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The effect of pH on the volatiles formed in an extruded starch–glucose–lysine model system

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Starch–glucose–lysine extrudates were produced using a twin-screw co-rotating machine. The final barrel zone and die temperatures were 150 ± 3°C and 150 ± 7°C, respectively. The residence time was 32 s, the moisture content of the feed was 18% (m m^{-1}) and the feed pH was 4.0, 5.0 or 7.7. Isolates of the volatile components were prepared using continuous steam distillation–solvent extraction with pentane:ether (9:1, v/v) as the solvent. Analysis by gas chromatography–mass spectrometry resulted in the identification of 32, 20 and 16 compounds, respectively, from each isolate. At pH 7.7, nitrogen compounds dominated the isolate and pyrazines accounted for over 80% (m m^{-1}) of the volatiles. At pH values of 5.0 and 4.0, the yield of pyrazines was less than 10% of that at pH 7.7 and furans comprised more than 80% (m m^{-1}) of these aroma isolates. The yield of total volatiles was more than 60% greater at pH 7.7 than at the other pH values. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Extrusion cooking is an important and versatile food process that can be used to manufacture foods as diverse as expanded and non-expanded breakfast cereals, snackfoods, modified starch, intermediate moisture petfoods, fishfoods and texturised protein (Harper, 1979; Frame, 1994). It is therefore surprising that there are relatively few reports on the volatile flavour components of extruded materials, particularly those derived from cereals. Most of the published work emanates from The State University of New Jersey at Rutgers: for example, Izzo *et al.* (1994) and Nair *et al.* (1994).

An important route to flavour development in extruded materials containing reducing sugars and amino compounds is the Maillard reaction, which is favoured by elevated temperature and intermediate moisture (Maga, 1989). One difficulty experienced with extruded cereal flours is the lack of flavour generated (Parliment, 1989). Although the material is at intermediate moisture during cooking, favouring the Maillard reaction, the cooking time is too short for substantial flavour development. For example, Shen and Hoseney (1995) reported that two compounds, 1-(2-furfuryl)pyrrole and 1-(2-furfuryl)-2-pyrrolealdehyde, played an important role in popped popcorn aroma but could not be detected among the aroma components of extruded corn meal. In addition, three pyrazines, 2-furfural and 4-vinyl-2-methoxyphenol were either absent or were present at a much lower level in the extruded corn meal.

Thermal degradation and loss of volatiles at the extruder die may also account for the inferior flavour of extruded cereals and Nair *et al.* (1994) studied differences between the volatiles released at the extruder die and those retained by the extrudate in order to gain a better understanding of the processes leading to flavour formation during extrusion of a corn flour.

Interest has been shown in supplementing the cereal feedstock with flavour precursors or intermediates to achieve the required flavour development. Izzo *et al.* (1994) studied the effect of adding the flavour precursors, pyruvaldehyde and ammonium bicarbonate, to wheat flour on the generation of Maillard aroma compounds. They showed that levels of pyrazines could be enhanced, leading to an extrudate with a more toasted character.

Various operating conditions and feed parameters, such as moisture content and pH, affect extrudate quality, including flavour, colour and degree of expansion. In a sensory study of corn extrudates, it was established that temperature and moisture both had significant effects on raw flour aroma and toasted corn aroma and flavour (Chen *et al.*, 1991).

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The Maillard reaction is so complicated that many studies have examined the chemistry taking place in model systems comprising a single reducing sugar and a single amino acid in water. In order to extend this approach to low moisture extruded systems, Bates *et al.* (1994) dispersed the reactant sugar and amino acid in a starch matrix. They established that, of the variables studied (i.e. temperature, time, pH and moisture), pH had the most significant effect on colour development in a starch–glucose–lysine model system. Extrudates showed more colour development and were less expanded as the pH increased. This paper reports the effect of modifying the extruder feed pH on the development of volatile compounds in this starch– glucose–lysine system.

MATERIALS AND METHODS

Materials

Wheat starch type A was obtained from ABR Foods (Corby, UK). D-(+)-glucose, L-lysine monohydrochloride, citric acid, sodium bicarbonate and calcium chloride were obtained from BDH (Poole, UK). The lysine was of biochemical grade and the other chemicals, including the glucose, were of analytical grade. Peroxide-free diethyl ether (AnalaR grade, Prolabo, Manchester, UK), pentane (AnalaR grade, Fisons, Loughborough, UK), and 1,2-dichlorobenzene (99%; Aldrich, Gillingham, UK) were used for the preparation of volatile extracts from the extrudates.

Methods

Preparation of extruded model system

Wheat starch (type A), D-(+)-glucose and L-lysine monohydrochloride were mixed (0.96:0.03:0.01) to give a homogeneous extruder feed. The pH values of the feeds were unadjusted (pH 5.0) or were adjusted to 4.0 and 7.7 by adding citric acid or sodium hydrogen carbonate to the mix. The feed was cooked in a twin screw co-rotating extruder, Model MPF50 (APV Baker Ltd, Peterborough, UK) to give an expanded product. Starch, without addition of sugar and amino acid and without adjustment of the pH (i.e. pH 5.0), was also extruded. The feed moisture content was 18% (m m^{-1}) and the residence time was 32 s. The temperatures in the final barrel zone and at the die were 150 ± 3°C and 150 ± 7°C, respectively. Measurements of pH were carried out using the AOAC (1990) method for flour. Full experimental details have been reported previously (Bates *et al.*, 1994).

Preparation of extracts of volatile compounds

Ground extrudate (75 g) was slurried with 1 litre of distilled water in the sample flask of a modified Likens and Nickerson apparatus (Nursten & Woolfe, 1972) fitted with a condenser cooled with circulating ethylene glycol at 4°C. Antibumping granules and 0.5 ml of antifoam agent were added. Pentane:diethyl ether (9:1, v/v, 30 ml) was the extraction solvent. Volatile components were extracted for 2 h. Extracts were cooled at −18°C overnight to remove water. 1,2-Dichlorobenzene (0.1%, v/v, in diethyl ether, 0.1 ml) was the internal standard added to each extract. Extracts were concentrated to 0.5 ml using a Kurdena–Danish apparatus with a waterbath at 45°C, prior to final reduction to 0.2 ml by blowing over a slow stream of nitrogen. A blank extract was prepared using only water in the sample flask. All concentrates were prepared in duplicate and were stored at 4°C prior to analysis.

Gas chromatography-mass spectrometry (GC-MS)

Samples were analysed in duplicate using an HP 5988 mass spectrometer equipped with an HP 59970 GC-MS workstation (revision 3.2) system (Hewlett-Packard, Bracknell, UK). The fused silica column (50 m×0.325 m i.d.) was coated with BPX-5 (0.5 μm film thickness), obtained from SGE Ltd. (Milton Keynes, Bucks, UK). The conditions of analysis were: helium, 1.66 ml min^{-1}; temperature programme, 50°C for 2 min followed by a ramp rate of 4°C min^{-1} to a final temperature of 250°C, held for 10 min; injector temperature, 200°C; injection volume, 1 μl; injection technique, Grob splitless; ionisation mode, electron impact; electron energy, 70 eV; ion source temperature, 250°C; ionisation current, 100 μA; accelerating voltage, 4 kV; scan speed, 1 s per decade; mass range, 33–400. Quantitative data were obtained from the mass spectral integration report. Experimental linear retention indices (LRI) were calculated with reference to the retention times of a series of standard alkanes, run under the same GC-MS conditions.

RESULTS AND DISCUSSION

During extrusion, the aromas of the pH 4.0 and 5.0 starch–glucose–lysine samples were similar and were described as cereal and toasted, with the pH 5.0 extrudate possessing a stronger aroma. In contrast, the aroma of the pH 7.7 sample was much stronger and was cooked, burnt, pungent and acrid. The starch extrudate gave almost no aroma during cooking.

The identifications and yields of volatile compounds formed from sugar and amino acid degradations from each isolate are given in Table 1. Blank extractions indicated that the antifoam agent gave rise to six peaks on the GC-MS trace, and these have been omitted from Table 1. A total of 30 compounds were fully identified and a further six were partially characterised. Thirty-two, 20 and 16 compounds, respectively, were identified from the starch–glucose–lysine system extruded at pH 7.7, 5.0 and 4.0. Only two of these compounds were identified from starch extruded at pH 5.0. Table 2 shows the yields of compounds by chemical class in each extrudate. Use of the Kurdena–Danish apparatus and final concentration with a slow stream of nitrogen resulted in a modified ratio of volatile reaction products, especially at very low concentration levels. These techniques were required to obtain components in sufficiently high concentrations for identification by GC-MS. Since all the aroma isolates were prepared in exactly the same way, it is reasonable to make comparisons between the levels of compounds present in each of them.

The data show that both the total yield and number of compounds are greater at pH 7.7, compared to pH 5.0 and 4.0, where total yields and numbers are fairly similar. At pH 7.7, the extrudate was dominated by pyrazines, which accounted for over 80% (m m^{-1}) of

Table 1. Yields[a] of volatile components identified in the extrudates

Compound	LRI		Starch–glucose–lysine			Starch
	Literature[b]	Experimental	pH 7.7	pH 5.0	pH 4.0	pH 5.0
Pyrazine			419	—	—	—
2-Methyltetrahydrofuran-3-one			115	—	—	—
Methypyrazine	**833**	**833**	5856	216	189	—
2-Furfural	**830**	**842**	1133	2368	3141	2043
2-Acetylfuran	**907**	**923**	—	400	413	—
2,5- and/or 2,6-Dimethylpyrazine	**925**	**926**	2536	—	—	—
Ethylpyrazine	**929**	**930**	560	413	472	—
2,3-Dimethylpyrazine	**930**	**932**	800	tr	344	—
Vinylpyrazine		**950**	440	147	128	—
5-Methylfurfural	**963**	**987**	1317	5640	5027	157
2-Ethyl-5- or -6-methylpyrazine	**1008**	**1007**	88	35	101	—
Trimethylpyrazine		**1013**	715	131	—	—
2-Ethyl-3-methylpyrazine		**1014**	tr	tr	—	—
2-Methyl-3-vinylpyrazine		**1029**	616	48	—	—
2-Methyl-5- or -6-vinylpyrazine		**1032**	24	—	—	—
2-Methyl-6- or -5-vinylpyrazine		**1035**	181	tr	tr	—
2-Ethyl-3,5-dimethylpyrazine	**1093**	**1085**	531	—	—	—
2-Ethyl-3,6-dimethylpyrazine		**1088**	27	—	—	—
2-Ethyl-5,6-dimethylpyrazine		**1094**	53	—	—	—
A dimethyl-2-vinylpyrazine		**1109**	141	—	—	—
?2-Ethyl-6-vinylpyrazine		**1122**	13	—	—	—
2-Ethyl-5- and/or -6-methylpyridine		**1143**	67	13	11	—
5-Methyl-6,7-dihydro-5(*H*)-cyclopentapyrazine		**1157**	37	37	24	—
3,5-Diethyl-2-methylpyrazine		**1163**	53	—	—	—
2,3-Dimethyl-5-propylpyrazine		**1166**	24	—	—	—
?1-(Dimethylfuranyl)-2-propanone		**1198**	-	16	—	—
A C_4 pyridine		**1211**	21	—	—	—
A methylbenzofuran		**1213**	—	67	59	—
A methylpyrrolo[1,2-*a*]pyrazine		**1225**	—	8	tr	—
Quinazoline		**1239**	29	—	—	—
5-Methyl-2-(5-methyl-2-furfuryl)furan		**1281**	35	75	88	—
1,2,3,4-Tetrahydroquinoline		**1285**	283	61	43	—
5-Methylquinoxaline		**1360**	32	—	—	—
145(100), 160 M(98), 159(79), 91(45), 118(43), 132(36)		**1392**	32	—	—	—
1,2-Dimethyl-2,3-dihydro-1*H*-indole or 2-methyl-1,2,3,4-tetrahydroquinoline		**1406**	21	—	—	—
158 M(100), 131(51), 130(45), 39(41), 90(37), 41(36), 63(35)		**1415**	29	—	—	—
41(100), 43(95), 39(81), 131(78), 95(73), 81(32), 174 M(44)		**1416**	35	19	11	—
2-(2-Furyl)-5,6-dimethylpyrazine		**1452**	35	—	—	—
173 M(100), 130(99), 122(96), 95(59), 77(39), 131(47), 158(35)		**1459**	24	48	—	—
An acetylmethyl-2,3-1*H*-pyrrolizine		**1540**	67	—	—	—
2-(5-Methyl-2-furfuryl)-5-furfural		**1541**	—	37	27	—

[a] Yields are quoted as ng g^{-1} extrudate. The figures given are the means of two independent runs and the precision is ±25%. (The precision was obtained from the means of the coefficient of variation of each figure.) –, the compound was not detected in this sample.

[b] Literature LRI values quoted were those found in the literature or obtained for the authentic compound using the same stationary phase.

Table 2. Yields[a] of compounds by chemical class

Chemical class	Starch—glucose—lysine			Starch
	pH 7.7	pH 5.0	pH 4.0	pH 5.0
Furans and derivatives	2600	8603	8755	2200
Pyridines and derivatives	371	74	54	—
Pyrazines and derivatives	13181	1035	1258	—
Miscellaneous	117	—	—	—
Unknowns	120	67	11	—
Total	16389	9779	10078	2200

[a]See footnote *a*, Table 1.

the volatile isolate. Several other nitrogen compounds (pyridines, quinazoline and an acetyl-2,3-1*H*-pyrrolizine) were also identified at this pH level. It is well documented that reactions between reducing sugars and amino acids leading to nitrogen-containing volatiles, particularly pyrazines, are favoured by increasing pH in aqueous systems (Leahy and Reineccius, 1989; Apriyantono & Ames, 1993). Leahy and Reineccius (1989) demonstrated that the activation energies for several alkylpyrazines decreased with increasing pH over the pH range 5–9 for a glucose–lysine system. The dominance of the volatiles from the pH 7.7 extrudate by pyrazines will contribute nutty, toasted and roasted character to this sample.

2,3-Dihydro-1*H*-pyrrolizines are normally associated with proline-containing systems (Tressl *et al.*, 1985). The formation of these compounds from lysine probably occurs via pyrrolidine as an intermediate and a possible mechanism has been proposed (Apriyantono & Ames, 1993).

At pH 5.0 and 4.0, total yields of furans were very similar. Most of the yield is due to furfural and 5-methylfurfural and these two compounds account for over 80% (m m^{-1}) of the total volatiles at pH 5.0 and 4.0 (Ames, 1990). The presence of both these furans in the starch extrudate indicates that the starch was not an inert matrix and was an additional source of carbohydrate precursor during extrusion. Four furans were identified at pH 7.7 with most of the total yield being accounted for by furfural and 5-methylfurfural, although these latter two compounds were present at much lower levels than in the pH 4.0 and 5.0 samples. It is well known that the routes to their formation in reducing sugar–amino acid systems are favoured by low pH. Their identification in the pH 7.7 sample is surprising but raises the question of the meaning of pH in such intermediate moisture systems. Another factor to consider is the possibility that interactions between pH and low moisture affect the course of the Maillard reaction.

A major degradation product of hexose sugars, including glucose, at low pH is 5-hydroxymethylfurfural (HMF). This compound was not identified in the volatile isolates since the procedure used to prepare the isolates of volatiles did not favour its extraction. Analysis of methanol extracts of the non-volatile compounds of these extrudates by HPLC has established that HMF accounted for more than 80% of the total peak area at 280 nm for the pH 4.0 extrudate, but was only detected in trace levels in the pH 7.7 sample (Bates, 1996).

The Maillard reaction is a major source of flavour and colour development in many heat-processed foods and it is worth considering the data presented in this paper together with the colour measurement data for extrudates prepared over the same pH range (Bates *et al.*, 1994). The L^* value decreased and the a^* and b^* values both increased with increasing pH. In contrast, the profiles of volatile compounds were very similar at pH 4.0 and 5.0 and very different at pH 7.7. Thus, it may be possible to steer colour and flavour development independently, at least to some extent, by careful control of the processing conditions.

CONCLUSION

Extrusion cooking of a starch–glucose–lysine model mixture at feed pH values of 4.0, 5.0 and 7.7 gives volatile isolates that are dominated by nitrogen compounds, especially pyrazines (at pH 7.7) and by furans (at pH 4.0 and 5.0). Yields of volatile compounds are also about 60% higher at pH 7.7 than at pH 4.0 and 5.0.

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