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The influence of pH on the non-volatile reaction products of aqueous Maillard model systems by HPLC with diode array detection

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Aqueous solutions of sugar (xylose or glucose) and amino acid (glycine or lysine monohydrochloride), one molal with respect to each reactant, were heated without control of the pH for up to 120 min. Total reaction products were analysed by HPLC with diode array detection and the data obtained were distinctive and different from those of the corresponding model systems maintained at pH 5 throughout heating. Less unresolved material and variations in its chromatographic behaviour suggested differences in the melanoidins formed. Resolved peaks from the xylose–lysine and glucose–lysine systems were grouped into spectral families, based on their diode-array spectra. For xylose–lysine, seven of these peaks were common to the systems heated both with and without pH control for 15 min (based on retention time and spectral matching). No peaks were common to both glucose–lysine systems heated for 120 min. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The Maillard reaction is greatly influenced by the pH of the system and, at lower pH values, the formation of furfural (from pentoses) or 5-hydroxymethylfurfural, HMF, (from hexoses) is favoured. Higher pH values favour the formation of furanones (Nursten, 1986).

Maillard model systems have been examined at constant pH values with control of the water activity (Fox *et al.*, 1983; Buera *et al.*, 1987), at constant pH values in aqueous solution (Wolfrom *et al.*, 1953; Ames *et al.*, 1993; Ames and Apriyantono, 1994). When the pH is not controlled, it falls throughout the reaction, giving a different profile of reaction products compared to the analogous system reacted at constant pH. In foods, the pH may drift or remain constant during processing and storage. Therefore, the modelling of either of these situations has practical implications.

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Aqueous xylose–lysine systems have been heated with control of the pH at 5 and without pH control and two compounds were common to both systems (Ames *et al.*, 1993; Ames and Apriyantono, 1994). The first structure of a three-ring compound isolated from a xylose–lysine system has recently been reported by Arnoldi *et al.* (1997). Investigations have been limited to one sugar–amino acid combination and no attempt was made to assign peaks to spectral families based on their diode-array spectra.

Bailey *et al.* (1996*a*) refluxed four aqueous sugar (xylose or glucose)–amino acid (glycine or lysine monohydrochloride) solutions with the pH maintained at 5, prior to analysis by reversed-phase HPLC with diode-array detection (HPLC–DAD). By analysing the systems with time of heating, a series of chromatograms was obtained which could be used to develop chromatographic maps of the systems. Both similarities and differences between the different sugar–amino acid combinations were apparent.

This paper compares and contrasts the HPLC–DAD data obtained for the same four sugar–amino acid systems refluxed without control of the pH with the systems maintained at pH 5 during heating, and assigns the compounds reponsible for some of the resolved peaks to families, based on their diode-array spectra.

MATERIALS AND METHODS

Model systems were prepared and analysed according to Bailey *et al.* (1996*a*), except that the pH of the systems was allowed to fall during heating. In summary, aqueous solutions (one molal with respect to the sugar and the amino acid) were refluxed for up to 120 min. The sugar was xylose (Xyl) or glucose (Glu) and the amino acid was glycine (Gly) or lysine monohydrochloride (Lys). The pH and absorbance at 460 nm of each system were measured after heating for predetermined times. After heating, the total reaction products were analysed by HPLC–DAD using a reversed-phase (Spherisorb 5 μm ODS2) column and a water–methanol gradient (50–850 ml litre^{-1} over 60 min). Background corrected spectra of reaction products and standard compounds were carried out using Hewlett Packard (Bracknell, UK) Chemstation software. Spectral matches of less than 999 were rejected, a spectral match of 1000 being perfect.

RESULTS AND DISCUSSION

The pH of the systems decreased with time of heating (Table 1). Absorbance at 460 nm is plotted against heating time for each system in Fig. 1 and increased with time of heating. (The relative average deviations of the uncorrected absorbance values were within 50%.)

Little browning was observed when Xyl or Glu were heated alone, but the pH dropped over 30 min from 4.3 to 3.7 and from 4.5 to 4.2, respectively. Heating Lys or Gly alone for 30 min caused the pH to drop from 5.7 to 4.7 and from 5.0 to 4.7, respectively, and gave small unretained peaks only, with no browning. The most complex chromatograms for each system were obtained after heating for 15 min for Xyl-Lys, and after 120 min heating for the other systems. Therefore, the discussion will focus on the chromatograms obtained after these heating times.

The chromatograms of the model systems showed four features, i.e. unretained material (retention time < 3 min), resolved peaks (retention times range from 3 to 30 min) and convex and tailing broad bands (eluting between 3 and 30 min; Bailey *et al.*, 1996*a*). Of the four wavelengths chosen to monitor the separations (254, 280, 360 and 460 nm), most detail was observed on the 280 nm chromatograms. The chromatograms of the systems heated without pH control were different from those of the systems reacted at pH 5. In order to facilitate a comparison of the systems, the 280 nm chromatograms of the four systems heated without pH control and with the pH maintained at 5 during heating are shown in Figs 2 and 3, respectively. The identities given for selected peaks in Fig. 2 were obtained by comparing the retention times and diode-array spectra with those of the standard compounds (Bailey *et al.*, 1996*a*). The systems are discussed by comparing and contrasting the similarities and differences observed in the four types of chromatographic behaviour, i.e. unretained material, resolved peaks and broad bands.

Table 1. pH of the model systems with time of heating

Reaction time (min)	Xyl-Lys	Glu-Lys	Xyl-Gly	Glu-Gly
0	5·5	5·5	5·2	5·2
6	5·0	4·5	4·7	4·5
15	4·0	4·5	4·6	4·5
30	3·5	4·3	4·5	4·5
60	3·0	4·0	4·2	4·3
90	3·0	3·7	n.d.	n.d.
120	2·9	3·5	3·9	4·1

n.d., not determined.

Unretained material

Unretained material, similar to that obtained from the analogous systems reacted with pH control, was observed when the pH was allowed to fall during heating. Diode-array spectra indicated that the material formed after 6 min absorbed in the visible region and was pale yellow in colour. After more than 6 min, this material absorbed further into the visible region and was brown (Bailey *et al.*, 1996*a*). The development of the unretained material with heating time was very similar for model systems heated both with and without pH control.

Resolved peaks

Either furfural (for the Xyl systems) or HMF (for the Glu systems) gave the largest resolved peak on the 280 nm chromatograms. This was in contrast to the systems heated at pH 5, where either no furfural or HMF or, in the case of Glu-Gly, a lower amount of HMF, was observed (Monti *et al.*, 1996).

The 280 nm chromatogram of Xyl-Lys, after 15 min with no control of the pH, showed many well resolved peaks (Fig. 2a), and peaks with similar diode-array spectra were grouped into five spectral families, A–E (Table 2). Seven of the 10 main peaks listed in Table 2 were common to the system at pH 5 (Fig. 3a). Some of the peaks that are considered to be the same in the system, heated with and without pH control and listed in Table 2, have slightly different λ_{max} values. These slight variations are due to minor differences in obtaining the background subtracted spectra using the diode-array detector software. These small differences did not present a problem for spectral matching.

The influence of the drifting pH on the reaction was more evident at longer heating times. For example, only three of the 10 main peaks were common to both systems after 90 min (8.02 min in Family A and 7.10 and 9.39 min in Family B) (Bailey *et al.*, 1996*b*). Small resolved peaks were observed on the 360 nm (six main peaks) and 460 nm (four main peaks) chromatograms after 15 min of reaction without pH control, and diode-array spectra showed these to be due to coloured (yellow) compounds.

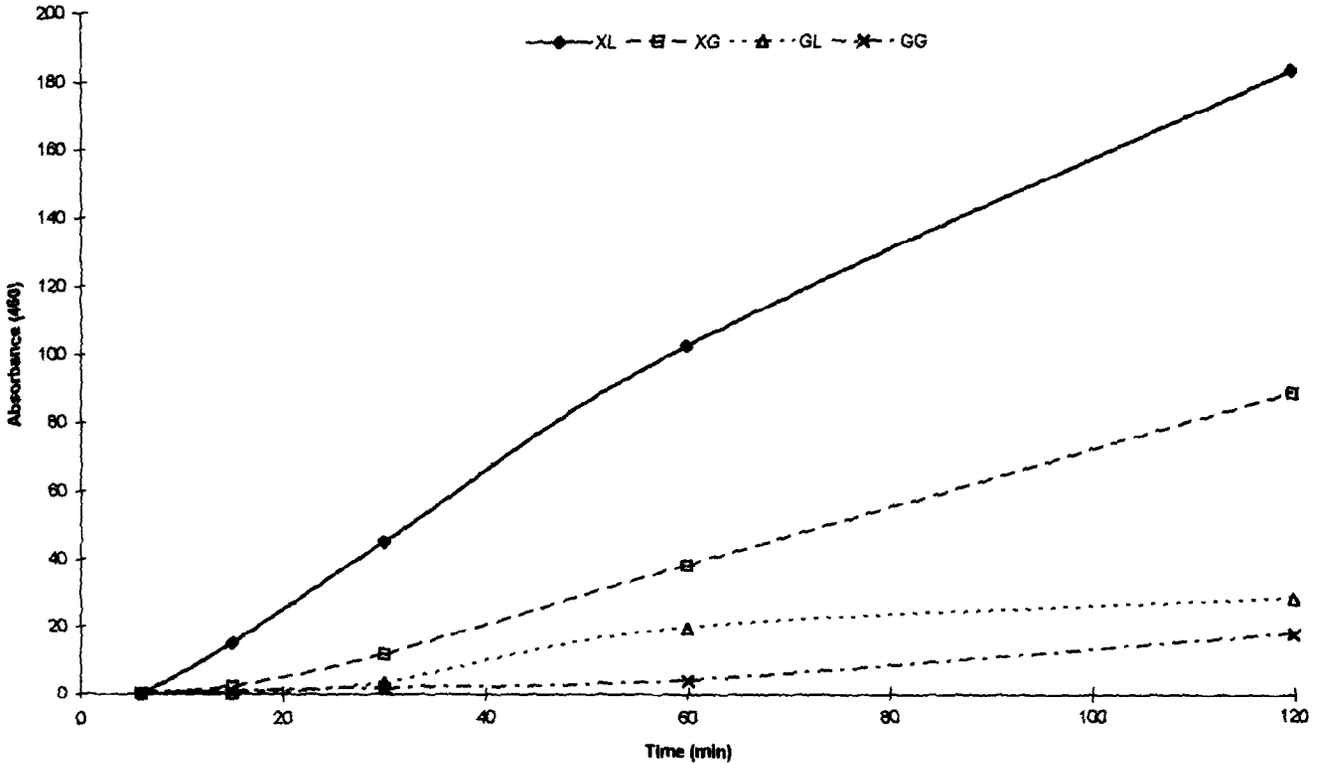

Fig. 1. Corrected absorbance × dilution values at 460 nm of aqueous sugar–amino acid model systems with time of heating without pH control. XL, Xyl-Lys; XG, Xyl-Gly; GL, Glu-Lys; GG, Glu-Gly.

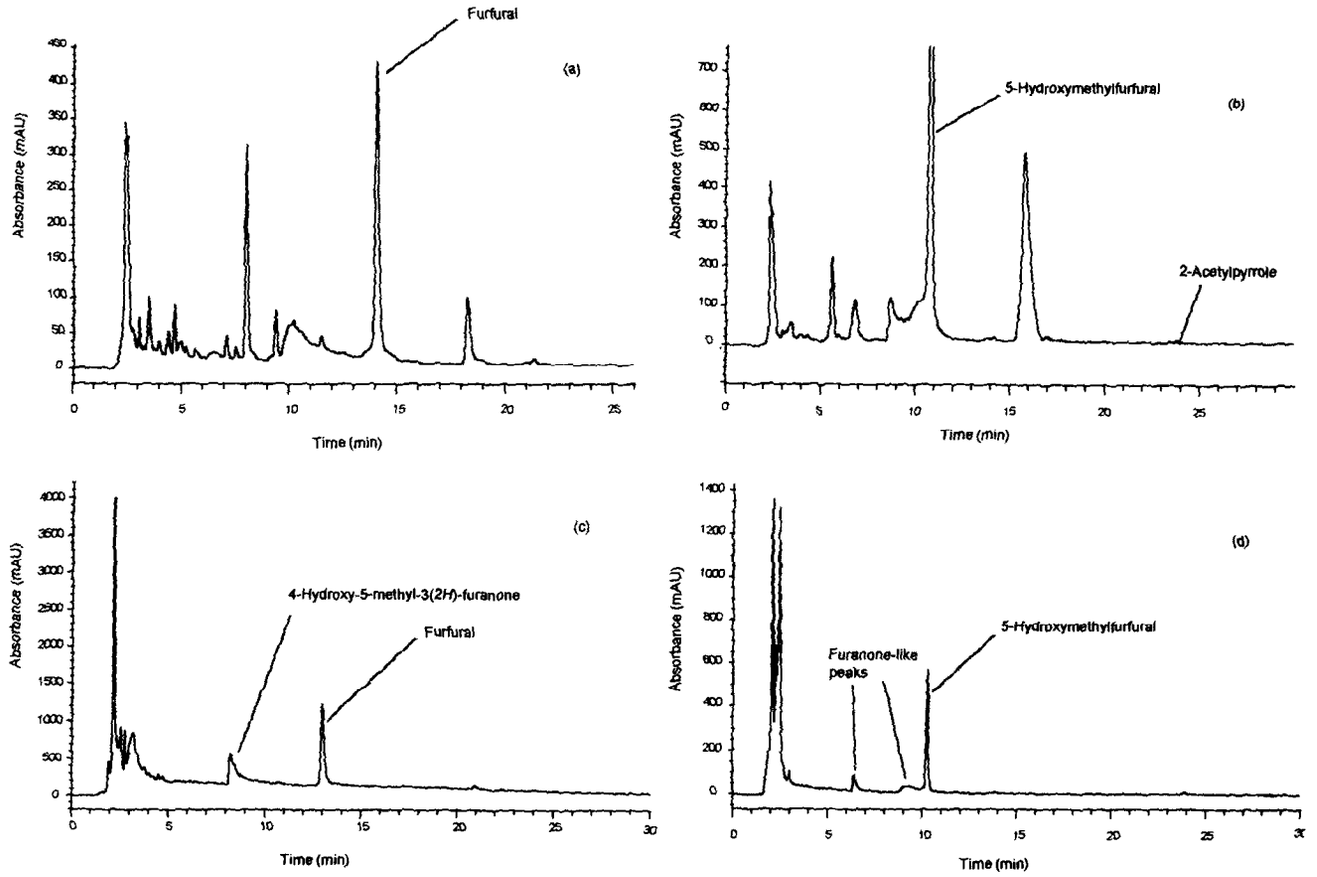

Fig. 2. Chromatograms (280 nm) of the model systems heated without pH control. (a) Xyl-Lys, 15 min, (b) Glu-Lys, 120 min, (c) Xyl-Gly, 120 min, and (d) Glu-Gly, 120 min.

Table 2. Diode array spectral matching of peaks from xylose–lysine model systems refluxed for 15 min without pH control and at pH 5

Spectral family	Without pH control			With pH control		
	Absolute retention time (min)	Relative retention time[a]	λ_{max} (nm)	Absolute retention time (min)	Relative retention time	λ_{max} (nm)
A	3·48	0·49[b]	271sh, 297	3·23	0·47[b]	271sh, 297
	4·40	0·62	271sh, 299	4·62	0·67[c]	269sh, 303
	4·68	0·66[c]	269sh,301	7·40	1·08	269sh, 299
	8·02	1·13[d]	267sh, 297	7·80	1·14[d]	267sh, 297
B	7·10	1·00[e]	269, 335	6·87	1·00[e]	269, 331
	9·39	1·32[f]	269, 331	9·16	1·33[f]	269, 331
	11·52	1·62[g]	267, 333	11·12	1·62[g]	267, 333
C	7·82	1·01	317	6·40	0·93	269sh, 319
				4·14	0·60	317
D	18·30	2·58[h]	261sh, 293	17·31	2·52[h]	261sh, 293
E	19·43	2·74	237, 351			

[a] Relative to the peak at 7·10 min in Family B.
[b–h] Peaks with the same superscript letter are considered to be identical compounds.
sh, shoulder.

Spectra from the 280 nm chromatogram of Glu-Lys after 120 min (Fig. 2b) gave many matches with the system at pH 5 (Fig. 3b, Table 3), but no retention time matches were found. Thus, although spectral families were common to the two systems, this sugar–amino acid combination was unique among the four systems in having no individual compounds that were present in the systems heated both with and without pH control. Both HMF and 2-acetylpyrrole were present after 120 min in the system in which the pH was allowed to fall. Small peaks were observed on the 360 and 460 nm traces when the pH was uncontrolled. The 460 nm chromatograms for the systems heated with and without maintaining the pH at 5 were very different (Fig. 4).

The 280 nm chromatogram of Xyl-Gly without pH control (Fig. 2c) showed two peaks, furfural and 4-hydroxy-5-methyl-3(2*H*)-furanone, HMFone, (HPLC–DAD spectra matched those of standard samples). Furfural could not be detected in the pH 5 system but HMFone was identified (Fig. 3c). A coloured

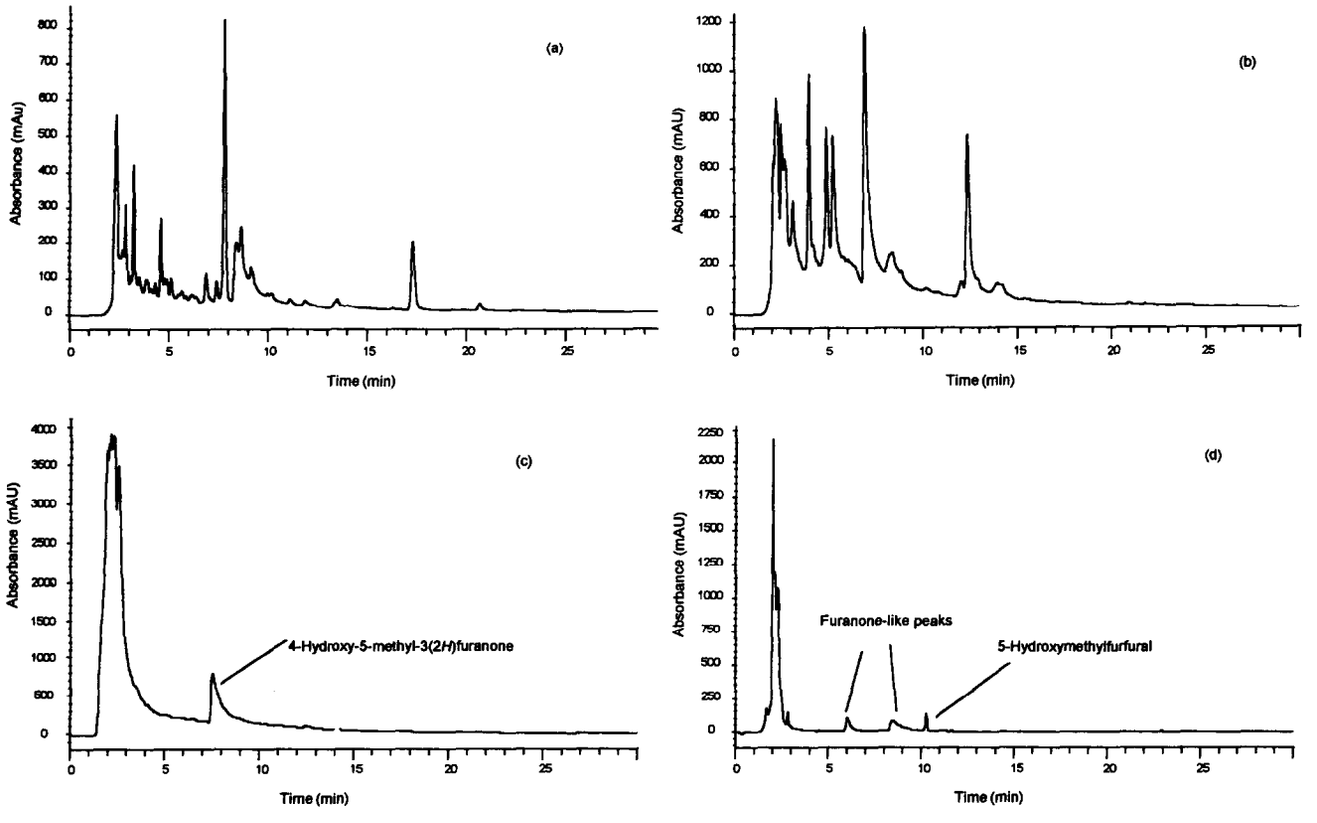

Fig. 3. Chromatograms (280 nm) of the model systems heated with the pH controlled at 5 throughout heating. (a) Xyl-Lys, 15 min, (b) Glu-Lys, 120 min, (c) Xyl-Gly, 120 min, and (d) Glu-Gly, 120 min.

Table 3. Diode array spectral matches of peaks from the glucose–lysine model systems refluxed for 120 min without pH control and at pH 5

Without pH control		With pH control	
Absolute retention time (min)	λ_{max} (nm)	Absolute retention time (min)	λ_{max} (nm)
4·74[a]	227	No matches	
5·04[a]	229	No matches	
5·62[b]	269sh, 297	3·96[b]	263sh, 297
6·86[b]	269sh, 297	3·96[b]	263sh, 297
8·70[e]	299	6·90[e]	297
11·2[c]	265sh, 295, 371	No matches	
11·50[c]	265sh, 295, 365	No matches	
15·33	293, 431	11·86	265sh, 297, 441
15·82[f]	261sh, 297	12·38[f]	261sh, 297
15·93[f]	261sh, 297, 393	12·51[f]	261sh, 297, 451
19·18[d]	279, 363, 433	No matches	
20·6	359, 459	15·28	287, 359
23·40[d]	259, 361, 431	No matches	

sh, shoulder.
[a–f] Compounds with the same superscript show the same spectral features.

compound was detected at 360 nm in the systems heated under both pH conditions.

The chromatograms of Glu-Gly heated with and without pH control, showed five peaks that were common (by both retention time and spectral matching) to the two systems. They were HMF and two peaks with furanone-like spectra on the 280 nm chromatograms (Figs 2, 3d) and two coloured peaks on the 360 nm chromatograms. The diode-array spectra from both the coloured peaks (at 9.48 and 10.65 min) gave high spectral matches with the spectrum of the coloured peak (at 10.44 min) of the Xyl-Gly system. They could be structurally similar.

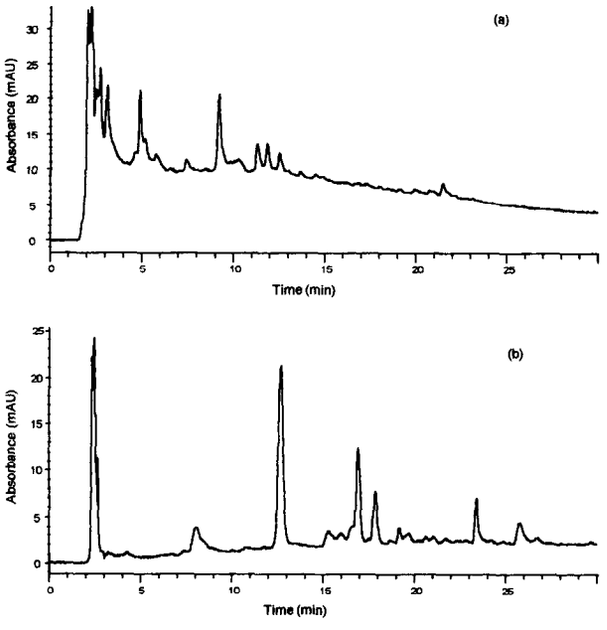

Fig. 4. Chromatograms (460 nm) of the Glu-Lys model system heated for 120 min (a) with pH control and (b) without pH control.

Unresolved broad bands

The broad bands were designated as brown melanoidins (Bailey *et al.*, 1996*a*) and were due to high molecular weight material being spread out on the column by the gradient. All the systems, except Glu-Gly, showed smaller unresolved broad bands when the pH was uncontrolled, compared to the pH 5 systems. However, differences in the chromatographic patterns of the

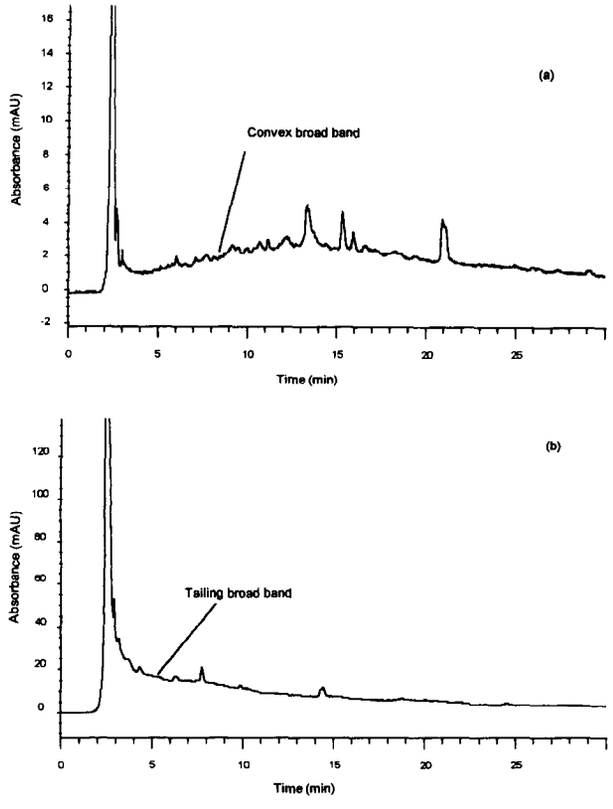

Fig. 5. Chromatograms (460 nm) of the Xyl-Lys system heated without pH control: (a) 15 min heating (b) 30 min heating.

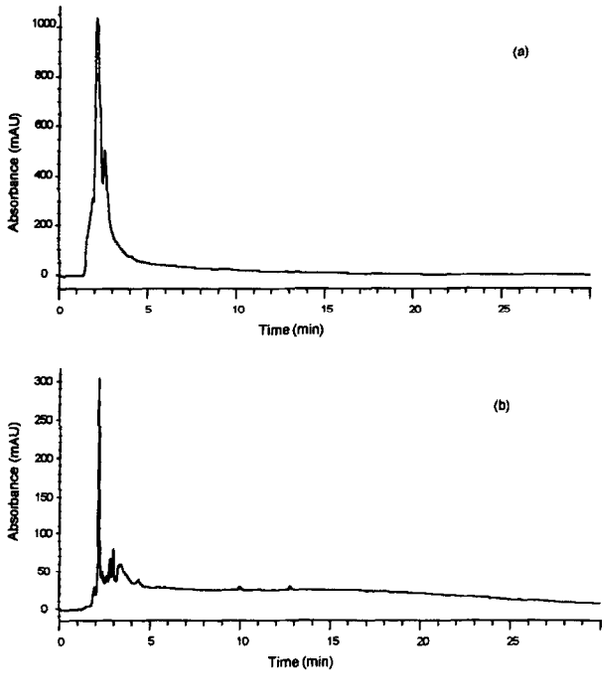

Fig. 6. Chromatograms (460 nm) of the Xyl-Gly system heated for 120 min (a) with pH control and (b) without pH control.

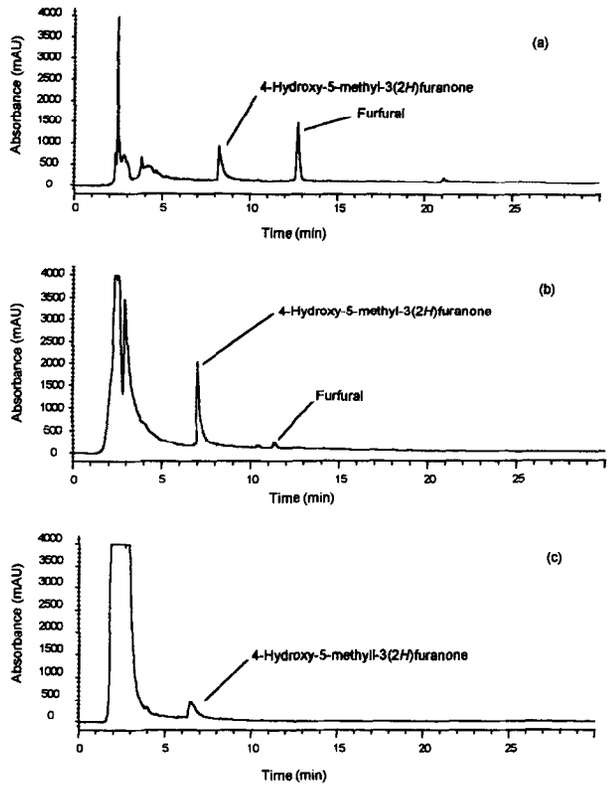

Fig. 7. Chromatograms (280 nm) of the Xyl-Gly system heated for 120 min (a) with the pH controlled at 3, (b) with the pH controlled at 4, and (c) with the pH controlled at 6.

broad bands appearing under different pH conditions suggest differences in the material formed. These differences could be reflected in the colour or other functional properties (e.g. antioxidant activity) of the melanoidins.

Compared to the system at pH 5, the Xyl-Lys system heated without pH control showed smaller tailing and convex broad bands. However, the main difference between the two systems was the change of band type with reaction time when the pH was uncontrolled, shown on the 460 nm chromatograms. Thus, a convex broad band was obtained after 15 min heating and at 60 min or longer reaction times while, after 30 min, a tailing broad band was observed (Fig. 5). At pH 5, only convex broad bands were observed, similar to that in Fig. 5a, the size and broadness of which increased with reaction time (Bailey *et al.*, 1996*a*).

The convex broad bands shown on the 460 nm chromatograms of the Glu-Lys system heated without pH control were smaller and slower to develop than at pH 5 (Fig. 4) and appeared only after 60 min heating.

No convex broad bands were observed on the chromatograms of Xyl-Gly heated without pH control, but tailing broad bands appeared at all wavelengths after 30 min reaction. However, the most significant difference between this system and the one heated at pH 5 was a broad band on the 460 nm chromatogram after 120 min reaction (Fig. 6), which was neither convex nor tailing, but of a third type, not observed previously. This band was almost flat between 5 and 18 min and then sloped towards the baseline at longer times. The different shapes of the broad bands suggest different interactions between the material responsible for them and the HPLC stationary phase that could be due to substances varying in structure and/or shape.

The chromatograms of the Glu-Gly system heated without pH control showed tailing broad bands at all wavelengths after 120 min reaction. In contrast, tailing

Table 4. Peak areas of the main resolved compounds in xylose–glycine model system heated for 120 min with fixed pH conditions

	Peak area (mAU*s)				
	Without pH control	pH 3	pH 4	pH 5	pH 6
4-Hydroxy-5-methyl-3(2*H*)-furanone	14 381	13 603	22 652	29 462	7 671
Furfural	11 265	15 892	1 548	a	a
Coloured peak	1 049	560	1 954	1 313	531

a, Below limit of detection.

broad bands were observed only at 280 and 360 nm when the system was maintained at pH 5. At pH 5, Glu-Gly was the only system to show no broad bands after 120 min.

The different chromatographic patterns observed in this work suggest differences in the composition of the high molecular weight material (melanoidins) in the examined model systems heated under different pH conditions. The chromatographic patterns found suggest a complexity in melanoidin formation in systems with a drifting pH, that is not revealed by simple absorbance measurements. This is no doubt due to the extent to which the pathways to melanoidins that are favoured by either high or low pH are operating within a given reaction time.

Xyl-Gly systems were also reacted for 120 min at pH 3, 4 and 6 (Fig. 7). The peak areas for furfural, HMFone and the coloured peak are shown in Table 4. The 280 nm chromatogram of the system at pH 3 (Fig. 7a) was closest to that of the system without pH control (Fig. 2c). The systems showed unretained peaks, furfural, HMFone, the coloured peak and, on the 460 nm chromatograms, the new broad band. However, the ratios of the peak areas of the resolved peaks in the systems at pH 3 and without pH control were different, so although the two systems were similar, they were not identical. The chromatograms of Xyl-Gly at pH 4, 5 (Fig. 3c) and 6 showed increasing amounts of unretained material, but the new broad band was absent.

CONCLUSION

The chromatographic behaviour of the four aqueous sugar–amino acid systems investigated varies dramatically depending on whether the pH is maintained at 5 during refluxing or if it is allowed to fall. The pH conditions affect the formation of unretained material, resolved peaks and broad bands observed on the chromatograms. The HPLC–DAD data may be used to classify the resolved peaks with similar spectra into spectral families and to see, more clearly, differences and similarities between the systems heated under different pH conditions. The types and sizes of the broad bands present depend on the initial reactants, the pH conditions and the time of heating.

The HPLC–DAD method used has given an insight into the differences and similarities of the total reaction products of aqueous Maillard model systems that has not been reported previously.

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