The pressure-induced increased rate of color development at alkaline pH in this model system has been explained (Hill et al., 1996; Hill, 1998). These incubation times gave volatile compound extracts that were sufficiently strong to yield good mass spectra. Few additional compounds were identified from system A when the incubation time was increased to 10 h.

Model Systems Incubated to the Same Degree of Absorbance. Table 2 lists the identities, LRI values, and amounts of the compounds isolated from systems A and B, the relative percentage yield (RPY) of each compound, expressed as the amount of compound detected in the pressurized sample relative to the amount detected in the atmospheric pressure sample (system A) and quoted as a percentage, the *p* values, and the level of significant difference between the amounts of each compound detected in both systems.

Six main peaks were detected in extracts of the blank model systems. They were attributed to solvent impurities or contamination from the polyethylene bags and are not included in Table 2. Another eight unidentified compounds, accounting for only ∼3% of the total area of system A, are not included in Table 2.

A total of 32 compounds from 8 eight chemical classes were identified in system A. LRI data were available for 13 of these compounds, confirming identifications made from library matching of mass spectra. However, LRI data were not available for the other compounds, the identifications of which are considered to be tentative.

Table 2 shows that the application of high pressure gives a substantial decrease in the yields of all the compounds listed, despite systems A and B having attained the same degree of browning. No compounds were detected in greater amounts in the pressure-treated samples, and many of the compounds were not detected in system B. There were significant differences between the amounts of all 18 compounds identified in both systems A and B, and these differences were significant at the 0.001 level for 16 of them.

Five classes had RPY values of <7%, whereas the remaining three were <15% (Table 3). The RPY of the grand total of all compounds listed in Table 2 was 3.8%. A comparison of the order of yields of compound classes in systems A and B shows that, despite compounds being present at far lower levels, the overall order of classes is very similar between the two systems, except for pyranones. Yields of pyranones are greatly sup-

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Table 2. Volatile Compounds Identified in the Solvent Extracts Prepared from Systems A and B[a]

compound	exptl LRI[b]	ref LRI	amount (µg/100 mL) A	B	RPY[c] (%)	p value[d]	L of S[e]
pyrazines							
methylpyrazine	835	833	256.6	2.7	1.1	0.0000	***
2,5/2,6-dimethylpyrazine	925	925	3950.9	110.4	2.8	0.0000	***
2,3-dimethylpyrazine	929	930	127.2	6.1	4.8	0.0000	***
2-ethyl-5/6-methylpyrazine	1011	1008	5.8	nd[f]	ao[g]	ao	ao
trimethylpyrazine	1013	1006	758.0	19.8	2.6	0.0000	***
2-ethyl-3,5/6-dimethylpyrazine	1087	1086	64.0	24.5	38.2	0.0002	***
3-ethyl-2.5-dimethylpyrazine	1093		28.1	nd	ao	ao	ao
2,6-diethylpyrazine	1096	1090	8.3	3.2	38.5	0.0122	*
2,5-dimethyl-3-(2-methylpropyl)pyrazine	1321		16.8	nd	ao	ao	ao
pyranones							
2,3-dihydro-5-hydroxy-6-methyl-(4H)-pyran-4-one	1104		51.0	3.4	6.6	0.0000	***
2,3-dihydro-3,5-dihydroxy-(4H)-pyran-4--one	1167		442.0	4.1	0.9	0.0000	***
pyridines							
2-acetyl-1,4,5,6-tetrahydropyridine	1061	1062	29.6	9.0	30.5	0.0004	***
2-acetyl-3-ethylidene-3,4,5,6-tetrahydropyridine	1472		5.3	nd	ao	ao	ao
2,5-diethylpyridine	1422		43.8	14.3	32.6	0.0001	***
3-methyl-2(1H)-pyridinone	1233		50.6	nd	ao	ao	ao
1,2,3,4,5,6-hexahydro-(7H)-cyclopentapyridine-7-one	1440		245.8	3.3	1.3	0.0000	***
6-methyl-1,2,3,4,5,6-hexahydro-(7H)-cyclopentapyridine-7-one	1457		21.2	nd	ao	ao	ao
3-acetyl-4-hydroxy-1,6-dimethyl-2(1H)-pyridinone	1672		70.9	nd	ao	ao	ao
furans and furanones							
5-methylfurfuryl alcohol	966		14.3	nd	ao	ao	ao
4,5-dihydro-2-methyl-3(2H)-furanone	820	820	13.8	3.4	25.0	0.0002	***
4-hydroxy-2,5-dimethyl-3(2H)-furanone	1076	1075	113.0	17.1	15.1	0.0002	***
pyrrolizines							
5-formyl-6-methyl2,3-dihydro-(1H)-pyrrolizine	1484		17.2	4.1	24.0	0.0012	**
7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine	1541		14.6	3.7	25.0	0.0001	***
5-acetyl-7-methyl-2,3-dihydro-(1H)-pyrrolizine	1634		11.0	nd	ao	ao	ao
7-acetyl-5,6-dimethyl-2,3-dihydro-(1H)-pyrrolizine	1778		78.3	9.6	12.2	0.0000	***
pyrroles							
2-acetylpyrrole	1084	1087	38.2	nd	ao	ao	ao
3-acetyl-1-methylpyrrole	1195		6.6	nd	ao	ao	ao
2-(1-pyrrolidinyl)-2-cyclopenten-1-one	1595		356.8	18.7	5.3	0.0000	***
alicyclic ketones							
3-methyl-1,2-cyclopentanedione	1043	1043	25.1	6.0	23.8	0.0009	***
2,5-dimethyl-2,5-cyclohexadiene-1,4-dione	1129	1129	6.5	nd	ao	ao	ao
2,3-dimethyl-2,5-cyclohexadiene-1,4-dione	1182		16.9	nd	ao	ao	ao
indolizines							
octahydroindolizine	1305		24.7	nd	ao	ao	ao

[a] Average of system A (eight replicate experiments) and system B (eight replicate experiments) data. Average coefficients of variation of peak areas <20% for both systems. [b] LRI values were calculated from retention data obtained using the BPX5 column and a standard solution of C_6-C_{22} n-alkanes. [c] RPY, relative percentage yield, i.e., yield from system B as a percentage of the yield from system A. [d] p values determined using Student's t test. [e] L of S, level of significant difference. *, <0.05; **, <0.01; ***, <0.001. [f] nd, not detected. [g] ao, detected only in system B.

Table 3. Yields of Individual Compound Classes Identified in the Glucose−Lysine Model Systems A and B

compound class	yield from system A µg/100 mL	%	yield from system B µg/100 mL	%	RPY[c] (%)
pyrazines	5215.7	75	166.6	63	3.2
pyranones	493.0	7.1	7.5	2.8	1.5
pyridines	467.2	6.8	26.6	10	5.7
pyrroles	401.6	5.8	18.7	7.1	4.7
furans and furanones	141.1	2.0	20.5	7.8	14.5
pyrrolizines	121.2	1.8	17.4	6.6	14.4
alicyclic ketones	48.5	0.7	6.0	2.3	12.4
indolizines	24.7	0.4	nd[a]	nd	ao[b]
total	6913.0		263.3		3.8

[a] nd, not detected. [b] ao, detected in system A only. [c] See footnote c, Table 2.

pressed under pressure (RPY value of 1.5%) and are lower than those of pyrrolizines in system B.

There are three possible explanations for the lower yields of volatile reaction products in system B compared to system A. First, the formation of volatile compounds could be retarded under pressure. Second, the formation of nonvolatile reaction products, including the high molecular weight end products of the Maillard reaction (melanoidins), under pressure may proceed via alternative pathways that produce fewer intermediate volatile compounds than the conventional ones. Third, subsequent formation of nonvolatile products from volatile compounds could be accelerated under pressure. A combination of these three possibilities may also be involved.

Model Systems Incubated at Atmospheric Pressure and Subsequently either at Atmospheric Pressure or at 600 MPa. Table 4 lists the RPY values for compounds identified in systems C and D, compared to system A, and the level of significant difference between the RPY values for each compound detected in both systems. The data indicate that subsequent incubation under pressure of the glucose−lysine model system containing a range of volatile compounds (system D) enhances the degradation or subsequent reaction of some compounds, which continue to be formed in the same system incubated at atmospheric pressure (system C). Compounds with the lowest RPY values in Table 4 usually possess an active carbonyl group and/or a

Table 4. Volatile Compounds of Model Systems C and D

compound	RPY[a]		*p* value[b,c]	L of S[c,d]
	C	D		
pyrazines				
methylpyrazine	131	74	0.0011	**
2,5- and/or 2,6-dimethylpyrazine	124	86	0.0042	**
2,3-dimethylpyrazine	142	98	0.0280	*
trimethylpyrazine	146	116	0.0060	**
2-ethyl-5-(and/or 6-)methylpyrazine	179	164	0.7540	ns
2-ethyl-3,5-(and/or 3,6-)dimethylpyrazine	169	173	0.5190	ns
3-ethyl-2,5-dimethylpyrazine	139	143	0.8958	ns
2,6-diethylpyrazine	167	176	0.8149	ns
pyranones				
2,3-dihydro-5-hydroxy-6-methyl-(4*H*)-pyran-4-one	153	32	0.0008	***
2,3-dihydro-3,5-dihydroxy-(4*H*)-pyran-4-one	209	21	0.0000	***
pyridines				
2-acetyl-1,4,5,6-tetrahydropyridine	110	265	0.0030	**
2,5-diethylpyridine	136	90	0.0031	**
3-methyl-2(1*H*)-pyridinone	131	104	0.0979	ns
1,2,3,4,5,6-hexahydro-(7*H*)-cyclopentapyridin-7-one	163	55	0.0008	***
6-methyl-1,2,3,4,5,6-hexahydro-(7*H*)-cyclopentapyridin-7-one	156	91	0.0147	*
3-acetyl-4-hydroxy-1,6-dimethyl-2(1*H*)-pyridinone	150	142	0.6357	ns
furans and furanones				
5-methylfurfuryl alcohol	310	75	0.0005	***
4,5-dihydro-2-methyl-3(2*H*)-furanone	200	43	0.0001	***
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	337	90	0.0009	***
pyrrolizines				
5-formyl-6-methyl-2,3-dihydro-(1*H*)-pyrrolizine	98	54	0.0152	*
7-acetyl-5-methyl-2,3-dihydro-(1*H*)-pyrrolizine	158	89	0.0606	ns
7-acetyl-5,6-dimethyl-2,3-dihydro-(1*H*)-pyrrolizine	171	86	0.0160	*

[a] RPY, relative percentage yield, i.e., yield from systems C and D, respectively, as a percentage of the yield from system A. [b] See footnote *d* to Table 2. [c] Values refer to differences in the RPY values for systems C and D. [d] See footnote *e* to Table 2.

methyl group and are thus potentially able to participate in aldol condensations.

Effect of High Hydrostatic Pressure on Yields of Compound Classes. The results obtained for all four model systems are discussed below by compound class.

Pyrazines. Quantitatively, pyrazines were the most important class of compounds identified in both systems A and B, which contained nine and six representatives, respectively. 2,5- and/or 2,6-dimethylpyrazine was the most abundant reaction product in both systems. The nine compounds identified in system A accounted for >75% of the total yield of identified compounds.

The formation of pyrazines in sugar–amino acid model systems under atmospheric conditions has been widely studied, and it is generally reported that significant pyrazine formation does not begin until 70 °C (Shibamoto and Bernard, 1976). In the current study, significant levels of pyrazines were formed at 60 °C, due to the high initial pH (10.1) of the system. This may be attributed to either increased reactivity of the amino groups of lysine toward the carbonyl group of glucose, resulting in an increase in the rearrangement and fragmentation of sugars (Koehler and Odell, 1970; Hwang et al., 1994), or to the increased rate of sugar mutarotation, as a result of high pH conditions (Spark, 1969).

Hwang et al. (1994) identified many pyrazines on heating an aqueous solution of glucose and lysine at 180 °C for 1 h at an initial pH of 8.5. Several pyrazines reported by them and in system A were the same, that is, the methyl, 2,5- and/or 2,6-dimethyl, 2,3-dimethyl, trimethyl, and 2-ethyl-5- (and/or 6-) methyl derivatives. The pyrazines possessing only methyl substitutents accounted for >97% of the pyrazines formed in system A, and pyrazines with longer chain substituents (ethyl or 2-methypropyl) were identified at much lower levels.
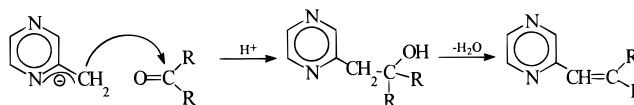

Figure 1. Proposed aldol-type condensation reaction of methylpyrazine with carbonyl compounds [based on Isaacs (1981)].

From Table 3 it can be seen that the RPY of pyrazines as a class in system B was only 3.2%. According to Table 2, the methyl, 2,5- and/or 2,6-dimethyl, 2,3-dimethyl, and trimethyl derivatives each have RPY values <5%, whereas formation of 2-ethyl-3,5- (and/or 3,6-) dimethylpyrazine and 2,6-diethylpyrazine was suppressed to a lesser extent in system B, RPY values being ~40%. Thus, the formation and/or degradation of these two ethylpyrazines is affected by the application of pressure to a lesser extent than that of the four methylpyrazines in system B. These differences could be due to the methyl substituents undergoing aldol-type condensations with carbonyl compounds, as shown in Figure 1. Base-catalyzed ionization of the methylpyrazine is generally favored by high pressure, as charge generation leads to pressure-favored electrostriction, resulting in a decrease in overall volume (Isaacs, 1981). The CH_2- moiety then undergoes nucleophilic attack on a carbonyl carbon, yielding the condensation product. As the reaction essentially involves dimerization of two compounds, this should show a negative volume of reaction and, therefore, be favored under pressure (Isaacs, 1981).

This explanation would support the lower reactivity of ethylpyrazines under pressure. Ionization of the ethyl group is less favored than that of the methyl group because ethyl hydrogen is less acidic than methyl hydrogen. The relatively high RPY value of 2-ethyl-3,5-(and/or 3,6-)dimethylpyrazine suggests that the presence of the ethyl substituent is conferring stability to the methyl substituents of the molecule. A possible

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explanation is that the ethyl substituent destabilizes the formation of CH_2- on the methyl groups, due to induction of negative charge from the ethyl group into the pyrazine ring.

Table 4 lists the RPY values for pyrazines identified in the model systems that were further incubated at either atmospheric pressure or 600 MPa (systems C and D). It can be seen that, of the eight pyrazines identified, four (all possessing only methyl substituents) had significantly higher RPY values in system C, and the methyl and dimethyl compounds all have RPY values of <100% in system D. This suggests that pressure is accelerating the further reaction of these compounds in system D, which continue to be formed in the atmospheric system (system C). In contrast, levels and RPY values of all the pyrazines that possess at least one ethyl substituent are not significantly different. This supports the hypothesis that ethyl substituents confer stability to the pyrazines at elevated pressure and suggests that the reduced levels of pyrazines possessing at least one ethyl substituent in system B (Table 2) is due to their formation being retarded under pressure.

Pyridines. Pyridines were the third largest class of compounds detected in system A, accounting for ~7% of the total yield of compounds. Pyridines also contained the second highest number of representatives (seven). 1,2,3,4,5,6-Hexahydro-(*7H*)-cyclopentapyridin-7-one accounted for over half of the total pyridine yield in system A.

Meynier and Mottram (1995) showed that pyridines were detected in significant amounts in a ribose–lysine system heated at 140 °C at pH values between 4.5 and 6.5, with yields increasing with pH.

Tressl et al. (1986) characterized 2-acetyl-1,4,5,6-tetrahydropyridine as a proline-specific Maillard reaction product, after identifying it in proline–monosaccharide systems. However, a formation pathway for pyrrolidine from lysine ARP has been proposed (Yaylayan and Sporns, 1989; Yaylayan et al., 1990). Attack by methylglyoxal at the nitrogen of pyrrolidine would produce reactive iminium ions, which could subsequently react via the mechanism proposed by Tressl et al. (1986) to form 2-acetyl-1,4,5,6-tetrahydropyridine, providing a possible explanation for its formation in a glucose–lysine model system in this study (Figure 2).

Incubation under pressure (system B) caused a decrease in yields of all pyridines, with an average RPY of only 5.7%, four pyridines being detected only in system A.

Table 4 shows the RPY values of two pyridines and four pyridinones identified in systems C and D. All of the representatives gave RPY values of >100% in system C, compared to system A. RPY values for both cyclopentapyridin-7-one derivatives were significantly lower in system D compared to system C, indicating that subsequent incubation at elevated pressure favored their degradation. This is supported by the results in Table 2, which show that these compounds were detected only at very low levels in system B. The RPY value for 2-acetyl-1,4,5,6-tetrahydropyridine was significantly higher in system D compared to system C. Its formation (outlined in Figure 2) is likely to be favored by pressure.

Pyrrolizines. The tentatively identified pyrrolizines accounted for ~2% of the total yield of compounds identified in system A. They are of interest because 2,3-dihydro-(*1H*)-pyrrolizines are generally considered to be
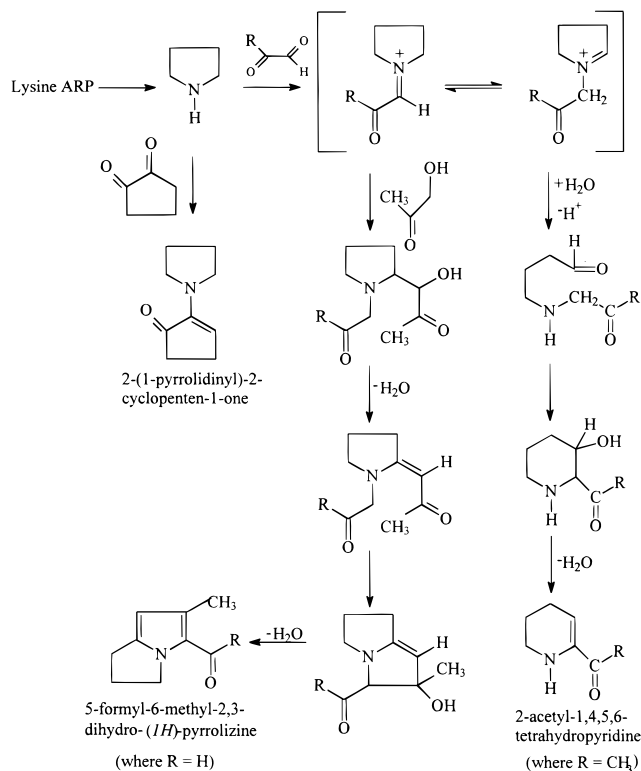

Figure 2. Proposed mechanism of formation for 2-acetyl-1,4,5,6-tetrahydropyridine, 5-formyl-6-methyl-2,3-dihydro-(*1H*)-pyrrolizine, and 2-(1-pyrrolidinyl)-2-cyclopenten-1-one from the lysine ARP [adapted from Tressl et al. (1985, 1986) and Ames and Apriyantono (1993)].

specific to proline systems (Tressl et al., 1985a, 1986), although Apriyantono and Ames (1993) tentatively identified 2,3-dihydro-(*1H*)-pyrrolizines in a xylose–lysine model system.

Incubation under pressure (system B) resulted in a decrease in yields of pyrrolizines and an RPY of 14.4% for the compound class, indicating that pyrrolizines, together with furans and furanones, were least affected by incubation under pressure.

It has been proposed (Apriyantono and Ames, 1993) that the formation of 2,3-dihydro-(*1H*)-pyrrolizines proceeds via pyrrolidine as an intermediate in lysine model systems in a pathway related to that concerned with the formation of 2-acetyl-1,4,5,6-tetrahydropyridine (Apriyantono and Ames, 1993), as shown in Figure 2. Thus, incubation under pressure may be expected to affect the formation of 2-acetyl-1,4,5,6-tetrahydropyridine and 2,3-dihydro-(*1H*)-pyrrolizines to the same extent. The RPY values reported in Table 2 for these compounds tend to support this.

All four pyrrolizines identified contain either an acetyl or a formyl substituent, which may undergo nucleophilic attack, for example, by the amino group of the amino acid or the methyl group of other compounds; for example, the pyrrolizine may undergo self-condensation. Such aldol-type condensation reactions are generally favored by high pressure.

Table 4 shows the RPY values of the three pyrrolizines identified in systems C and D. It can be seen that the two acetyl-2,3-dihydro-(*1H*)-pyrrolizines identified continued to be formed during subsequent incubation under atmospheric conditions, while 5-formyl-6-methyl-2,3-dihydro-(*1H*)-pyrrolizine remained at the same level. All three pyrrolizines gave RPY values of <100% in

system D, indicating that their subsequent reaction was favored by high pressure. However, levels of only two representatives were significantly different ($p = 0.05$) between systems C and D.

Pyrroles. Only three pyrroles are reported in system A, and two of these were not identified in system B. Under pressure, they may undergo pressure-favored reactions, for example, aldol condensation, due to the presence of active methyl and carbonyl substituents on all compounds. For example, they could undergo base-catalyzed aldol condensation with themselves.

The only report of the identification of 2-(1-pyrrolidinyl)-2-cyclopentenones in Maillard systems is that of Tressl et al. (1985b), who identified eight representatives in proline/pyrrolidine−monosaccharide model systems, reacted for 90 min at 150 °C. This is the third class of compound, initially characterized as proline-specific (Tressl et al., 1985a,b, 1986) reported in this study. Again, it is possible that pyrrolidine acts as an intermediate and subsequently reacts to form 2-(1-pyrrolidinyl)-2-cyclopentenones (Tressl et al., 1985a), as shown in Figure 2.

Pyranones. Pyranones were the second largest class of compound detected in system A, despite the identification of only two representatives (Table 2). Yields of pyranones were reduced to the greatest extent in system B, with a total RPY of only 1.5%. The huge reduction in the amounts of pyrazines in system B may be due to pressure favoring subsequent reactions involving them. As the rate of color development in system B was greater than that in system A, it is possible that intermediate compounds, such as these pyranones, act as precursors of colored components. Ledl and Severin (1982) proposed that sugar degradation compounds possessing an acidic methyl group are able to react with active carbonyl groups to yield colored compounds. Also, the pyranones identified could rapidly undergo aldol condensation, which would be favored by elevated pressure (Isaacs, 1981), which could also account for a low RPY value in system B.

Table 4 shows the RPY values of the two pyranones in systems C and D. The data for both compounds are similar and indicate a large increase in levels after continued incubation at atmospheric pressure (system C) but a highly significant ($p < 0.001$) reduction in the amounts detected in the pressurized system (D). The RPY values for both compounds in system D are much less than 100%. Therefore, while pressure may be inhibiting the formation of these compounds (system B), it is definitely favoring their degradation via subsequent reactions, accounting for their low levels in system D.

Furans and Furanones. Three representatives were identified, accounting for ∼2% of the total yield of compounds identified in system A. They included the important flavor compound 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (Furaneol). System B contained lower amounts of all three compounds, which gave a total RPY of 14.5%. Despite the fact that this value is still very small, reflecting the huge reduction in the amounts of these three compounds in the pressure-treated system, it was the largest RPY value among all of the compound classes. The methyl groups of 4,5-dihydro-2-methyl-3(*2H*)-furanone and 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone may react with carbonyl compounds as described above for pyranones.

Table 4 shows the RPY values of the three furan and furanone compounds identified in systems C and D. RPY values for all three compounds in system D were <100%. In contrast, the RPY values in system C were all >100%, indicating that these compounds continued to be formed under further incubation at atmospheric pressure. The significant differences observed between the RPY values for systems C and D for these compounds are attributed to pressure favoring subsequent reactions involving them.

In conclusion, pressure greatly suppressed the yields of all the identified volatile compounds formed in the glucose−lysine system of initial pH 10.1. Many of the identified compounds may form by, or subsequently react via, aldol condensation. It has been reported that high pressure can increase the rate of aldol condensations (Matsumoto et al., 1985). This helps to explain differences in yields of many compounds in the systems studied, including the very low RPY values in systems B and D, for pyrazines subsituted with methyl groups (which undergo aldol condensation) and the relatively high RPY values for compounds that may form by aldol condensation, such as 2-acetyl-1,4,5,6-tetrahydropyridine and 5-formyl-6-methyl-2,3-dihydro-(1*H*)-pyrrolizine, in system B.

Table 4 clearly indicates that the relative composition of the volatile compounds can be significantly modified by applying hydrostatic pressure in system D, compared to system C. This finding could be of importance to the flavor industry. More research should be done to better understand how hydrostatic pressure affects the Maillard reaction in foods.

ABBREVIATIONS USED

ACKNOWLEDGMENT

LITERATURE CITED

Apriyantono, A.; Ames, J. M. Xylose-lysine model systems: The effect of pH on the volatile reaction products. *J. Sci. Food Agric.* **1993**, *61*, 477−484.

Bristow, M. J. The effect of high pressure on the Maillard reaction. Ph.D. Dissertation, The University of Reading, 1998.

Hayashi, R., Balny, C., Eds. *High-Pressure Bioscience and Biotechnology*; Elsevier Science: Amsterdam, The Netherlands, 1996.

Hill, V. M. The effect of high-pressure processing on the Maillard reaction in a glucose-lysine model system. Ph.D. Dissertation, The University of Reading, 1998.

Hill, V.; Ledward, D. A.; Ames, J. M. Influence of high hydrostatic pressure and pH on the rate of Maillard browning in a glucose−lysine system. *J. Agric. Food Chem.* **1996**, *44*, 594−598.

Hill, V. M.; Ames, J. M.; Ledward, D. A.; Royle, L. The use of capillary electrophoresis to investigate the effect of high hydrostatic pressure on the Maillard reaction. In *The Maillard Reaction in Foods and Medicine*; O'Brien, J., Nursten, H. E., Crabbe, M. J. C., Ames, J. M., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1998; pp 121−126.

Huang, T.-C.; Fu, H.-Y.; Ho, C.-T. Mechanistic studies of tetramethylpyrazine formation under weak acidic conditions and high hydrostatic pressure. *J. Agric. Food Chem.* **1996**, *44*, 240−246.

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Hwang, H. T.; Hartman, T. G.; Rosen, R. T.; Lech, J.; Ho, C. T. Formation of pyrazines from the Maillard reaction of glucose and lysine-α-amine-^{15}N. *J. Agric. Food Chem.* **1994**, *42*, 1000–1004.

Isaacs, N. S. *Liquid-Phase High-Pressure Chemistry*; Wiley: Chichester, U.K., 1981.

Isaacs, N. S.; Coulson, M. Effect of pressure on processes modelling the Maillard reaction. *J. Phys. Org. Chem.* **1996**, *9*, 639–644.

Koehler, P. E.; Odell, G. V. Factors affecting the formation of pyrazine compounds in sugar-amine reactions. *J. Agric. Food Chem.* **1970**, *18*, 895–898.

Ledl, F.; Severin, T. Browning reaction of pentoses with amines. *Z. Lebensm. Unters. Forsch.* **1982**, *167*, 410–413.

Ledward, D. A., Earnshaw, R. G., Johnson, D. E., Hasting, A. P. M., Eds. *High-Pressure Processing of Foods*; Nottingham University Press: Loughborough, U.K., 1995.

Matsumoto, K.; Sera, A.; Uchida, T. Organic synthesis under high pressure. I. *Synthesis* **1985**, 1–26.

Meynier, A.; Mottram, D. S. The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chem.* **1995**, *52*, 361–366.

Shibamoto, T.; Bernard, R. A. Effect of time, temperature and reactant ratio on pyrazine formation in model systems. *J. Agric. Food Chem.* **1976**, *24*, 847–851.

Spark, A. A. Role of amino acids in nonenzymic browning. *J. Sci. Food Agric.* **1969**, *20*, 308–316.

Tamaoka, T.; Itoh, N.; Hayashi, R. High-pressure effects on Maillard reaction. *J. Agric. Food Chem.* **1991**, *55*, 2071–2074.

Tressl, R.; Rewick, D.; Helak, B.; Kamperschroer, H.; Martin, N. Formation of 2,3-dihydro-(*1H*)-pyrrolizines as proline specific Maillard products. *J. Agric. Food Chem.* **1985a**, *33*, 919–923.

Tressl, R.; Helak, B.; Koppler, H.; Renwick, D. Formation of 2-(1-pyrrolidinyl)-2-cyclopentenones and cyclopent(*b*)azepin-8(*1H*)-ones as proline-specific Maillard products. *J. Agric. Food Chem.* **1985b**, *33*, 1132–1137.

Tressl, R.; Helak, B.; Martin, N.; Renwick, D. Formation of proline specific Maillard products. In *Amino-Carbonyl Reactions in Food and Biological Systems*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Developments in Food Science 13; Elsevier: Amsterdam, The Netherlands, 1986; pp 235–243.

Yaylayan, V.; Sporns, P. Intrmolecular cyclisations of lysine Amadori rearrangement products. *Food Chem.* **1989**, *33*, 81–91.

Yaylayan, V.; Jocelyn Pare, J. R.; Laing, R. Intramolecular nucleophilic substitution reactions of tryptophan and lysine Amadori rearrangement products. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R. Eds.; Birkhäuser: Basel, Switzerland, 1990; pp 115–120.