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The effect of pH on the formation of volatile compounds in meat-related model systems

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The effect of small pH changes on volatile compounds produced in the Maillard reaction was investigated using aqueous model systems. The reaction mixtures consisted of four amino acids, glycine, lysine, cysteine and methionine, heated individually with ribose at five pH values between 4·5 and 6·5. The colour, the overall aroma and the nature of the volatile compounds were all influenced by pH. At low pHs, 2-furfural was a major product of all the model systems, but its concentration decreased as the pH was raised. Nitrogen-containing compounds, such as pyrazines were detected at higher pHs, with the lysine model system producing the largest quantities of nitrogen-containing heterocyclic compounds. The major products of the methionine systems were dimethyl disulphide and 3-(methylthio)propanal and, as the pH increased, the latter compound showed a small decrease in concentration, while an increase in the disulphide was observed. The cysteine model system led to a large number of sulphur-containing compounds, including 2-methyl-3-furanthiol, a compound with a strong meaty aroma, whose formation was greatly favoured by lower pH.

INTRODUCTION

The Maillard reaction is an important route to many of the aroma volatiles found in cooked meat. In model systems, it has been established that the reaction is affected by pH; as the pH increases, the quantities of coloured and polymeric compounds increase (El'Ode *et al.*, 1966; Shu *et al.*, 1985). High pH values also favour the formation of certain aroma volatiles, but others are only formed under acid conditions. Previous studies on the effect of pH on the Maillard reaction have involved relatively large pH differences (2–5 pH units or more) often without buffers. It has been established that, in unbuffered model systems containing amino acids and sugars, pH variation of 3 or more units may occur during the heating (Whitfield *et al.*, 1988; Wong & Bernhard, 1988) and this may affect both the rate and the pathways of formation of volatile and coloured compounds.

Moreover, any study of meat-like model systems must take into account the high buffering capacity of meat, where the pH of meat, before cooking, is commonly in the range 5·5–6·0 and pH variations during cooking do not exceed 0·2–0·5 pH units. Thus, to study the influence of pH on the Maillard reaction, it is necessary to maintain a constant pH during the reaction. Phosphate and pyrophosphate buffers exhibit convenient buffering capacity. Phosphate buffers have been reported to catalyse the reaction (Potman & van Wijk, 1989), but, provided that the overall phosphate concentration remains constant, this should not prevent the comparison of reactions carried out at various pHs.

Sulphur-containing components have been reported to be key aroma compounds in meat (Werkhoff *et al.*, 1990; Mottram, 1991; Schutte, 1974). The importance of pyrazines in the overall aroma of roasted foods has also been well established (Maga, 1982).

This work was undertaken using four different amino acids: glycine and lysine, which are involved in the formation of heterocyclic nitrogen-containing compounds such as pyrazines, and cysteine and methionine, which are precursors of sulphur-containing compounds. The reducing sugar chosen was ribose, because of its high reactivity and its relevance as a flavour precursor in meat systems. The effect of small changes in pH, within the range 4·5–6·5, has been investigated on selected volatile components.

MATERIALS AND METHODS

Materials

Glycine, L-lysine, L-cysteine, L-methionine and D(–)-ribose were all purchased from Sigma. Disodium dihydrogen pyrophosphate, tetrasodium pyrophosphate and

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diethyl ether (AnalaR grade) were purchased from BDH. Pyrophosphate buffer was used instead of phosphate buffer because of its better buffering capacity in the chosen range of pHs (4·5–6·5). Reference chemicals were either purchased from reliable sources or obtained as gifts from flavour laboratories.

Preparation of the reaction mixtures

For each model system, an aqueous solution containing the amino acid (100 mM) and ribose (70 mM) was prepared in disodium pyrophosphate (0·2 M). Five portions of 25 ml were placed in 50 ml volumetric flasks, and the pH was adjusted to 4·5, 5·0, 5·5, 6·0 or 6·5 using tetrasodium pyrophosphate (0·2 M). The final volumes were then adjusted to 50 ml with pyrophosphate buffer of the appropriate pH. The reaction mixtures were heated in sealed glass ampoules at 140°C under pressure for 1 h (Whitfield *et al.*, 1988). The mixtures were cooled to room temperature, and the pH was measured.

Isolation of volatiles

Aliquots (2 ml) of each reaction mixture were diluted in 50 ml of glass-distilled water and the volatiles were extracted during 2 h with 20 ml of diethyl ether using a Likens–Nickerson simultaneous distillation extraction apparatus. Before the extraction, 100 μl of octadecane in *n*-hexane (50 ng/μl) was added to the diethyl ether as internal standard. The diethyl ether was dried over anhydrous sodium sulphate, and the extract was concentrated by fractional distillation over a Vigreux column to a final volume of approximately 100 μl.

Gas chromatography

Analyses were performed using a Hewlett-Packard 5890 gas chromatograph fitted with an on-column injector and a WCOT fused silica capillary column (30 m × 0·32 mm i.d., 1 μm film thickness) coated with DB5 (J&W Scientific). The helium carrier gas was set at a flow rate of 1·5 ml/min. The initial temperature of 40°C was maintained for 5 min and then increased to 220°C at 4°C/min, where it was held for 15 min. In order to calculate the linear retention index (LRI) of each component, a solution containing C-8 to C-20 *n*-alkanes in diethyl ether was also analysed. Individual components in the volatile extracts were quantified by comparison of GC peak area with the area of the internal standard. Since the response factors of each component were not determined, this provided only approximate concentrations.

Gas chromatography–mass spectrometry

A Carlo Erba 4200 gas chromatograph, equipped with a split–splitless injector was coupled with a Finnigan 4000 mass spectrometer. Analyses were carried out with the same capillary column as used for the GC analyses, with the end connected directly into the ion source heated at 250°C. The flow rate of the carrier gas (helium) was set at 1 ml/min. The initial column temperature of 60°C was maintained for 5 min and then increased to 220°C at 4°C/min) where it was held for 15 min. The mass spectrometer was operated in the electron impact mode with an electron energy of 40 eV. A continuous scan mode was employed with a scan time of 1 s over the mass range 33–400 amu. All GC–MS data were monitored, stored and processed using an INCOS 2100 data system. Components were identified by comparison of the mass spectra with known spectra from compounds analysed in this laboratory or from spectra in reference collections (Heller & Milne, 1978; ten Noever de Brauw *et al.*, 1980). Whenever possible, identification was confirmed by comparing the LRI of sample peaks with those of authentic standards, run under similar conditions.

RESULTS AND DISCUSSION

The chromatograms from the four reaction mixtures were complex. Previous studies have identified over 70 components of the glycine–ribose reaction and 120 from the cysteine–ribose reaction mixtures (Salter *et al.*, 1988; Farmer *et al.*, 1989). The amounts of selected volatiles are shown in Tables 1–4. The data were obtained from triplicate analyses, and coefficients of variation were below 20%, except for a few compounds present in low concentrations. The pyrophosphate buffer proved to be an efficient buffer in the pH range 4·5–6·5 since no change of more than 0·2 pH unit occurred after heating. Both qualitatively and quantitatively, the Maillard reaction products were strongly affected by pH, and the volatiles could be classified into three groups: those which exhibited a higher concentration as pH increased (pyrazines and pyridines), those showing lower concentration at high pH (furans and some sulphur-containing compounds) and, finally, those which were not affected by pH variations (some heterocyclic sulphur components).

Colour and overall aroma

For all the model systems, colour as well as overall aroma were affected by pH variations. The solutions became browner as pH increased. The colour of the reaction mixtures depended on the amino acid. Glycine and lysine-containing solutions were yellow–brown at pH 4·5 and brown at pH 6·5. Cysteine and methionine solutions were yellow–orange at low pH and tended to be red–brown at high pH values. These observations are in good agreement with the literature (El'Ode *et al.*, 1966; Shu *et al.*, 1985). For the overall aroma, glycine- and lysine-containing model systems were described as caramel-like at pH 4·5, which became sweet, nutty and roasted as pH increased. The cysteine model system was described as strongly sulphurous, unpleasant at pH 4·5 and became more roasted-meat-like and less sulphurous at higher pH values. Finally, the methionine-

Table 1. Approximate quantities (μg/10 mg ribose) of selected volatiles formed in the reaction of glycine and ribose at pHs between 4·5 and 6·5. Each value is the mean of 3 replicates

Compound	LRI	pH				
		4·5	5·0	5·5	6·0	6·5
Pyrazines						
Methylpyrazine	824	nd	nd	0·3	0·5	0·7
2,5-dimethylpyrazine	912	nd	nd	0·5	3·0	4·0
Furans						
2-Furfural	831	151·0	10·1	3·6	0·3	tr
2-Furylmethanol	855	1·2	3·0	2·9	4·3	3·4
4,5-Dihydro-2-methyl-3(2*H*)-furanone	805	nd	0·4	0·6	1·5	1·2
4-Hydroxy-5-methyl-3(2*H*)-furanone	1042	12·9	5·2	5·5	1·2	0·8

LRI: linear retention index.
nd: not detected (limit of detection *c.* 10 ng/10 mg ribose).
tr: trace (<0·1 μg/10 mg ribose).

Table 2. Approximate quantities (μg/10 mg ribose) of selected volatiles formed in the reaction of lysine and ribose at pHs between 4·5 and 6·5. Each value is the mean of 3 replicates

Compound	LRI	pH				
		4·5	5·0	5·5	6·0	6·5
Pyrazines						
Methylpyrazine	824	nd	0·4	1·3	4·1	4·6
2,5-Dimethylpyrazine	912	nd	0·1	0·6	2·8	5·0
Pyridines						
2-ethyl-4-methylpyridine[a]	1134	0·9	1·4	2·5	3·4	5·4
A dimethylpyridine[a]	1262	0·9	1·2	1·8	5·6	6·5
Furans						
2-Furfural	831	10·7	4·0	tr	nd	nd
2-Furylmethanol	855	1·4	1·0	1·4	1·9	1·9
4-Hydroxy-5-methyl-3(2*H*)-furanone	1042	8·1	7·1	7·3	1·4	1·3

LRI: linear retention index.
nd: not detected (limit of detection *c.* 10 ng/10 mg ribose).
tr: trace (<0·1 μg/10 mg ribose).
[a]Tentative identification based on comparison of mass spectra with literature spectra.

containing mixture was burnt, cabbage-like at pH 4·5 and, as pH increased, the aroma was described as cabbage, potato-like and less unpleasant.

Pyrazines and pyridines

Some alkyl pyrazines were found in the model system from all the amino acids at pH 5·0 and above, but their quantities were greater in the lysine- and glycine-containing systems. Lysine provided the highest concentration, and methionine the lowest. Pyridines were detected in significant amounts in the lysine model system. Both the nature and quantities of these nitrogen-containing heterocycles were affected by the amino acid structure.

Alkyl pyrazines are commonly found in roasted and toasted foods. Several researchers have proposed mechanisms for alkyl pyrazine formation in various carbohydrate/amine systems (Koehler *et al.*, 1969; Shibamoto & Bernhard, 1977; Rizzi, 1988; Wong & Bernhard, 1988). The carbohydrate was shown to provide the carbon atom while amino acids mostly furnished only nitrogen to the pyrazine molecule (Koehler *et al.*, 1969). Pentoses were reported to be more reactive than hexoses (Shibamoto & Bernhard, 1977). Moreover, it appeared that aldoses, such as ribose, gave rise to pyrazines more readily than did ketoses (Koehler & Odell, 1970). One of the main routes to pyrazines is via the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compound, and the condensation of two of these molecules results in an alkyl pyrazine. The mechanism is initiated by the condensation of the amino acid with the carbonyl group, and the ease of the nucleophilic attack of the amino nitrogen on the carbon of the carbonyl would be influenced by the structure of the amino acid. Consequently, each amino acid would be expected to give a different product distribution. Our results corroborate this hypothesis.

It has been established previously that a basic pH favours pyrazine formation in the Maillard reaction

Table 3. Approximate quantities (μg/10 mg ribose) of selected volatiles formed in the reaction of cysteine and ribose at pHs between 4·5 and 6·5. Each value is the mean of 3 replicates

Compound	LRI	pH				
		4·5	5·0	5·5	6·0	6·5
Pyrazines						
Methylpyrazine	824	nd	nd	nd	0·9	3·6
Dimethypyrazine	912	nd	nd	nd	0·3	1·5
Furans						
2-Furfural	831	3·9	1·5	1·0	0·6	tr
2-Furylmethanol	855	nd	1·3	1·5	0·8	0·9
4,5-Dihydro-2-methyl-3(2*H*)-furanone	805	nd	0·2	0·6	0·8	0·7
4-Hydroxy-5-methyl-3(2*H*)-furanone	1042	10·6	6·1	5·2	2·6	3·0
2-Methyl-3-furanthiol	868	15·2	6·3	1·7	1·0	tr
2-Furylmethanethiol	909	8·5	6·0	3·1	1·5	1·4
Thiophenes						
2-Formylthiophene	1000	2·9	2·7	2·9	1·9	3·6
2-Formyl-5-methylthiophene	1125	1·5	2·0	2·4	2·6	4·0
2-Propionylthiophene	1188	3·0	3·7	3·2	1·5	1·8
4,5-Dihydro-3(2*H*)-thiophenone	948	0·4	0·7	1·6	1·5	2·8
2-Methyl-4,5-dihydro-3(2*H*)-thiophenone	987	5·7	6·7	6·0	4·8	5·1
2-Thiophenethiol[a]	969	3·8	3·4	3·9	1·5	1·7
Thieno[2,3-*b*]thiophene	1205	1·3	0·8	0·3	nd	nd
Other sulphur compounds						
3-Mercapto-2-pentanone	903	5·4	5·0	3·3	0·5	tr
Thiazole	715	nd	nd	2·4	1·9	2·3
2-Acetylthiazole	1018	nd	0·3	0·5	1·6	4·4
1,2-Dithian-4-one[a]	1169	1·4	1·0	1·2	0·9	2·0
3-Methyl-1,2-dithian-4-one[a]	1312	tr	0·3	0·7	0·9	2·0

LRI: linear retention index.
nd: not detected (limit of detection *c.* 10 ng/10 mg ribose).
tr: trace (<0·1 μg/10 mg ribose).
[a]Tentative identification based on comparison of mass spectra with literature spectra.

Table 4. Approximate quantities (μg/10 mg ribose) of selected volatiles formed in the reaction of methionine and ribose at pHs between 4·5 and 6·5. Each value is the mean of 3 replicates

Compound	LRI	pH				
		4·5	5·0	5·5	6·0	6·5
Pyrazines						
Methylpyrazine	824	nd	nd	nd	nd	tr
Furans						
2-Furfural	831	92·4	10·5	3·2	2·1	1·9
2-Furylmethanol	855	3·1	3·4	3·6	4·0	4·9
4-hydroxy-5-methyl-3(2*H*)-furanone	1042	3·1	2·1	1·1	1·2	1·8
2-Furylmethyl methyl sulphide	1002	2·5	3·2	5·5	7·6	7·5
Aliphatic sulphur compounds						
Dimethyl disulphide[a]	727	16·2	22·2	24·82	8·6	41·9
3-(Methylthio)propanol	903	167·2	98·6	138·2	109·1	90·0
Dimethyl trisulphide[a]	965	0·5	0·6	0·6	0·5	0·5

LRI: linear retention index.
nd: not detected (limit of detection *c.* 10 ng/10 mg ribose).
tr: trace (<0·1 μg/10 mg ribose).
[a]Tentative identification based on comparison of mass spectra with literature spectra.

(Leahy & Reineccius, 1989), which may be explained by decreased reactivity of the amino group at lower pH due to its protonation. Shu & Ho (1988) studied the effect of a large variation in pH on the formation of aroma compounds in a model system containing cysteine and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. Pyrazines were detected at pH 7·1, but not at 5·1 or 2·2, and they suggested that the amino group of cysteine was more reactive at pH 7·1. Our results are in good agreement with this hypothesis. Nevertheless, the reaction mixture containing methionine, which has a lower $pK_{a(NH_3)}$ value than cysteine (9·21 instead of 10·28), gave

much less pyrazines than the cysteine system. This suggests that the protonation of the amino group is not the only factor involved in the formation of pyrazines.

In the lysine-containing model system, alkyl substituted pyridines exhibited the same pH dependence, and the availability of unprotonated amino groups may again offer an explanation.

Furans derivatives

Considerably more 2-furfural was obtained from the glycine and methionine reaction than from lysine or cysteine, and larger amounts were formed at the lower pHs with the greatest variations occurring between pH 4·5 and pH 5·0. Moreover, most of the other furan derivatives exhibited the same pH dependence.

Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by a deamination and dehydration. This tautomerism is believed to be favoured by lower pH, while 2,3-enolization is more dependent on higher pH (Nursten, 1980). This may provide an explanation for the larger quantities of furfural at pH 4·5. In the glycine model system, furfural was the major compound at pH 4·5, while it was produced in lower quantities in the lysine system. This difference could be due to the extra amino group of lysine, which is able to react with the aldehyde group of furfural. This reaction leads to the formation of coloured compounds. The reaction of aldehyde with an amino group could increase with pH as the concentration of unprotonated amine increased. The four reaction mixtures showed much more browning as pH increased, and this was particularly noticeable for lysine. In the case of cysteine, the reaction of furfural with hydrogen sulphide, formed via the Strecker degradation, provides a further loss of furfural, leading to lower concentrations in the final reaction mixture when compared with the systems containing the other amino acids.

Another furan compound, namely 4-hydroxy-5-methyl-3(2*H*)-furanone, also showed a decrease in concentration with increasing pH, but the effect was not as marked as for furfural. This furanone is particularly interesting because of its importance in meat and roasted flavours in foods where it is produced by the thermal degradation of ribose. Shu *et al.* (1985) have studied the degradation of one analogue, namely, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. The compound was more stable at pH 5·1 than 2·2 or 7·1 and they found that more volatile compounds were produced at the lower pH.

Sulphur compounds

Sulphur-substituted furans are particularly interesting because some of them have been shown to possess meat-like aroma (MacLeod, 1986; Mottram, 1991). A number of such compounds were found in the cysteine/ribose reaction mixtures, the major ones being 2-methyl-3-furanthiol and 2-furylmethanethiol. Their concentrations were markedly pH-dependent (as pH increased, the quantities in the reaction mixtures decreased). 2-Methyl-3-furanthiol is an important meaty aroma compound and it can be formed by the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with hydrogen sulphide (van den Ouweland & Peer, 1975; Whitfield *et al.*, 1993). The concentration of 2-methyl-3-furanthiol dropped markedly between pH 4·5 and 5·0. The decrease continued between pH 5 and 5·5 with only low concentrations at the higher pHs. A similar effect of pH was observed with trace quantities of disulphides, derived from 2-methyl-3-furanthiol, which were isolated from a similar reaction mixture containing cysteine and ribose (Farmer & Mottram, 1990). The variation of 2-methyl-3-furanthiol concentration with pH was of importance in regard to meat flavour. This compound possessed a 'burnt rubber' odour when it was concentrated and a 'meaty, beef broth' aroma on dilution. Its odour threshold value has been reported to be very low, 5–10 μg/kg (Gasser & Grosch, 1988). The flavour intensity of meat is known to increase with decreasing pH, and the marked pH dependency of the generation of this compound may offer an explanation for the relationships between pH and meat flavour which have been observed (Dransfield *et al.*, 1985; Lawrie, 1992). 2-Furylmethanethiol was also preferentially formed at lower pHs. It is believed to be important in coffee aroma as well as being found in roast meat. It is probably formed by the reaction of 2-furfural or 2-furylmethanol and hydrogen sulphide. The higher concentrations of both these furan derivatives at lower pH may be tentatively explained by the availability of hydrogen sulphide, whose formation from cysteine, by hydrolysis or via the Strecker degradation, could be favoured by lower pH.

Other sulphur compounds, such as thiophenes, thiazoles, 3-mercapto-2-pentanone and sulphur-heterocyclics, were identified in the cysteine reaction mixture. The concentration of thiazoles increased with pH, which could be due to the increased availability of ammonia at higher pH (Mulders, 1973). For other compounds the variations of their concentration with pH were not apparent, probably because their formations involved multistep reaction and various precursors.

The methionine reaction mixture produced other sulphur-containing compounds, including 3-(methylthio)-propanal (methional, the Strecker aldehyde from methionine), dimethyl di- and tri-sulphides and 2-furylmethyl methyl sulphide. With the exception of the small quantities of dimethyl trisulphide, the formation of these sulphur compounds also showed pH dependence. Methional was the most abundant volatile from this reaction mixture and it decreased slightly in concentration as the pH increased, while the dimethyl disulphide increased. Methanethiol is produced in the Strecker degradation of methionine and this was responsible for these sulphides by oxidation or by reaction with furfural or furylmethanol.

CONCLUSIONS

The Maillard reaction between four amino acids and sugars in buffered medium was pH-dependent. The in-

tensity of browning increased with pH, and the overall aroma of the four model systems varied with pH. For small pH variation ($\Delta pH = 0{\cdot}5$), the concentrations of several compounds were highly modified. Pyrazines were formed at pHs above 5·0. The lysine-containing model system gave the largest amounts of pyrazines, and pyridines were only formed in this system. The model system containing cysteine produced a large number of sulphur compounds including some which possessed meaty or roasted aromas. The formation of meat aroma compounds such as 2-methyl-3-furanthiol was favoured by low pH value (pH 4·5). The changes in volatiles with pH could be explained by changes in the concentration of one or more of the intermediates in the Maillard reaction. It seems that the protonation of the functional group of the amino acid was also important. This study clearly shows that control of pH is a very important parameter for the Maillard reaction in foods and model systems.

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