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Comparison of the non-volatile ethyl acetate-extractable reaction products formed in a xylose–lysine model system heated with and without pH control

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Aqueous solutions of xylose (1 M) and lysine monohydrochloride (1 M), initial pH 5.27, were refluxed for 1 h, either without pH control (final pH 2.83) or by maintaining the pH at 5 throughout heating by the addition of sodium hydroxide solution. The ethyl acetate-extractable components were obtained from each system and analysed by TLC and HPLC (with diode array detection). The study illustrates the effect of pH during heating on the TLC and HPLC separation patterns obtained for the non-volatile reaction products. None of the colourless compounds and only two of the coloured compounds detected were common to both systems. One of these coloured compounds is 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone (FHMF). Compounds U1(1) and U2(2) are both coloured and were isolated from the system without pH control. Their similar electronic absorption and PMR spectra indicate certain common structural features, including the presence of two terminal furan rings, each substituted in the 2-position.

INTRODUCTION

pH has an important influence on the profile of products formed during the Maillard reaction (Ames, 1990). As far as volatile compounds are concerned, the yields of various classes and individual compounds may reach maxima at specific pH values; e.g, pyrazine production increases with pH over the pH range 5–9 (Leahy & Reineccius, 1989; Mottram & Leseigneur, 1990). The effect of pH on the volatile reaction products identified in the xylose–lysine monohydrochloride model system heated either with the pH controlled at 5 or without pH control (final pH 2.83) as studied in the current paper, has already been reported (Apriyantono & Ames, 1993; Ames & Apriyantono, 1994).

The rate of Maillard browning, as measured by absorbance at a single wavelength in the range 420–490 nm, increases with pH over the pH range 4–8 (Kawashima *et al.*, 1980; Westphal *et al.*, 1988). When the pH range is extended up to pH 12, the rate of browning in sugar–amino acid model systems shows a maximum at a pH of about 10 (Ashoor & Zent, 1984). Relatively little work has been carried out on the coloured compounds formed during the Maillard reaction, although they may be divided into 2 classes, the melanoidins and the low molecular weight structures (Ames, 1992). The melanoidins possess molecular weights of several thousand daltons, while all the coloured low molecular weight compounds identified to date have relative molecular masses of less than 500 Da (Ames *et al.*, 1993). Only one previous study has reported the effect of pH on the formation of non-volatile Maillard reaction products (Miller *et al.*, 1984). An aqueous solution of glucose and glycine was refluxed for 30 h either without pH control (pH dropped from 4.9 to 3.1 during heating) or at a constant pH of 4.5. An additional reaction product, 5-(3,4,5,6-tetrahydropyrid-3-ylidene-methyl)-2-furanmethanol, was reported when the pH was kept at 4.5 throughout heating. No mention was made of the colour properties of the model systems or of any individual compounds identified. The current paper reports a comparison of the low molecular weight non-volatile reaction products obtained from selected fractions of a xylose–lysine monohydrochloride model system heated with and without pH control.

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Table 1. Solvent systems used for the separation of the coloured bands obtained from the ethyl acetate–extractable components of the model systems

Band code	R_f value[a,b]	Solvent system
U1	0·09	Methyl acetate : water (8 : 1)
U2	0·23	Methyl acetate : water (8 : 1)
U3	0·30	Ethyl acetate : toluene (2 : 1)
U4	0·43	Ethyl acetate : toluene (5 : 1)
U5	0·54	Ethyl acetate : toluene (1 : 1)
U6	0·66	Methyl acetate : water (8 : 1)
C2	0·49	Ethyl acetate : toluene (1 : 1)
C3	0·54	Dichloromethane : acetronitrile (4 : 1)

[a] R_f = retardation factor.

[b] Solvent system used for the ethyl acetate–extractable components was chloroform : ethyl acetate : 95% ethanol in the ratio 15 : 1 : 1 (for the system without pH control) and 20 : 1 : 1 (for the system with pH control).

MATERIALS AND METHODS

Materials

All chemicals used were the purest available. D-(+)-Xylose (99+%, Gold Label) was obtained from Aldrich Chemical Company Ltd, Gillingham, Dorset, UK. L-Lysine monohydrochloride (chromatographically homogeneous) and analytical-grade solvents were obtained from BDH Chemicals Ltd, Poole, Dorset, UK. HPLC-grade methanol was obtained from Rathburn Chemicals Ltd, Walkerburn, UK and HPLC-grade water was prepared in the laboratory using a Purite Labwater RO50 unit (Purite Ltd, High Wycombe, UK).

Methods

Aqueous model systems consisting of xylose (0.5 mol) and lysine monohydrochloride (0.5 mol) in 500 ml degassed distilled water, i.e. one molal solutions, were heated either with control of the pH at 5 or without pH control (final pH 2.83±0.14) to give the total browning products (TBP). The ethyl acetate-extractable browning products (EABP) were isolated and HPLC and TLC separations were performed. PMR, IR, mass and electronic absorption spectra and absorbance measurements of selected fractions and purified components were obtained. Full experimental details are given in Ames *et al.* (1993). Solvent systems used for the TLC separation of the coloured bands obtained on TLC of EABP from the systems heated with pH control (Bands C2 and C3) and without pH control (Bands U1–U6) are given in Table 1.

RESULTS AND DISCUSSION

A comparison of the data obtained for the non-volatile components of the model systems with and without pH control was made and the findings are summarised in Table 2. Both model systems changed colour during heating from pale yellow to dark brown. The absorbance of TBP was higher in the system with the pH maintained at 5, and this was expected since, in general, the rate of brown colour development during the Maillard reaction increases with increasing pH (Kawashima *et al.*, 1980; Westphal *et al.*, 1988).

Table 2. Comparison of data obtained for the non-volatile components of the model systems with and without pH control

Property	Without pH control	With pH control
Absorbance units at 420 nm[a]	0·102	0·160
Yield of EABP[b]	0·36 ± 0·05	0·41 ± 0·05
Number of TLC bands[c]	7[d]	4[e]
Colourless compounds[f]	17	29
Coloured compounds[f]	14[g]	5[g]
Presence of FHMF	Detected[h]	Detected[i]
Presence of U1(1) and U2(2)	Detected[j]	Not detected
Presence of 2-furfural	Detected[i]	Not detected
Presence of HMFone	Not detected	Detected[i]

[a] TBP diluted 1 : 1250 in distilled water.

[b] Percent (m/m) of the initial reactants.

[c] Obtained from EABP, separated using chloroform : ethyl acetate : 95% ethanol (15 : 2 : 2) as the mobile phase.

[d] Six bands were coloured.

[e] Two bands were coloured.

[f] Detected by HPLC with diode array detection from EABP and fractions obtained by TLC.

[g] Two compounds were common to both systems.

[h] Identified by IR and MS.

[i] Detected by HPLC with diode array detection.

[j] Analysed by NMR.

Extraction with ethyl acetate gave EABP which accounted for <0.5% (m/m) of the initial reactants in both systems. The yield of ethyl acetate-extractable components was higher in the system with pH control although the numbers of coloured TLC bands and coloured compounds detected by HPLC with diode array detection were lower. TLC of EABP resulted in two coloured bands (Bands C2 and C3) and in six coloured bands (Bands U1–U6), respectively, for the systems heated with and without pH control. In addition, two colourless bands were observed in the system with the pH maintained at 5, while a single colourless band was present on TLC of the system in which the pH was allowed to drop.

Analysis of the coloured bands by further TLC resulted in the separation of seven and seventeen bands, respectively, from the systems with and without pH control and several of them were yellow. Analysis of all the coloured TLC bands by HPLC with diode array detection resulted in the separation of several colourless and coloured peaks. (Absorption spectra were reconstructed from the diode array data and components were considered to be coloured when absorbance occurred in the visible region.) The data obtained are summarised in Table 2.

The separation patterns obtained on TLC and HPLC of EABP from the two model systems illustrate how different the reaction products of Maillard systems can be after heating the same mixture with and without pH control. Analysis of the reconstructed diode array data showed that none of the colourless HPLC peaks and only two of the coloured compounds reported in this study were present in both systems.

An important compound present in the system in which the pH was allowed to drop was 2-furfural. This is the major compound formed on degradation of the pentose Amadori Rearrangement Product (ARP) via 1,2-enolisation, the route favoured by low pH (Hodge, 1967). 2-Furfural accounted for more than 99% (m/m) of the total volatiles of this xylose–lysine monohydrochloride system heated without pH control (Apriyantono & Ames, 1993). This compound could not be identified in the system in which the pH was maintained at 5 (by HPLC with diode array detection) but it was identified at a level of 52% (m/m) of the total volatiles (Apriyantono & Ames, 1993). A Likens and Nickerson apparatus was used for continuous collection of the volatile compounds during heating and this minimised further reactions involving 2-furfural, leading to the presence of relatively high levels of the compound in the volatile fraction.

4-Hydroxy-5-methyl-3(2*H*)-furanone is formed on degradation of the ARP via 2,3-enolisation, the route favoured at neutral and higher pH (Hodge, 1967), and was identified in the system with the pH maintained at 5. It was also identified in trace amounts among the volatiles of this system (Apriyantono & Ames, 1993) but could not be detected in the system in which the pH was allowed to drop. No attempt was made to identify the other colourless compounds summarised in Table 2; however, their structural elucidation is likely to provide clues regarding the structures of coloured Maillard reaction products as well as possible routes to their formation.

The different pH conditions used during heating also affected the numbers of coloured compounds detected by HPLC with diode array detection (see Table 2). The presence of a lower number of coloured compounds in appreciable amounts in the ethyl acetate extract of the system with pH control could be due to the formation of a more limited range of compounds in this system or to certain compounds reacting to give more polar compounds which were not extracted by the ethyl acetate. The former explanation seems the more likely, since the yield of EABP was higher in the system with the pH maintained at 5. The possible formation of ethyl acetate-extractable melanoidins must also be taken into account. Melanoidins are final products of the Maillard reaction and they are coloured. Since the rate of colour production increases with pH, it is likely that melanoidins are present in higher amounts in the system with pH control. The possible presence of ethyl acetate-extractable melanoidins was indicated by the presence of a 'hump' on the HPLC chromatograms of EABP from both model systems and this type of chromatogram is characteristic of a polydisperse polymer (Snyder *et al.*, 1983). However, such chromatograms may also be due to unresolved complex mixtures of strongly absorbing compounds of low molecular weight.

Table 3. PMR data for Compound U2(2)

Chemical shift (ppm)	Multiplicity[a]	Coupling constants, *J* (Hz)	Inference
1·78	s	—	3H
5·86	dt	2·0 and 6·0	1H
6·24	d	3·5	1H
6·42	dd	3·5 and 2·0	1H
6·77	dd	3·5 and 2·0	1H
7·19	d	3·5	1H
7·31	d	2·0	1H
7·45	s	—	1H, no vicinal H
7·60	d	2·0	1H
8·02	s	—	1H, no vicinal H
8·09	d	2·0	1H

[a] s, singlet; d, doublet; dd, double doublet; dt, double triplet.

Two of the coloured compounds detected are present in both model systems. The first was identified as 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone (FHMF) and has been discussed by Ames *et al.* (1993). The second compound was not identified. No attempt was made to quantify FHMF in this study but this compound is one of the major low molecular weight coloured compounds formed in the system without pH control, while it was detected in smaller amounts in the system with the pH maintained at 5. It would seem that, in the system with pH control, either the availability of 2-furfural limits the formation of FHMF or FHMF may take part in further reactions, e.g. with carbonyl compounds.

Selected compounds were collected and purified from the model system without pH control, prior to structural analysis. The analysis of one of these, Compound U1(1), has been discussed in detail (Ames *et al.*, 1993). Compound U1(1) was isolated from TLC Sub-band U1M3. It has an empirical formula of $C_{25}H_{19}O_7N$, a λ_{max} in methanol at 406 nm with a subsidiary maximum at 334 nm, and probably has four rings including two terminal furan rings substituted in the 2-position. A second compound, Compound U2(2) was isolated from TLC Band U2 by further TLC and semi-preparative HPLC. It has a retention time of 49.23 min (compared to a retention time of 42.39 min for Compound U1(1)) on analysis by HPLC with the single wavelength detector, and its purity was 88%, based on detection at 420 nm. Only UV-visible and PMR spectra were obtained for Compound U2(2). Its electronic absorption spectrum in methanol shows a λ_{max} at 406 nm, a subsidiary maximum at 334 nm and a shoulder at 242 nm. The spectrum is very similar to that of Compound U1(1) (Ames *et al.*, 1993), suggesting that the two compounds have very similar structures. The PMR data for Compound U2(2) (see Table 3) also show many similarities to those obtained for Compound U1(1) (see Ames *et al.*, 1993), again suggesting that the structures of the two compounds are very similar. The signals obtained account for 13 protons and no aldehydic group is indi-

Table 4. Comparison of PMR data obtained for terminal furan rings (substituted in the 2-position) for U1(1) and U2(2)

Compound U1(1)[a]			Compound U2(2)			Assignment[c,d]
Chemical shift (ppm)	Multiplicity[b]	Coupling constant *J* (Hz)	Chemical shift (ppm)	Multiplicity[b]	Coupling constant *J* (Hz)	
6·78	dd	1·8, 4·0	6·77	dd	2·0, 3·5	C4
7·17	d	4·0	7·19	d	3·5	C3
8·04	d	1·8	8·09	d	2·0	C5
6·42	dd	1·9, 3·2	6·42	dd	2·0, 3·5	C4'
6·25	d	3·2	6·24	d	3·5	C3'
7·59	d	1·9	7·60	d	2·0	C5'

[a] Ames *et al.* (1993).

[b] d, doublet; dd, double doublet.

[c] C3, C4, C5, C3', C4' and C5' refer to carbon atoms 3, 4, 5, 3', 4' and 5', respectively, on the appropriate furan ring.

[d] References for similar compounds: Bhacca *et al.* (1962), O'Reilly (1982).

cated. Two terminal furan rings, each substituted in the 2-position, appear to be present in both compounds and relevant PMR data for both compounds are given in Table 4. Compounds U1(1) and U2(2) show several other signals and coupling constants at very similar values, as seen in Table 3 and in Ames *et al.* (1993). Compounds U1(1) and U2(2) were only detected in the model system with a pH drop. These compounds each contain two terminal furan rings which are likely to derive from 2-furfural, which was formed in large amounts in this system.

CONCLUSIONS

Although the EABP of the aqueous xylose–lysine model system heated with and without pH control accounts for <0.5% (m/m) of the initial reactants, analysis by TLC and HPLC with diode array detection shows a large number of components to be present. The profile of EABP is greatly affected by pH and only two of the compounds detected were common to both systems. The role of the low molecular weight coloured compounds in the Maillard reaction is not clear. They may be end-products in their own right or intermediates in reactions which ultimately lead to the formation of melanoidins.

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