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An analysis of Maillard reaction products in ethanolic glucose–glycine solution

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Abstract

The present study compared the Maillard reaction products in ethanolic and aqueous glucose–glycine solutions. The pH 4.3, 0.2 M glucose–0.2 M glycine solutions were prepared and heated. HPLC-DAD (diode array detection) was then used to analyse the browned solutions, the ethyl acetate extracts of the solutions, and the coloured bands in TLC of the extracts. HMF (5-hydroxymethyl-2-furaldehyde) was found in both of the browned aqueous and ethanolic solutions, while 2-hydroxymethylfuran was found only in the browned ethanolic solution. HMF and 2-acetylpyrrole appear in the ethyl acetate extract of both systems, whereas 2-acetyl-1-methylpyrrole is present only in the extract of the ethanolic solution. The difference in profile of products in the aqueous and the ethanolic solutions indicates that the mechanisms of Maillard reaction in these two systems are not exactly the same.

Keywords: Maillard reaction; Ethanolic; Diode array detection; 5-hydroxymethyl-2-furaldehyde; Glucose–glycine

1. Introduction

Studies on the Maillard reaction usually use water as the solvent. Only a limited number of reports have dealt with non-aqueous systems. Among them, Baltes, Franke, Hortig, Otto, and Lessig (1981) reported that ethanol may react with Amadori rearrangement products to form *O*-ethylglycosides and ethoxy derivatives of hydroxymethylfurans in the Maillard reaction between glucose and *p*-chloroaniline in water/ethanol (1:1) model solutions. Ledl and Severin (1982) reported the occurrence of browning on heating of piperidinohexosereductone and furfural in acetic acid and ethanol solution. Ledl, Hiebl, and Severin (1983) heated xylose or glucose with glycine in pure methanolic or acqueous methanolic solutions, and reported that some Maillard reaction products are more stable in methanolic solution than in aqueous solution. Hofmann (1998) heated xylose, alanine and furan-2-carboxaldehyde in aqueous and methanol/water (2:1) solutions. He found the presence of (2*R*)-4-oxo-3,5-bis[(2-furyl)-methylidene]tetrahydropyrrolo-[1,2-*c*]-5(*S*)-(2-furyl)-oxazolidine and its 5(*R*)-(2-furyl)-oxazolidine diastereomer, (1*R*,8*aR*)-4-(2-furyl)-7-[(2-furyl)-methylidine]-2-methoxy-2*H*,7*H*,8*aH*-pyrano-[2,3-*b*]-pyran-3-one and its (1*S*,8*aR*) diastereomer, and (*E*)- and (*Z*)-2-methoxy-4-[(2-furyl)-methylene]-2*H*-pyran-3-one only in the methanol/water solution. His explanation was that methanol reacted with some Maillard reaction intermediates and produced these new colour-active compounds. Cutzach, Chatonnet, and Dubourdieu (1999) suggested that ethanol reacts with HMF to form 5-ethoxymethylfurfural in the aging of sweet fortified wines. Miksik, Struzinsky, Macek, and Deyl (1990) identified oxo compounds as their 2,4-dinitrophenylhydrazones in heated glucose–glycine ethanol/glacial acetic acid solution.

Coloured Maillard reaction products may be divided into two classes: the high molecular weight melanoidins and the low molecular weight compounds (Rizzi, 1997). The mechanism and the profile of products of Maillard

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reaction are very complicated. Therefore, researchers commonly use model systems to limit the scope. New Maillard reaction products in model systems are continually being identified. Glucose–glycine solution is among the most frequently used model systems. The intermediate Maillard reaction products identified in aqueous glucose–glycine solutions include quinoxalinone, alkyl-substituted pyrazinones, 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone (Keyhani & Yaylayan, 1997; Yaylayan, Matni, Pare, & Belanger, 1997), pyrrole-like or furanone-like compounds (Bailey, Ames, & Monti, 1996), and 2-acetyl-6-hydroxy-7-hydroxymethyl-1,5,6,7-tetrahydro-4*H*-azepinone (Ames, Bailey, & Mann, 1999). Yaylayan and Kaminsky (1998) were the only team who worked on the glucose–glycine system in a solution other than pure water. They found $C_7H_{11}N_1O_4$ and glucose polymers in a refluxed water/methanol (1:2; v/v) solution of glucose–glycine.

Many alcoholic beverages, such as beer, sake, rice wine, some fortified wines and liqueur, are ethanolic solutions that contain a noticeable amount of Maillard reaction substrates. In a previous study (Shen & Wu, 2004), we proved the effect of ethanol in accelerating Maillard browning. The browning extent and HMF content in ethanolic glucose–glycine solution were found to increase with an increase in ethanol concentration. A hypothesis based on the difference between Maillard browning mechanisms in ethanolic and aqueous systems was proposed, but no further evidence was provided.

The present study is to evaluate the profiles of Maillard reaction products in both alcoholic and aqueous glucose–glycine solutions that have been browned to a similar degree to elucidate the different Maillard browning mechanisms in the two solvent systems.

2. Materials and methods

2.1. Reagents

Ethanol (95% v/v) was purchased from Taiwan Tobacco & Liquor Co. (Taipei, Taiwan). Glucose, glycine, anhydrous sodium sulfate, and HPLC standard compounds, including ethanedial (glyoxal), 2-acetylfuran, 2-acetyl-5-methylfuran, 2-hydroxymethylfuran (furfuryl alcohol), 2-acetylpyrrole, 2-acetyl-1-methylpyrrole, pyrrole-2-carboxaldehyde, pyrazine, 2-methylpyrazine, pyrazine-2-carboxylic acid, 3-hydroxypyridine, 3-hydroxy-2-methyl-4-pyrone (maltol), 4-hydroxyl-5-methyl-3(2*H*)furanone, 4-hydroxy-2,5-di-methyl-3(2*H*)furanone, and 5-hydroxymethyl-2-furaldehyde, were the products of Sigma-Aldrich Co. (St. Louis, MO). Succinic acid was the product of Merck Co. (Darmstadt, Germany). Chloroform, ethyl acetate and methanol were purchased from J. T. Baker Co. (Phillipsburg, NJ).

2.2. Preparation of browned solutions

Among all the aqueous model systems for the Maillard reaction, hexose- and pentose-glycine systems are most frequently used. The concentrations of glucose and glycine are each usually prepared between 0.1 M and 2.0 M (Wedzicha & Kaputo, 1992). Glucose and glycine are important ingredients in alcoholic beverages, such as sake, the pH value of sake being around 4.3 (Kizaki, Fukuda, & Takahashi, 1998). Therefore, 0.2 M glucose–0.2 M glycine (G–G) model solutions, buffered at pH 4.3, were used in the present study.

We prepared aqueous and 50% (v/v) ethanolic G–G solutions buffered to pH 4.3 in 0.05 M succinic acid–sodium hydroxide. Aliquots of 4 ml of the aqueous G–G solution were transferred to 4.5 ml glass vials, hermetically capped, and heated in boiling water for 36 h as an accelerated storage test to obtain the browned aqueous solution. The browned ethanolic solution was obtained by following a similar procedure, except that the heating time was 18 h. The $A_{420\,nm}$ values of the browned solutions were measured using a UV/VIS 8500 double beam spectrophotometer (Lab Alliance, State College, PA). The degrees of browning in the heated aqueous solution and the heated ethanolic solution, as indicated by $A_{420\,nm}$ values, 10.7 and 10.8, respectively, were close to each other. The profiles of the browning products in these two solutions should be very much alike if their Maillard reaction mechanisms are the same. On the other hand, some noticeable deviation in the profiles of products in these solutions would be detected if the browning mechanisms were different.

2.3. Extraction for browning products

A portion of the browned ethanolic solution was evaporated under vacuum at 40 °C to remove alcohol and to reduce the volume to 50%. Each 500 ml aliquot of the browned aqueous solution or 250 ml aliquot of the browned ethanolic solution concentrate was extracted with 200 ml of ethyl acetate. The extraction was repeated six times in total. The six batches of extract from a sample of browned solution were pooled, dehydrated with anhydrous sodium sulfate, and evaporated under vacuum at 40 °C. The residue was dissolved in 20 ml of ethyl acetate, dehydrated, and vacuumed to obtain the dark-brown ethyl acetate-extractable component. The component was then dissolved in methanol for TLC or HPLC-DAD analysis.

2.4. TLC analysis

Separation of the browning products in the ethyl acetate extract from either aqueous or ethanolic G–G solution was performed on 0.2 mm thick F_{654} silica gel-coated 20 cm × 20 cm aluminium plates and glass plates (Merck Co., Darmstadt, Germany). The mobile phase was chloroform:ethyl acetate:95% ethanol = 15:2:2 by volume. The coloured bands on the TLC plate were recovered by exhaustive elution with methanol, concentrated using a rotary evaporator, and stored at −20 °C before HPLC-DAD analysis.

2.5. HPLC-DAD analysis

Each sample of the browned solutions, the ethyl acetate extractables, and the TLC bands was filtered through a Millex-HN nylon clarification kit, 0.45 μm pore size (Millipore, Bedford, MA), and then analysed using an HPLC-DAD system which consisted of a SphereClone 5 μm ODS (2) 250 × 4.6 mm column (Phenomenex, Torrance, CA), an Alliance 2990 pump and a 996 diode array detector (Waters Co., Milford, MA). The injection volume was 20 μl. The mobile phase was a linear gradient of 100% water to 100% methanol over 60 min at a flow rate of 1.0 ml/min. Absorption spectra, in the range 200–500 nm, were recorded. Background corrected spectra of reaction products and standard compounds were compared using Waters Millennium 32° software. Spectral matches of less than 999 were rejected, a spectral match of 1000 being perfect.

3. Results and discussion

3.1. HPLC-DAD analysis of standard compounds

Table 1 shows retention time and wavelength of maximum absorbance (λ_{max}) in HPLC-DAD analysis of the standard compounds, as representative products, in Maillard browning. The furans have a λ_{max} between 273 and 288 nm, with a smaller absorption peak at 224–225 nm. The λ_{max} is between 284 and 286 nm for the furanones, 289 and 291 nm for the pyrroles, and 260 and 267 nm for the pyrazines.

3.2. HPLC-DAD chromatograms of browned solutions

Bailey et al. (1996) classified the HPLC-DAD chromatographic behaviour of Maillard reaction products into four groups, namely, (1) unretained peaks, (2) convex broad band, (3) tailing broad band, and (4) resolved peaks. We applied their classification in the following discussion.

Figs. 1 and 2 are HPLC-DAD chromatograms of the browned aqueous solution and the browned ethanolic solution, respectively. The absorbance at 280 nm (Figs. 1A and 2A) is essentially for colourless substances, while that at 360 and 420 nm (Figs. 1B,C and 2B,C) indicates coloured substances. HPLC-DAD spectra at 280 nm show a major resolved peak at a retention time of about 10 min in both of the chromatograms for browned aqueous and ethanolic solutions (Figs. 1A and 2A). The DAD showed 283 nm to be the λ_{max} of this colourless compound. We identified it as HMF after comparing with standard compounds in retention time and λ_{max} (Table 1). Tressl, Nittka, Kersten, and Rewicki (1995) reported that hexoses may react with amino acids in pH 4.3 aqueous solution to form Amadori rearrangement products, which then undergo 1,2-enolisation to form HMF as a key intermediate in the Maillard reaction. The same pathway for HMF formation may exist in the present pH 4.3 aqueous and ethanolic model solutions as well.

The unretained peaks on the chromatogram represent material excluded from the column, either on the basis of molecular size or polarity (Bailey et al., 1996). The retention times of the unretained peaks, for both the aqueous and ethanolic solutions, are less than 5 min with a stronger absorbance at 360 nm, or yellow in colour, than at 460 nm, or brown in colour. Similar results for a chromatogram of pH 5.0 aqueous glucose/glycine solution had been reported previously by Bailey et al. (1996).

Both the convex broad band and tailing broad band are unresolved bands. Snyder, Stadalius, and Quarry (1983) suggested that these bands be designated as unresolvable polymers. There is no tailing broad band in the chromatogram of either ethanolic or aqueous solution in the present study. A convex broad band appears in the 460 nm spectra (Figs. 1C and 2C), suggesting the existence of melanoidin polymer in both of the browned aqueous and ethanolic G–G solutions. Bailey et al. (1996) reported neither convex broad bands nor tailing broad bands in the chromatogram of heated pH 5.0 aqueous glucose/glycine (1 M/1 M) solution. Monti, Bailey, and Ames (1998) reported the occurrence of tailing broad bands in the chromatogram of heated aqueous glucose/glycine (1 M/1 M) solution without pH buffer. The discrepancy in the presence of broad band between our observation and the above-mentioned reports could be attributed to the difference in the pH of reaction mixture and the heating time–temperature history.

Table 2 presents the retention times and λ_{max} of major products of Maillard reaction in the HPLC-DAD analysis of the browned aqueous and ethanolic solutions. We found three compounds in the aqueous solution, and six in the ethanolic solution. Among them, only two compounds were present in both solutions ($t_R = 3.34$ and 10.04 min, and $\lambda_{max} = 296$ and 285 (229) nm in the aqueous solution, while $t_R = 3.31$ and 10.14 min, and $\lambda_{max} = 296$ and 283 (229) nm in the ethanolic solution). The one with a

Table 1
HPLC retention times and λ_{max} values of standard compounds

Compounds	Retention time (min)	λ_{max} (nm)
Pyrrole-2-carboxaldehyde	14.37	291
2-Acetylpyrrole	19.29	289
2-Acetyl-1-methylpyrrole	28.68	289
3-Hydroxypyridine	19.70	211, 247, 276, 315
Pyrazine-2-carboxylic acid	2.07	267
Pyrazine	13.12	260, 301
2-Methylpyrazine	19.86	265
3-Hydroxy-2-methyl-4-pyrone (maltol)	1.70	210, 278
2-Acetylfuran	18.37	273, 225
2-Acetyl-5-methylfuran	24.70	288, 224
2-Hydroxymethylfuran (furfuryl alcohol)	14.30	276, 225
4-Hydroxy-2,5-dimethyl-3(2*H*)furanone	15.30	286
4-Hydroxy-5-methyl-3(2*H*)furanone	9.96	284
5-Hydroxymethyl-2-furaldehyde	9.91	283, 229
Ethanedial (glyoxal)	1.77	250

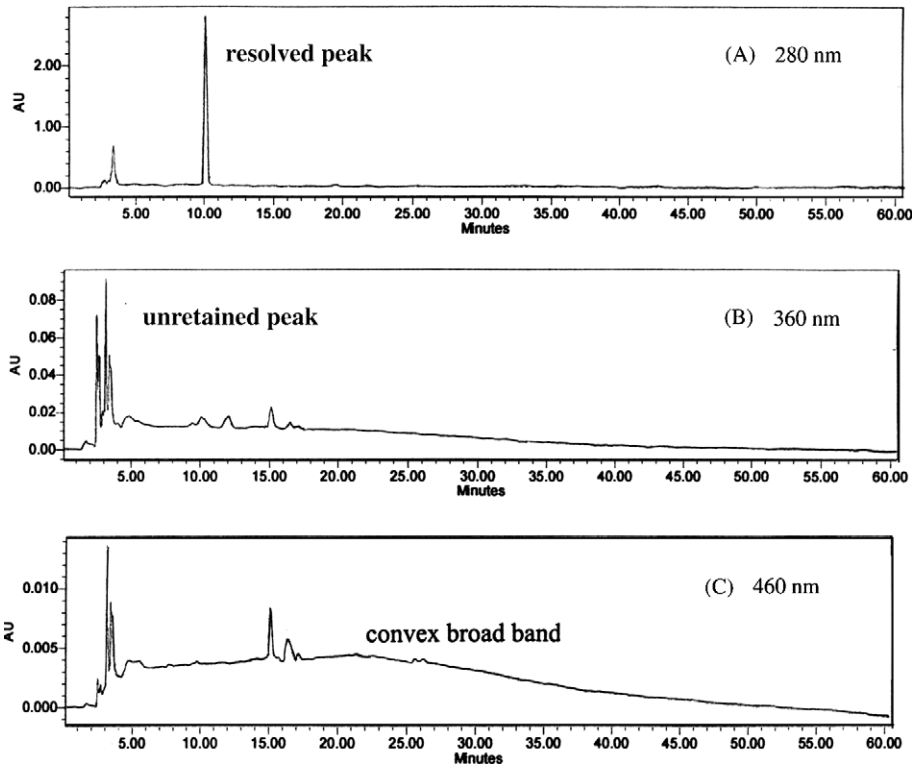

Fig. 1. HPLC-DAD chromatograms of aqueous 0.2 M glucose–0.2 M glycine solution buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide and heated in a boiling water for 36 h.

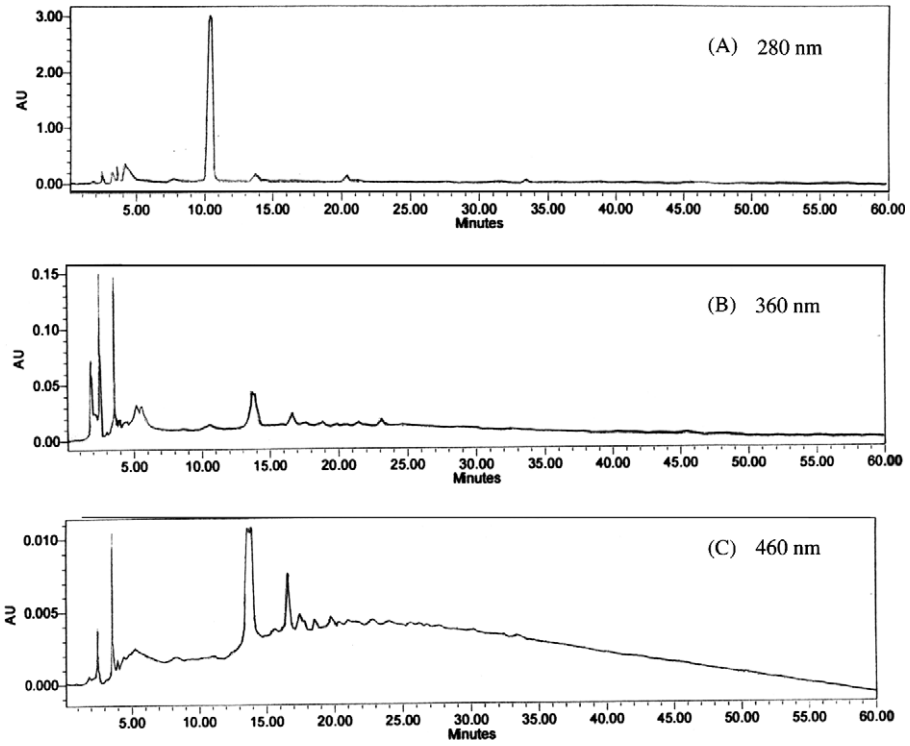

Fig. 2. HPLC-DAD chromatograms of 50% ethanolic 0.2 M glucose–0.2 M glycine solution buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide and heated in a boiling water for 18 h.

Table 2
Retention times and λ_{max} values for major peaks in the HPLC-DAD chromatogram of browned 0.2 M glucose–0.2 M glycine solutions buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide

Aqueous G–G solution heated at 100 °C for 36 h		Ethanolic (50%) G–G solution heated at 100 °C for 18 h	
t_R (min)	λ_{max} (nm)	t_R (min)	λ_{max} (nm)
2.68	293, 236	3.31	295
3.34	296	3.66	222, 309
10.04[a]	285, 229	4.80	355
		8.06	296
		10.14[a]	283, 229
		14.07[b]	276, 227

[a] A comparison of the retention time and diode array data with those for the standard compound indicates that this compound is 5-hydroxymethyl-2-furaldehyde.
[b] A comparison of the retention time and diode array data with those for the standard compound indicates that this compound is 2-hydroxymethylfuran.

retention time of about 10 min was identified as HMF. The other compound remains unknown. Furfuryl alcohol, 2-hydroxymethylfuran, was detected only in the browned ethanolic solution (Table 2), indicating some difference in Maillard reaction mechanism between the aqueous and ethanolic solutions.

3.3. HPLC-DAD chromatograms of ethyl acetate extractables

The HPLC-DAD chromatograms for the ethyl acetate extractables show no tailing broad bands (data not shown).

Table 3
Retention times and λ_{max} values for major peaks in the HPLC-DAD chromatogram of ethyl acetate extractables from browned 0.2 M glucose–0.2 M glycine solutions buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide

Ethyl acetate extractables of aqueous G–G solution heated at 100 °C for 36 h		Ethyl acetate extractables of 50% ethanolic G–G solution heated at 100 °C for 18 h	
t_R (min)	λ_{max} (nm)	t_R (min)	λ_{max} (nm)
2.70	295	2.51	295
4.60	294	5.74	272, 325
7.17	295	7.29	296
11.22[a]	284, 232	10.76[a]	282, 229
17.92	273	12.88	295
18.50[b]	290	14.61	294
25.07	282, 222	18.64[b]	289
		19.06	295
		28.77[c]	289
		32.54	293

[a] A comparison of the retention time and diode array data with those for the standard compound indicates that this compound is 5-hydroxymethyl-2-furaldehyde.
[b] A comparison of the retention time and diode array data with those for the standard compound indicates that this compound is 2-acetylpyrrole.
[c] A comparison of the retention time and diode array data with those for the standard compound indicates that this compound is 2-acetyl-1-methylpyrrole.

Convex broad bands appear at 360 and 460 nm, confirming the presence of coloured polymers in the ethyl acetate extractables of both the aqueous and the ethanolic browned solutions.

Table 3 presents the retention times and λ_{max} of major products of the Maillard reaction in the HPLC-DAD analysis of ethyl acetate extractables of the browned solutions. We found seven compounds in the extract from the aqueous solution and ten from the ethanolic solution. Among them, four compounds are present in both systems ($t_R = 2.70$, 7.17, 11.22, and 18.50 min and $\lambda_{max} = 295$, 295, 284(232), and 290 nm in the aqueous solution, corresponding to $t_R = 2.51$, 7.29, 10.76, and 18.64 min and $\lambda_{max} = 295$, 296, 282(229) and 289 nm in the ethanolic solution). These data show the presence of HMF and 2-acetylpyrrole in both the browned aqueous and ethanolic solutions, while 2-acetyl-1-methylpyrrole is present in the ethanolic solution only.

3.4. TLC chromatograms of ethyl acetate extractables

The result of TLC analysis is shown in Table 4. Each system contains the same number of coloured bands. However, the sets of coloured bands differ from each other, reconfirming that the Maillard reaction products are not all the same in the aqueous and ethanolic solutions. There are three deep-coloured bands, namely orange (W1, $R_f = 0.13$), grey (W6, $R_f = 0.44$) and yellow (W9, $R_f = 0.69$) on the plate for ethyl acetate extractables from the aqueous solution. All the other six bands on the same plate are light in colour. No deep-coloured band was found in the TLC analysis of ethyl acetate extractables from the ethanolic solution.

HPLC-DAD analysis showed the presence of more than one compound in each coloured band on the TLC plate (data not shown). This means that TLC can only be used for preparatory separation of ethyl acetate extractables, but not the ultimate purification.

3.5. HPLC-DAD chromatograms of TLC-prepared ethyl acetate extractables

The 280 nm absorbance HPLC-DAD chromatogram of TLC-prepared ethyl acetate extractables from either the aqueous or the ethanolic solutions shows a resolved peak at retention time of about 10 min, representing HMF (data not shown).

A convex broad band at 360 and 460 nm appears in the sample prepared from the aqueous solution. A convex broad band at 460 nm also appears in that from the ethanolic solution (data not shown).

Table 5 presents the retention time and λ_{max} of the coloured products of Maillard reaction in the HPLC-DAD analysis of the TLC-prepared ethyl acetate extractables from the browned aqueous and ethanolic solutions. We found eight coloured compounds in the extract from the aqueous solution and five from the ethanolic solution.

Table 4
R_f values and colour of bands on TLC of ethyl acetate extractables from browned 0.2 M glucose–0.2 M glycine solutions buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide

Ethyl acetate extractables of aqueous G–G solution heated at 100 °C for 36 h			Ethyl acetate extractables of 50% ethanolic G–G solution heated at 100 °C for 18 h		
Code	R_f value	Colour	Code	R_f value	Colour
W1	0.13	Brown to yellow	E1	0.07	Light brown
W2	0.21	Pale yellow	E2	0.20	Pale brown
W3	0.22	Pale yellow	E3	0.27	Light brown
W4	0.31	Light brown	E4	0.34	Light brown
W5	0.34	Light brown	E5	0.40	Pale yellow
W6	0.44	Grey	E6	0.46	Pale yellow
W7	0.50	Pale yellow	E7	0.50	Pale brown
W8	0.55	Pale brown	E8	0.58	Light yellow
W9	0.69	Yellow	E9	0.72	Light yellow

Table 5
Retention times and λ_{max} values for major peaks in the HPLC-DAD chromatogram of TLC-prepared ethyl acetate extractables from browned 0.2 M glucose–0.2 M glycine solutions buffered at pH 4.3 with 0.05 M succinic acid–sodium hydroxide

TLC-prepared ethyl acetate extractables of aqueous G–G solution heated at 100 °C for 36 h		TLC-prepared ethyl acetate extractables of 50% ethanolic G–G solution heated at 100 °C for 18 h	
t_R (min)	λ_{max} (nm)	t_R (min)	λ_{max} (nm)
25.31	418, 244	22.29	368
27.62	233, 298, 401	23.14	368, 247
28.16	383, 243	24.30	333, 224
29.18	406, 244	25.85	368, 242
30.51	397	28.00	358,222
31.10	365		
34.91	228, 289, 387		
38.98	217, 287, 380		

None of them is present in both systems, again confirming that the mechanism of Maillard browning in ethanolic systems differs from that in aqueous ones.

4. Conclusions

The present study compares the Maillard browning products in aqueous and ethanolic glucose–glycine solutions, buffered to pH 4.3 and heated to a similar degree of browning. The HPLC-DAD analysis shows significant differences in the browned mixtures, the ethyl acetate extractables, and the TLC coloured fractions. The difference between the profiles of products in the aqueous and the ethanolic systems indicates that the mechanisms of the Maillard reaction in these two systems are not the same.

The Maillard reaction in aqueous systems has for a long time been used in the manufacture of food pigments and flavours. The present study suggests the feasibility of obtaining new compounds with potentially desirable characteristics through the Maillard reaction in ethanolic systems. Further investigation in this area is likely to be valuable.

References

Ames, J. M., Bailey, R. G., & Mann, J. (1999). Analysis of furanone, pyranone, and new heterocyclic colored compounds from sugars – glycine model Maillard systems. *Journal of Agricultural and Food Chemistry, 47*, 438–443.

Bailey, R. G., Ames, J. M., & Monti, S. (1996). An analysis of the non-volatile reaction products of aqueous Maillard model systems at pH 5, using reversed-phase HPLC with diode-array detection. *Journal of the Science of Food and Agriculture, 72*, 97–103.

Baltes, W., Franke, K., Hortig, W., Otto, R., & Lessig, U. (1981). Investigations on model systems of Maillard reactions. *Progress in Food and Nutrition Science, 5*, 137–145.

Cutzach, I., Chatonnet, P., & Dubourdieu, D. (1999). Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *Journal of Agricultural and Food Chemistry, 47*, 2837–2846.

Hofmann, T. (1998). Studies on the influence of the solvent on the contribution of single Maillard reaction products to the total color of browned pentose/alanine solutions – a quantitative correlation using the color activity concept. *Journal of Agricultural and Food Chemistry, 46*, 3912–3917.

Keyhani, A., & Yaylayan, V. A. (1997). Glycine specific novel Maillard reaction products: 5-hydroxy-1,3-dimethyl-2(1H)-quinoxalinone and related compounds. *Journal of Agricultural and Food Chemistry, 45*, 697–702.

Kizaki, Y., Fukuda, H., & Takahashi, K. (1998). Changes in componments of Kijou-shu, a type of sake, during aging. *Journal of the Brewing Society Japan, 93*(2), 148–152.

Ledl, F., & Severin, T. (1982). Formation of coloured compounds from hexoses (in German). *Zeitschrift fur Lebensmitteluntersuchung und - Forschung A, 175*, 262–265.

Ledl, F., Hiebl, J., & Severin, T. (1983). Formation of coloured β-pyranones from pentoses and hexoses (in German). *Zeitschrift fur Lebensmitteluntersuchung und -Forschung A, 177*, 352–355.

Miksik, I., Struzinsky, R., Macek, K., & Deyl, Z. (1990). Liquid column chromatography of oxo compounds as their 2,4-dinitrophenylhydrazones with special reference to glucose–glycine Maillard reaction products. *Journal of Chromatography, 500*, 607–613.

Monti, S. M., Bailey, R. G., & Ames, J. M. (1998). The influence of pH on the non-volatile reaction products of aqueous Maillard model systems by HPLC with diode array detection. *Food Chemistry, 62*, 369–375.

Rizzi, G. P. (1997). Chemical structure of coloured Maillard reaction products. *Food Reviews International, 13*, 1–28.

Shen, S. C., & Wu, J. S. B. (2004). Maillard browning in ethanolic solutions. *Journal of Food Science, 69*, 273–279.

Snyder, L. R., Stadalius, M. A., & Quarry, M. A. (1983). Gradient elution in reversed-phase HPLC separation of macromolecules. *Analytical Chemistry, 55*, 1412A–1430A.

Tressl, R., Nittka, C., Kersten, E., & Rewicki, D. (1995). Formation of isoleucine-specific Maillard products from [1-^{13}C]-D-glucose and [1-^{13}C]-D-fructose. *Journal of Agricultural and Food Chemistry, 43*, 1163–1169.

Wedzicha, B. L., & Kaputo, M. T. (1992). Melanoidins from glucose and glycine: Composition, characteristics and reactivity towards sulfite ion. *Food Chemistry, 43*, 359–367.

Yaylayan, V. A., & Kaminsky, A. (1998). Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model systems. *Food Chemistry, 63*, 25–31.

Yaylayan, V., Matni, G., Pare, J. R. J., & Belanger, J. (1997). Microwave-assisted synthesis and extraction of selected Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 149–152.