Effect of Hexanal and Iron on Color Development in a Glucose/Phenylalanine Model System

Biagio Fallico[†] and Jennifer M. Ames*

Department of Food Science and Technology, The University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom

Aqueous glucose/phenylalanine (0.1 M with respect to each reactant) systems were heated in an autoclave for 30 min at 140 °C, in the presence of hexanal (0.04, 0.1, and 0.2 M) or $FeCl_2$ (0.01 M). Results show that hexanal significantly inhibited color development at pH 5 and 6 and led to an increase of 5-(hydroxymethyl)furfural. Iron addition had similar effects at pH 5, but only small effects at pH 6. The reaction routes proposed give key roles to the α-dicarbonyl compounds, to the Strecker aldehyde, and to the Schiff base formed by the reaction between hexanal and phenylalanine.

Keywords: *Maillard reaction; color; glucose; phenylalanine; hexanal; iron; 5-(hydroxymethyl)-furfural*

INTRODUCTION

The Maillard reaction, a type of nonenzymatic browning, occurs when the carbonyl group of a reducing sugar condenses with a free amino group, typically of an amino acid. It is one of the most important and widely studied reactions both in food chemistry (O'Brien et al., 1998) and in the food industry (Ames, 1998). It is responsible for the formation of the color that develops during the thermal treatment of many foods (Baltes, 1986).

In recent years, progress has been made concerning the structure of colored compounds formed during the Maillard reaction (Ames et al., 1993, 1997; Ames and Apriyantono, 1994; Arnoldi et al., 1997; Rizzi, 1997). All of these studies on the colored compounds have involved component model systems with two reactive components comprising a sugar (or sugar degradation product) and an amino compound.

In addition to reducing sugars, other carbonyl compounds, particularly lipid oxidation products, react with amino groups, producing browning in foods (Karel, 1984; Belitz and Grosch, 1987; Eriksson, 1987). Various lipid aldehyde−amino compound systems have been studied in some detail. Lysine can react with (*E*)-4,5-epoxy-(*E*)-2-heptenal to develop color on heating (Hidalgo and Zamora, 1993) or to form pyrrole derivatives, by heating in a microwave oven (Hidalgo and Zamora, 1995), and pH, time of irradiation, and lipid/amino acid ratio affect the development of such products (Zamora and Hidalgo, 1995). The formation of 2-pentylpyridine from 2,4-decadienal and an amino acid may involve reaction of the aldehyde with the amino acid directly or with ammonia formed from it (Kim et al., 1996). Hexanal is one of the most abundant aldehydes formed on autoxidation of linoleic acid (Belitz and Grosch, 1987) and has been used as a shelf-life marker (Jeon and Basette, 1984; Matè et al., 1996). Although its concentration in foods is usually only of a few parts per million, concentrations of ∼100−300 ppm have been found in fried chips (Jeon and Basette, 1984) and of ∼5000 ppm have been found in oxidized pork phospholipids extracts (Meynier et al., 1998). Hexanal reacts with tryptophan, both in model systems and in protease-digested soybeans (Arai et al., 1971) to give a condensation product involving two molecules of tryptophan (Kaneko et al., 1989). The reaction between hexanal and lysine leads to the formation of a pyridinium betaine (Kato et al., 1986). Last, Tashiro et al. (1985) have shown the ability of hexanal to alter unmodified and chemically modified lysozyme.

Studies involving three-component Maillard systems, that is, sugar, amino acid, and lipid, are less common, and they have been focused on flavor rather than color generation. Farmer and co-workers (Farmer et al., 1989; Farmer and Mottram, 1990, 1992) studied a phospholipid/amino acid/sugar system, and Arnoldi and Corain (1996) investigated the effect of addition of corn oil or extra virgin olive oil to a sugar/amino acid system. No data are available on the effect of lipid, or lipid degradation products, on color development in such three-component systems. In a different three-component system, Vasiliauskaite and Wedzicha (1997) investigated the role of formaldehyde, produced during the Strecker degradation of glycine, on the formation of melanoidins from glucose and glycine. Formaldehyde inhibited browning, and the melanoidins formed in the presence of formaldehyde are spectrally different from those formed in its absence.

The rate of browning is influenced by metal ions. They can enhance it by promoting oxidation reactions to form dicarbonyl compounds (Wolff, 1996) or by forming complexes able to catalyze browning (Kato et al., 1981; O'Brien and Morrissey, 1997). However, in some cases, metal ions are able to suppress browning (Yaylayan and Huyghues-Despointes, 1994) or make possible the coagulation of melanoidins (Gomyo and Horikoshi, 1976).

In recent years, phenylalanine alone (Papadopoulou and Ames, 1994) and sugar/phenylalanine systems (Kunert-Kirchhoff and Baltes, 1990; Keyhani and Yay-

* Author to whom correspondence should be addressed (telephone +44 118 931 8730; fax +44 118 931 0080; e-mail j.m.ames@afnovell.reading.ac.uk).

[†] Permanent address: Istituto di Industrie Agrarie, Università di Catania, Via S. Sofia 98, 95123 Catania, Italy.

10.1021/jf981191g CCC: $18.00
Published on Web 05/13/1999

Table 1. Codes and Composition of Model Systems

	A	B	C	D	E	F	G	H	I	J	K	L	M
glucose (0.1 M)	•			•		•	•	•	•	•	•	•	•
phenylalanine (0.1 M)		•			•	•	•	•	•	•	•	•	•
hexanal (0.04 M)							•				•		
hexanal (0.1 M)			•	•	•			•				•	
hexanal (0.2 M)									•				•
$FeCl_2$ (0.01 M)										•	•	•	•

layan, 1996; Yaylayan and Matni, 1997) have been studied. Well-known sugar degradation products and nitrogen-containing compounds, some of them specific reaction products of phenylalanine, have been identified. Ge and Lee (1997) determined the kinetic constants and activation energies for the Schiff base and Amadori rearrangement products in sugar/phenylalanine systems.

The aim of the work described here was to investigate the role of hexanal and iron on the development of colored compounds in an aqueous glucose/phenylalanine model system, both at pH 5 and at 6.

EXPERIMENTAL PROCEDURES

Materials. D-(+)-Glucose (ACS), $FeCl_2 \cdot 4H_2O$ (ACS), hexanal (98%), L-phenylalanine (99%), and 5-(hydroxymethyl)furfural (5-HMF) (98%) were obtained from Aldrich Chemicals (Gillingham, U.K.). $Na_4P_2O_7 \cdot 10H_2O$ and $Na_2H_2P_2O_7$ (ACS) were both from Sigma Chemicals (Gillingham, U.K.). HPLC grade water and methanol were obtained from Rathburn Chemicals Ltd. (Walkerburn, U.K.).

Methods. *(a) Preparation of Model Systems*. All experiments were conducted using an aqueous pyrophosphate buffer (0.2 M), both at pH 5 and at pH 6. Reagents were used at the following concentrations: glucose (0.1 M), phenylalanine (0.1 M), hexanal (0.04, 0.1, and 0.2 M), and $FeCl_2 \cdot 4H_2O$ (0.01 M). The compositions of the systems are given in Table 1. Two series of model systems were prepared, one at pH 5 (A_5-M_5) and the other at pH 6 (A_6-M_6). Model systems were heated in autoclavable glass bottles (50 mL, Duran Schott, Germany) in an autoclave (Certoclav, Traun, Austria) at 140 °C for 30 min and cooled in an ice bath. They were immediately diluted in methanol. All samples were prepared in quadruplicate.

(b) Spectrophotometry. Visible spectra were obtained for model systems diluted in methanol (30- and 40-fold, for F_5-M_5 and F_6-M_6, respectively) over the range 380−700 nm. Absorbance readings were taken at 380, 420, 460, and 520 nm.

(c) HPLC. The apparatus comprised a Hewlett-Packard (Bracknell, U.K.) 1050 quaternary pump, equipped with a diode array detector (DAD). Data analysis was carried out using Hewlett-Packard Chemstation software. HPLC was carried out using a 25 cm, 0.49 cm i.d., 5 μm Spherisorb ODS2 column, fitted with a guard cartridge packed with the same stationary phase (Hichrom Ltd., Theale, Reading, U.K.), with a linear methanol/water gradient, 50−850 mL L^{-1} methanol over 60 min (Bailey et al., 1996). At the end of the gradient the column was washed with methanol (15 min) before the mobile phase was returned to its starting composition. The injection volume was 20 μL, and chromatograms were monitored at 254, 280, 360, and 460 nm. Spectra of each peak were collected from 190 to 600 nm. The amount of 5-HMF in each system was determined using the same HPLC method by measuring the signal at 280 nm and using an external calibration curve (10−250 mg/L; $r^2 = 0.99998$). The HPLC signals at 360 and 460 nm were used to investigate the profile of colored compounds, the signal at 360 nm being chosen because many of the lightly colored compounds showed a λ_{max} between 350 and 380 nm.

(d) Statistical Analysis. ANOVA analysis and the Kruskal−Wallis test were performed to determine which samples differed significantly (confidence level = 95%), using Statgraphics Plus software for Windows (Manugistic Inc., Rockville, MD).

RESULTS

The glucose/phenylalanine system changed from uncolored to brown over the 30 min heating period, and the brown color was darker in the pH 6 system than in the pH 5 one.

Spectrophotometry. Data for systems F−M in Table 2 show a reduction in absorbance at all wavelengths at both pH values in the presence of hexanal and/or iron (systems G−M), compared to glucose/phenylalanine (F) alone. Absorbance values for F_5 were significantly higher ($P < 0.05$) compared to those for G_5-M_5. System F_6 gave absorbance values that were always significantly higher ($P < 0.05$) than for H_6, I_6, L_6, and M_6. Statistical analysis showed many significant differences among systems at both pH values (Table 2).

5-HMF. Levels of 5-HMF in each system are given in Table 3. 5-HMF levels were much higher at pH 5 than pH 6 for all systems. Although at pH 5 there was a good yield of 5-HMF also in the heated system containing glucose alone (A_5; 15 mg/L), at pH 6 there was a measurable yield of 5-HMF only when the amino acid was present also. Glucose/phenylalanine systems gave 148 mg/L of 5-HMF at pH 5 (F_5) and 9 mg/L at pH 6 (F_6).

Addition of hexanal led to an increase of the 5-HMF level in all systems at pH 5 (D_5; G_5-I_5). For example, Figure 1 shows the chromatograms at 280 nm of the F_5 and H_5 systems. Addition of hexanal had a similar effect at pH 6, when it was added at higher concentrations (H_6, I_6). At pH 6, addition of iron to glucose/phenylalanine, with or without hexanal (K_6-M_6, J_6), had no effect on 5-HMF levels (Table 3). At pH 5, however, addition of iron to glucose/phenylalanine system (J_5) or to glucose/phenylalanine/hexanal systems (K_5-M_5) led to further increases in 5-HMF levels compared to systems F_5-I_5. The 5-HMF level at pH 5 is between 10 and 38 times as great as that at pH 6, for all systems. Levels of 5-HMF increased linearly with the amount of hexanal; the equations of the lines and r^2 for each pH value, in the presence and absence of iron, are given in Table 4.

In Table 5 are reported the equations and r^2 values for the correlations among the absorbance values at the different wavelengths (280, 420, 460, and 520 nm) and yields of 5-HMF for the systems. As an example, Figure 2 shows the relationship between the absorbance values at 420 nm and yields of 5-HMF for the systems J_5, K_5, and L_5. The data show, both in Figure 2 and in Table 5, that the higher the 5-HMF level, the lower are the absorbance values.

HPLC. Heating phenylalanine (B_5, B_6) or hexanal (C_5, C_6) alone gave no peaks on the 360 and 460 nm chromatograms (i.e., visible region), but many on the 254 nm chromatograms. Heating glucose alone (A_5, A_6) and with hexanal (D_5, D_6) and heating phenylalanine/hexanal (E_5, E_6) resulted in little browning, the 360 and 460 nm chromatograms showing only small colored peaks eluting with the solvent front (unretained peaks). The chromatograms at 254 nm of E_5 showed many peaks, of which two were also found when hexanal was added to the glucose/phenylalanine systems at pH 5 (G_5-I_5) (data not shown).

Heating the glucose/phenylalanine system led to more complex chromatograms for the four measured wavelengths, both at pH 5 and at pH 6. As examples, Figure 3a reports the chromatograms at 360 nm of the systems F_5, H_5, F_6, and H_6 and Figure 3b those at 460 nm for systems F_6 and H_6. According to the classification of

Table 2. Absorbance Values for Model Systems[a]

λ (nm)	F_5	G_5	H_5	I_5	J_5	K_5	L_5	M_5
380	1.38 (0.01)^{a}	1.08 (0.08)^{b}	0.95 (0.09)^{cde}	1.07 (0.08)^{b}	1.05 (0.05)^{bc}	1.01 (0.05)^{bcd}	0.90 (0.04)^{f}	0.94 (0.02)^{da}
420	0.64 (0.05)^{a}	0.46 (0.05)^{bc}	0.38 (0.04)^{d}	0.40 (0.02)^{cd}	0.48 (0.03)^{b}	0.46 (0.04)^{bc}	0.40 (0.01)^{d}	0.38 (0.07)^{d}
460	0.38 (0.01)^{a}	0.28 (0.03)^{bc}	0.20 (0.03)^{d}	0.23 (0.03)^{cd}	0.28 (0.03)^{b}	0.28 (0.03)^{b}	0.22 (0.01)^{cd}	0.21 (0.04)^{d}
520	0.13 (0.01)^{a}	0.09 (0.01)^{bc}	0.07 (0.02)^{d}	0.08 (0.01)^{cd}	0.11 (0.01)^{b}	0.11 (0.01)^{b}	0.09 (0.01)^{bc}	0.08 (0.02)^{cd}
λ (nm)	F_6	G_6	H_6	I_6	J_6	K_6	L_6	M_6
380	1.63 (0.10)^{a}	1.59 (0.02)^{a}	0.98 (0.10)^{d}	1.31 (0.17)^{c}	1.41 (0.06)^{bc}	1.60 (0.03)^{a}	1.33 (0.10)^{bc}	1.44 (0.06)^{b}
420	0.80 (0.02)^{a}	0.72 (0.02)^{bc}	0.44 (0.03)^{f}	0.51 (0.08)^{e}	0.74 (0.00)^{ab}	0.77 (0.03)^{a}	0.65 (0.05)^{d}	0.67 (0.03)^{cd}
460	0.50 (0.01)^{a}	0.45 (0.01)^{b}	0.26 (0.03)^{e}	0.36 (0.06)^{d}	0.47 (0.01)^{ab}	0.48 (0.02)^{a}	0.38 (0.03)^{cd}	0.41 (0.01)^{c}
520	0.17 (0.01)^{a}	0.14 (0.01)^{bc}	0.06 (0.02)^{e}	0.10 (0.02)^{d}	0.17 (0.02)^{a}	0.16 (0.01)^{a}	0.13 (0.02)^{c}	0.14 (0.01)^{c}

[a] Mean of four replicate systems (with standard deviations in parentheses). Dilutions in methanol are 30- and 40-fold, respectively, for F_5-M_5 and F_6-M_6. Means in the same row followed by a different letter are significantly different ($P < 0.05$).

Table 3. Mean Concentration of 5-HMF (Milligrams per Liter)[a]

system	5-HMF	system	5-HMF
A_5	15 (2.0)	A_6	nd
B_5	nd[c]	B_6	nd
C_5	nd	C_6	nd
D_5[b]	32 (2.0)	D_6	nd
E_5	nd	E_6	nd
F_5	148 (14)^{a}	F_6	9 (1.0)^{a}
G_5	185 (5)^{b}	G_6	10 (1.0)^{a}
H_5	234 (21)^{c}	H_6	22 (3.0)^{b}
I_5	346 (53)^{e}	I_6	30 (4.0)^{c}
J_5	263 (11)^{cd}	J_6	7 (0.0)^{a}
K_5	290 (15)^{d}	K_6	11 (0.2)^{a}
L_5	370 (5)^{f}	L_6	25 (4.0)^{b}
M_5	485 (16)^{g}	M_6	32 (5.0)^{c}

[a] Mean of four replicate systems (with standard deviations in parentheses). Means in the same column followed by a different letter are significantly different ($P < 0.05$). [b] The same system plus 0.01 M $FeCl_2$ gave 49 mg/L of 5-HMF. [c] nd, not detected.

Bailey et al. (1996) and Monti et al. (1998), these systems show unretained material, resolved peaks, and unresolved broad bands. The chromatograms at 360 nm for systems F_5 and F_6 (Figure 3a) confirm higher production of lightly colored compounds in F_6 than in F_5. The two systems show many common peaks (Figure 3a and Table 6) but also some distinctive peaks; for example, peak 12 was detected only in F_5.

Addition of hexanal to the systems (H_5, H_6) led to a decrease in the number of resolved peaks at 360 and 460 nm (Figure 3). For the largest peaks, Table 6 reports the retention times, the spectral characteristics, and the systems in which they were detected. The majority of these peaks, at both pH values, disappeared when ≥0.1 M hexanal was added (H, I, L, and M systems). At pH 5, even at the lowest concentration of hexanal (G_5), all but one of these peaks disappeared. At pH 6, the change was not as drastic, most of the peaks surviving in the presence of 0.04 M hexanal (G_6). These results are in accord with the spectrophotometric and 5-HMF data (Tables 2 and 3, respectively). Addition of iron to the systems (F_6−I_6) had little effect (J_6−M_6). Some of the peaks in Table 6 showed the same spectral characteristics. The spectra of peaks 1, 5, and 10 gave very high computer fits with unknown colored compounds isolated from different sugar/amino acid systems, for example, glucose/glycine (Bailey et al., 1996; Monti et al., 1998), suggesting that they possessed similar structures. Peaks 6, 7, and 11 gave spectra typical of substituted rings containing an enone group. The first part of these spectra (250−300 nm) had a good fit with those obtained for maltol or cyclopentenone, and also with spectra for pyrazines. The last have been found as products from the α-aminoketones formed by Strecker degradation (Kunnert-Kirchhoff and Baltes, 1990). The spectra of peaks 2 and 4 gave very good computer matches with spectra of standard furanones and pyrroles, respectively (Ames, 1998). System H_6 showed a peak at 5.9 min on the 460 nm chromatogram (Figure 3b). This peak was not observed in the F_6 system but was found also in G_6 and I_6.

DISCUSSION

The data presented show that addition of hexanal to the glucose/phenylalanine system partially inhibits browning reactions, at both pH values.

Spectrophotometric analysis showed the absorbance values to decrease, not linearly, with the amount of hexanal added (0.04 and 0.1 M), although the highest concentration of hexanal (0.2 M) was not more effective than sample containing 0.1 M. Vasiliauskaite and Wedzicha (1997), studying the effect of formaldehyde in the browning of a glucose/glycine system, found that absorbance values decreased and that the melanoidins formed in the presence of formaldehyde differed spectrophotometrically from those formed in its absence.

Addition of iron to the glucose/phenylalanine system had only a small effect on absorbance values in all systems at pH 6 (J_6-M_6). Its addition at pH 5 led to a decrease of absorbance values, compared to system F_5, but there was no difference among systems with hexanal and with hexanal/iron.

Addition of hexanal had a great effect on the level of 5-HMF, both at pH 5 and at pH 6, whereas addition of iron to systems F−I had a significant effect at pH 5 only (Table 3). Absorbance values and their correlations with 5-HMF levels (Figure 2 and Table 5) confirm that the addition of hexanal had no special effect on the formation of particular colored compounds but does inhibit the general pathway leading to them. The higher the 5-HMF level, the lower are all of the absorbance values. Nevertheless, the high correlation values between absorbance values and 5-HMF levels do not imply that an increase in the 5-HMF level was directly responsible for a decrease in colored development. One or more unidentified pathways may occur for the suppression of color formation in the presence of hexanal or iron. Systems I and M are exceptions (Table 2), probably because the concentration of hexanal in the system reached a plateau level (Vasiliauskaite and Wedzicha, 1997) or because the residual hexanal no longer gave a homogeneous solution for spectrophotometry.

Understanding how hexanal acts in the system to enhance the level of 5-HMF may help to explain the negative correlation between 5-HMF and color development. It is known that hexanal is able to give aldoliza-

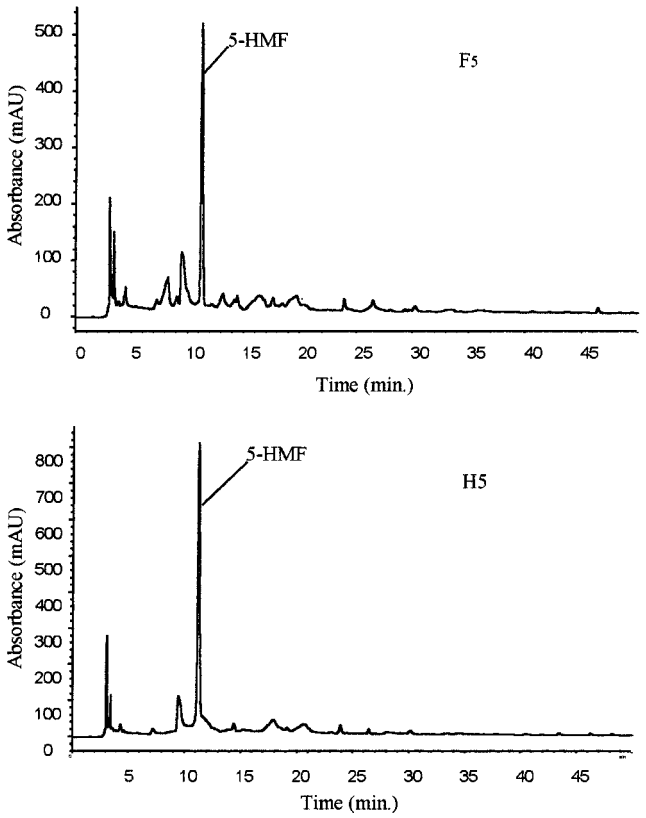


Figure 1. Chromatograms at 280 nm of systems F_5 and H_5.

Table 4. Equations for the Relationship between the Concentrations of 5-HMF and Hexanal

system	equation	r^2
F_5-I_5	5-HMF = 148.0 + 885.6 (hexanal)	0.9353
J_5-M_5	5-HMF = 255.2 + 1142.1 (hexanal)	0.9784
F_6-I_6	5-HMF = 8.22 + 104.7 (hexanal)	0.9087
J_6-M_6	5-HMF = 7.44 + 121.9 (hexanal)	0.9166

Table 5. Equations for the Relationships between the Concentration of 5-HMF and Absorbance Values (Abs)

λ (nm)	system	equation	r^2
380	F_5, G_5, H_5	Abs = 5.04 − 1.71 × log(5-HMF)	0.8377
	J_5, K_5, L_5	Abs = 3.94 − 1.88 × log(5-HMF)	0.9289
	F_6, G_6, H_6	Abs = 3.02 − 1.45 × log(5-HMF)	0.9171
	J_6, K_6, L_6	Abs = 2.38 − 0.77 × log(5-HMF)	0.8807
420	F_5, G_5, H_5	Abs = 2.59 − 0.93 × log(5-HMF)	0.8440
	J_5, K_5, L_5	Abs = 1.75 − 0.53 × log(5-HMF)	0.9491
	F_6, G_6, H_6	Abs = 1.52 − 0.78 × log(5-HMF)	0.9659
	J_6, K_6, L_6	Abs = 1.05 − 0.27 × log(5-HMF)	0.8217
460	F_5, G_5, H_5	Abs = 1.84 − 0.69 × log(5-HMF)	0.8443
	J_5, K_5, L_5	Abs = 1.23 − 0.39 × log(5-HMF)	0.9085
	F_6, K_6, H_6	Abs = 0.99 − 0.15 × log(5-HMF)	0.9616
	J_6, K_6, L_6	Abs = 0.75 − 0.27 × log(5-HMF)	0.8842
520	F_5, G_5, H_5	Abs = 0.48 − 0.17 × log(5-HMF)	0.7471
	J_5, K_5, L_5	Abs = 0.27 − 0.07 × log(5-HMF)	0.7735
	F_6, G_6, H_6	Abs = 0.36 − 0.21 × log(5-HMF)	0.9210
	J_6, K_6, L_6	Abs = 0.25 − 0.09 × log(5-HMF)	0.8168

tion reaction products (Pokorny et al., 1987) or to react with amino acids to give condensation products (Arai et al., 1971; Tashiro et al., 1985; Kaneko et al., 1989). Compared with system F_5, systems B_5 and E_5 (phenylalanine and phenylalanine/hexanal) produced a negligible amount of color and no 5-HMF. In contrast, system A_5 gave a small amount of 5-HMF, and its level increased with hexanal addition and still further in the presence of iron (D_5, Table 3). Nevertheless, these levels

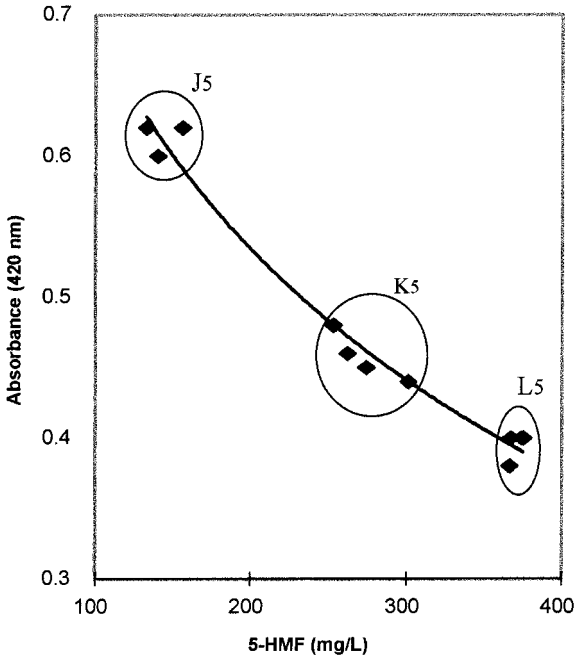


Figure 2. Relationship between absorbance values and the concentration of 5-HMF for systems J_5, K_5, and L_5.

are much lower than those in the glucose/phenylalanine system. Furthermore, addition of hexanal (G_5-I_5) or hexanal and iron (J_5-M_5) to the glucose/phenylalanine system is much more effective in producing additional amounts of 5-HMF than when these compounds are added to glucose alone. As phenylalanine/hexanal (E_5), as expected, did not produce any 5-HMF, the increased level of 5-HMF in G_5-M_5 cannot be ascribed to the direct reaction between the amino acid and hexanal. To explain the effect of formaldehyde on the glucose/glycine system, Vasilauskaite and Wedzicha (1997) suggested that it reacted with intermediates of the reaction. 5-HMF is the most abundant product of the rearrangement reaction of 3-deoxyglucosone, deriving from 1,2-enolization of the Amadori rearrangement product. When an excess of primary amino groups is present, the formation of 5-HMF is suppressed, pyrrole derivatives or pyridinium betaines resulting instead (Yayalayan and Huyghues-Despointes, 1994). Piloty and Baltes (1979a,b) demonstrated the ability of phenylalanine and other amino acids to react with dicarbonyl compounds to form heterocyclic compounds. These products are able to react further, leading to brown products. Hexanal added to the glucose/phenylalanine system could enhance the level of 5-HMF by reacting with the phenylalanine, decreasing the probability of reaction between 3-deoxyglucosone and the amino acid and so promoting the transformation of the 3-deoxyglucosone to 5-HMF (Scheme 1a).

Furthermore, the reaction between dicarbonyl compounds and amino acids leads to the so-called Strecker degradation (Yayalayan and Huyghues-Despointes, 1994). The importance of this reaction for glucose/phenylalanine has been pointed out by Kunnert-Kirchhoff and Baltes (1990). They showed that phenylacetaldehyde, because of its acidic hydrogen in the α-position, had the ability to give condensation products with aldehydes. 5-HMF is one of the most abundant aldehydes produced at high temperature, and phenylacetaldehyde reacts with it to give 3-[5′-(hydroxymethyl)-2′-furyl]-2-phenyl-2-propenal (Kunnert-Kirchhoff and Baltes, 1990). However, when an aliphatic aldehyde, for ex-

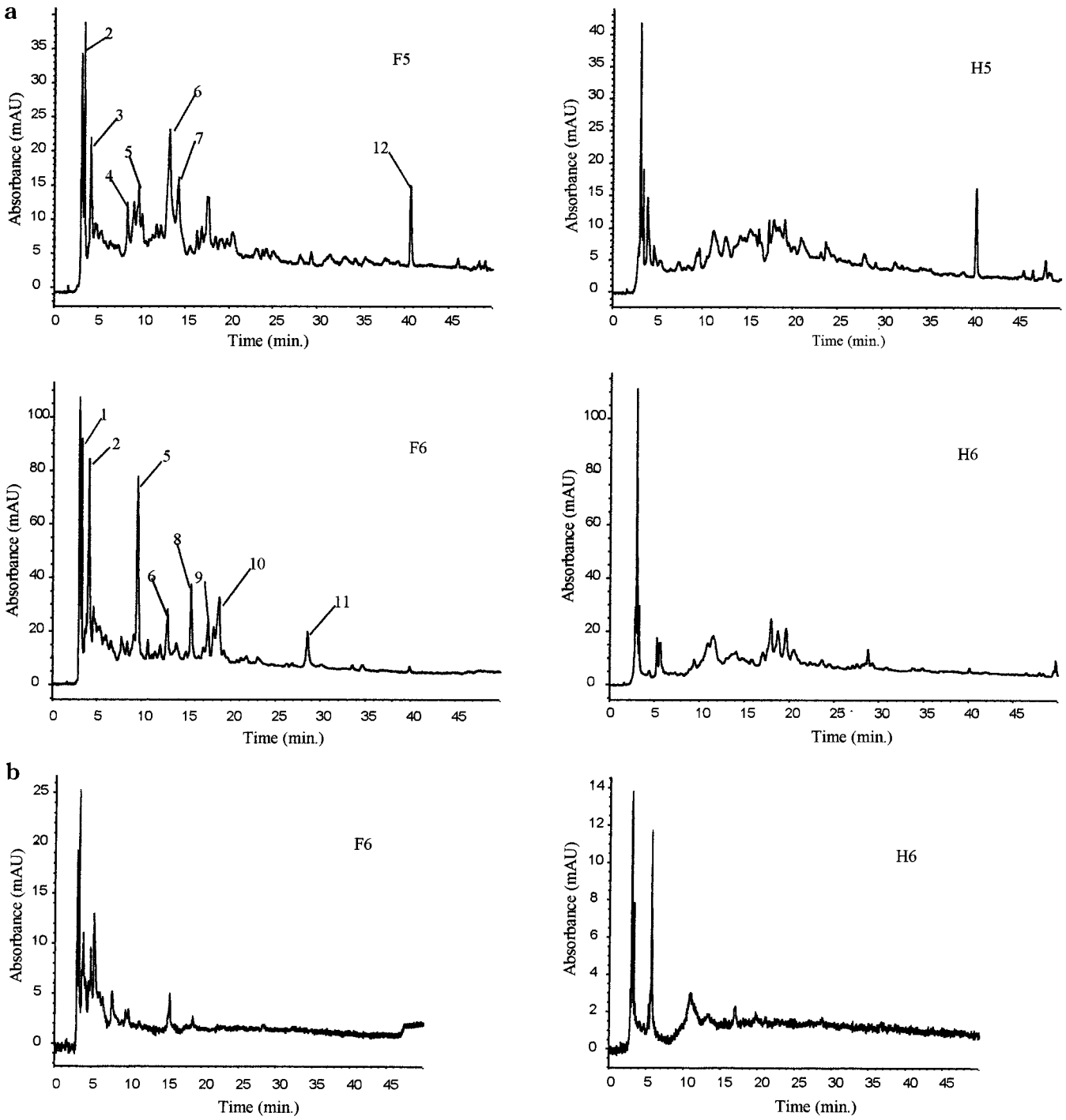


Figure 3. (a) Chromatograms at 360 nm of systems F_5, H_5, F_6, and H_6. (b) Chromatograms at 460 nm of systems F_6 and H_6.

ample, hexanal, is present, the phenylacetaldehyde can react with it in place of 5-HMF (Scheme 1b).

Thus, addition of hexanal to the glucose/phenylalanine system can have two effects: (a) impeding the reaction of dicarbonyl compounds with an amino group to form heterocyclic compounds and the Strecker degradation products by removing one of the reactants, phenylalanine, and (b) reacting with the Strecker aldehyde, preventing its condensation with 5-HMF. Furthermore, reactions between hexanal and reaction intermediates cannot be excluded.

It is known that the presence of an amino acid during heating promotes the formation of dicarbonyl compounds via ARP, and, due to the high E_a of the latter, their formation is highly favored at very high temperature (Ge and Lee, 1997). The greater effect of hexanal addition to the glucose/phenylalanine system compared to glucose alone could be due to a higher level of dicarbonyl compounds in the former system.

At pH 6 (F_6), the level of 5-HMF was lower, indicating, at this pH value, either that routes leading to 5-HMF, for example, 1,2-enolization, are less favored and/or that 5-HMF was used up at a greater rate in subsequent reactions compared to the rate at pH 5 (F_5). Nevertheless, addition of hexanal at pH 6 (I_6, J_6) led to an increase of 5-HMF levels and a decrease in color.

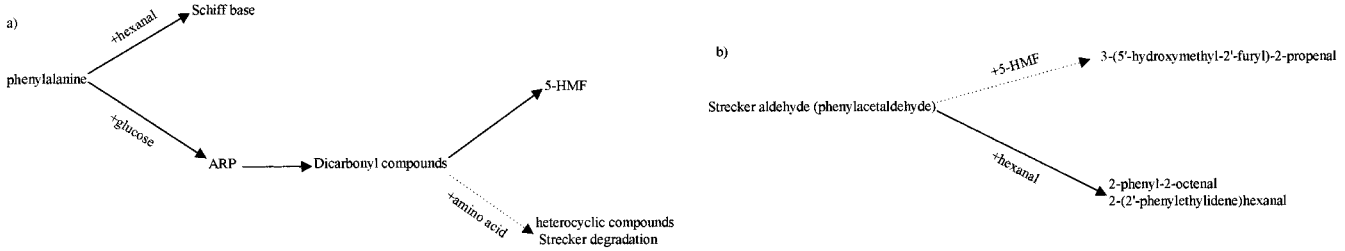
HPLC profiles confirm the findings for both pH values, as many of the peaks that disappeared at both pH values have UV−vis spectra which are pyrrole-, furanone-, maltol-, or cyclopentenone-like (Table 6).

Table 6. Retention Times (t_R) and λ_{max} Values of the Largest Colored Peaks (360 nm Chromatograms)[a,b]

peak	t_R (min)	λ_{max} (nm)	F_5	G_5	H_5	I_5	J_5	K_5	L_5	M_5	F_6	G_6	H_6	I_6	J_6	K_6	L_6	M_6
1	3.3 (F_6)	260; 400									+	d	−	−	+	−	−	−
2	3.4 (F_5)	300	+	d	−	−	d	−	−	−								
3	4.1 (F_5, F_6)	340	+	−	−	−	−	−	−	−	+	+	−	−	d	d	−	−
4	8.3 (F_5, F_6)	295; 260 sh	+	−	−	−	+	−	−	−	+	+	−	−	+	+	−	−
5	9.5 (F_5, F_6)	250; 380[a]	+	−	−	−	+	−	−	−	+	+	−	−	+	+	−	−
6	13.0 (F_5, F_6)	260; 360[b]	+	−	−	−	d	−	−	−	+	−	−	−	+	−	−	−
7	14.2 (F_5)	260; 360[b]	+	−	−	−	d	−	−	−								
8	15.5 (F_6)	287; 360[c]									+	−	−	−	+	+	−	−
9	17.4 (F_6)	287; 360[c]									+	+	−	−	+	+	−	−
10	18.7 (F_6)	250; 380[a]									+	d	−	−	+	+	−	−
11	28.5 (F_6)	260; 360[b]									+	+	d	d	+	+	d	−
12	40.6 (F_5)	355	+	+	+	+	+	+	+	+								

[a] +, peak detected; −, peak not detected; d, peak decreased compared to F systems. [b] λ values followed by the same letter showed the same UV−vis spectrum.

Scheme 1. Proposed Mechanisms Routes: Likely More Favored (→) and Less Favored (· · ·>) Routes in Glucose/Phenylalanine/Hexanal Systems



Iron had very little effect on the pH 6 systems, but it enhanced the level of 5-HMF and decreased color formation in the pH 5 systems. Onditi Ouma and Dart (1995), studying the effect of some metal ions on 5-HMF in honey, have found similar effects. Pokorny et al. (1988) obtained the same effect on browning by the addition of $FeCl_2$ and L-cysteine to a sugar/amino acid model system. In systems at pH 5, iron had the same effect as hexanal addition. Even though there are many examples of the influence of metal ions on the Maillard reaction, the mechanisms involved are almost unknown. The Maillard reaction products are able to complex metal ions (O'Brien and Morrissey, 1997), and many of these complexes are able to catalyze the formation of α-dicarbonyl compounds (Wolff, 1996). Also, the use of iron ions (Fe^{2+} or Fe^{3+}) has been found to promote browning reactions (Kato et al., 1981). Addition of iron to systems G_5−I_5 could promote the formation of 3-deoxyglucosone, which, in the presence of hexanal, instead of reacting with the amino acid, leads to the formation of 5-HMF. This hypothesis does not explain the behavior of system J_5 (glucose/phenylalanine/iron). In this system there is no hexanal, so the higher levels of 3-deoxyglucosone, according to literature data (Kato et al., 1981), should lead to an increase of color development, yet, compared to F_5, it gave lower absorbance values and fewer HPLC peaks. Unless, as reported by Gomyo and Hirokoshi (1976), in the J_5 system, there has been a precipitation of melanoidins due to the presence of iron.

Results showed that addition of hexanal to the glucose/phenylalanine system at both pH values, or the addition of $FeCl_2$ at pH 5, had a great effect both on color development and on 5-HMF level. Furthermore, this study has confirmed that 5-HMF has a role in browning reactions but that in some pathways it represents an alternative product to colored compounds. To verify the mechanistic hypothesis proposed in this paper and to elucidate the structure of the compounds involved in the systems under study, further investigations are in progress.

The concentrations of the reactants in the model systems were much higher than those present in most, if not in all, foods; nevertheless, some comments may be made of relevance to foods. The results suggest that, for systems in which the content of hexanal or related compounds is increased (e.g., when the amount of oxidized linoleic acid is increased), the development of color via Maillard browning could be suppressed. Also, when 5-HMF is used as an indicator of degree of thermal processing, an increase in the level of hexanal could result in an increase in 5-HMF level and thus an overestimation of the thermal treatment.

ABBREVIATIONS USED

DAD, diode array detector; 5-HMF, 5-(hydroxymethyl)furfural; ARP, Amadori rearrangement product.

ACKNOWLEDGMENT

We thank Professor H. E. Nursten for his comments on the manuscript and Professor E. Maccarone and Dr. Richard Bailey for useful discussions.

LITERATURE CITED

Ames, J. M. Applications of the Maillard reaction in the food industry. *Food Chem.* **1998**, *62*, 431−439.

Ames, J. M.; Apriyantono, A. Comparison of the nonvolatile ethyl acetate-extractable reaction products formed in a xylose-lysine model system heated with and without pH control. *Food Chem.* **1994**, *50*, 289−292.

Ames, J. M.; Apriyantono, A.; Arnoldi, A. Low molecular weight coloured compounds formed in xylose-lysine model systems. *Food Chem.* **1993**, *46*, 121−127.

Ames, J. M.; Arnoldi, A.; Bates, L.; Negroni, M. Analysis of the methanol-extractable nonvolatile Maillard reaction products of a model extrusion-cooked cereal product. *J. Agric. Food Chem.* **1997**, *45*, 1256−1263.

Arai, S.; Abe, M.; Yamashita, M.; Kato, H.; Fujimaki, M. Applying proteolytic enzymes on soybean. Part VIII. Formation of an indole derivative by condensation between tryp-

tophan and n-hexanal. *Agric. Biol. Chem.* **1971**, *35*, 552−559.

Arnoldi, A.; Corain, E. A. In *Flavour Science: Recent Developments*; Taylor, A. J., Mottram, D. S., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1996; pp 217−220.

Arnoldi, A.; Corain, E. A.; Scaglioni, L.; Ames, J. M. New colored compounds from the Maillard reaction between xylose and lysine. *J. Agric. Food Chem.* **1997**, *45*, 650−655.

Bailey, R. G.; Ames, J. M.; Monti, S. M. An analysis of the nonvolatile reaction products of aqueous Maillard model systems at pH 5, using reversed-phase HPLC with diode-array detection. *J. Sci. Food Agric.* **1996**, *72*, 97−103.

Baltes, W. Reactions of the Maillard-type in foods. *Food Chem.* **1986**, *20*, 213−214.

Belitz, H. D.; Grosch, W. *Food Chemistry*; Springer: Berlin, Germany, 1987; pp 164−167.

Eriksson, C. E. Oxidation of Lipids in Food Systems. In *Autoxidation of Unsaturated Lipids*; Chan, H. W. S., Ed.; Academic Press: London, U.K., 1987; pp 207−231.

Farmer, L. J.; Mottram, D. S. Interaction of lipid in the Maillard reaction between cysteine and ribose: the effect of a triglyceride and three phospholipids on the volatile products. *J. Sci. Food Agric.* **1990**, *53*, 505−525.

Farmer, L. J.; Mottram, D. S. Effect of cysteine and ribose on the volatile thermal degradation products of a triglyceride and three phospholipids. *J. Sci. Food Agric.* **1992**, *60*, 489−497.

Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.* **1989**, *49*, 347−368.

Ge, S. J.; Lee, T. C. Kinetic significance of the Schiff base reversion in the early-stage Maillard reaction of phenylalanine-glucose aqueous model system. *J. Agric. Food Chem.* **1997**, *45*, 1619−1623.

Gomyo, T.; Horikoshi, M. On the interaction of melanoidin with metallic ions. *Agric. Biol. Chem.* **1976**, *40*, 33−40.

Hidalgo, F. J.; Zamora, R. Nonenzymatic browning and fluorescence development in a (*E*)-4,5-epoxy-(*E*)-2-heptenal/lysine model system. *J. Food Sci.* **1993**, *58*, 667−670.

Hidalgo, F. J.; Zamora, R. Characterization of the products formed during microwave irradiation of the nonenzymatic browning lysine/(*E*)-4,5-epoxy-(*E*)-2-heptenal model system. *J. Agric. Food Chem.* **1995**, *43*, 1023−1028.

Jeon, I. J.; Basette, R. Analysis of *n*-pentanal and n-hexanal as indices of potato chip shelf life. *J. Food Qual.* **1984**, *7*, 97−106.

Kaneko, S.; Okitani, A.; Hayase, F.; Kato, H. Novel cross-linking compounds formed through the reaction of N^{α}-acetyltryptophan with hexanal. *Agric. Biol. Chem.* **1989**, *53*, 2679−2685.

Karel, M. Chemical effects in food stored at room temperature. *J. Chem. Educ.* **1984**, *61*, 335−339.

Kato, H.; Kaneko, S.; Okitani, A.; Ito, M.; Hayase, F. Modification of lysine residues to alkyl-substituted pyridiniums on exposure of proteins to vaporized hexanal. *Agric. Biol. Chem.* **1986**, *50*, 1223−1228.

Kato, Y.; Watanabe, K. Sato, Y. Effect of some metals on the Maillard reaction of ovalbumin. *J. Agric. Food Chem.* **1981**, *29*, 540−543.

Keyhani, A.; Yaylayan, V. Pyrolysis/GC/MS analysis of N-(1-deoxy-D-fructos-1-yl)-L-phenylalanine: Identification of novel pyridine and naphthalene derivatives. *J. Agric. Food Chem.* **1996**, *44*, 223−229.

Kim, Y.-S.; Hartman, T. G.; Ho, C. T. Formation of 2-pentylpyridine from the thermal interaction of amino acids and 2,4-decadienal. *J. Agric. Food Chem.* **1996**, *44*, 3906−3908.

Kunert-Kirchhoff, J.; Baltes, W. Model reactions on roast aroma formation. VII. Specific products of phenylalanine after cooking L-phenylalanine with D-glucose in a laboratory autoclave. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 9−13.

Matè, J. I.; Saltveit, M. E.; Krochta, J. M. Peanut and walnut rancidity: effects of oxygen concentration and relative humidity. *J. Food Sci.* **1996**, *61*, 465−468, 472.

Meynier, A.; Genot, C.; Gandemer, G. Volatile compounds of oxidized pork phospholipids. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1−7.

Monti, S. M.; Bailey, R. G.; Ames, J. M. The influence of pH on the nonvolatile reaction products of aqueous Maillard model systems by HPLC with diode array detection. *Food Chem.* **1998**, *62*, 369−375.

O'Brien, J.; Morrissey, P. A. Metal ion complexation byproducts of the Maillard reaction. *Food Chem.* **1997**, *58*, 17−27.

O'Brien, J., Nursten, H. E., Crabbe, M. J. C., Ames, J., Eds. *The Maillard Reaction in Foods and Medicine*; Royal Society of Chemistry: Cambridge, U.K., 1998.

Onditi Ouma, A.; Dart, R. K. Influence of metal ions on hydroxymethylfurfural formation in honey. *Anal. Proc.* **1995**, *32*, 515−517.

Papadopoulou, K.; Ames, J. M. Nonvolatile compounds formed on the thermal degradation of phenylalanine. *J. Agric. Food Chem.* **1994**, *42*, 1104−1109.

Piloty, M.; Baltes, W. Investigations on the reaction of amino acids with α-dicarbonyl compounds—reactivity of amino acids in the reaction with α-dicarbonyl compounds. *Z. Lebensm. Unters. Forsch.* **1979a**, *168*, 368−373.

Piloty, M.; Baltes, W. Investigations on the reaction of amino acids with α-dicarbonyl compounds—Volatile products of the reaction of amino acids with diacetyl (2,3-butanedione). *Z. Lebensm. Unters. Forsch.* **1979b**, *168*, 374−380.

Pokorny, J.; Janitz, W.; Viden, I.; Valentova, H.; Davidek, J. Reaction of oxidized lipids with protein, part 14. Aldolization reactions of lower alkanals in the presence of nonlipidic substances. *Nahrung* **1987**, *31*, 63−70.

Pokorny, J.; Pilkova, L.; Davidek, J.; Valenova, H. Effect of Amadori rearrangement products on the nonenzymic browning in model system. *Nahrung* **1988**, *32*, 767−776.

Rizzi, G. P. Chemical structure of coloured Maillard Reaction products. *Food Rev. Int.* **1997**, *13*, 1−28.

Tashiro, Y.; Okitani, A.; Utsunomiya, N.; Kaneko, S.; Kato, H. Changes in lysozyme due to interaction with vaporized hexanal. *Agric. Biol. Chem.* **1985**, *49*, 1739−1747.

Vasiliauskaite, R.; Wedzicha, B. L. The incorporation of formaldehyde into melanoidins from the browning of glucose and glycine. *Food Chem.* **1997**, *59*, 229−235.

Wolff, S. P. Free radicals and glycation theory. In *The Maillard Reaction. Consequences for Chemical and Life Science*; Ikan, R. I., Ed.; Wiley: Chichester, U.K., 1996; pp 72−88.

Yaylayan, V. A.; Huyghues-Despointes, A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

Yaylayan, V.; Matni, G. Microwave-assisted synthesis and extraction of selected Maillard reaction products. *J. Agric. Food Chem.* **1997**, *45*, 149−152.

Zamora, R.; Hidalgo, F. J. Influence of irradiation time, pH, and lipid/amino acid ratio on pyrrole production during microwave heating of a lysine/(*E*)-4,5-epoxy-(*E*)-2-heptenal model system. *J. Agric. Food Chem.* **1995**, *43*, 1029−1033.

Received for review November 2, 1998. Revised manuscript received March 23, 1999. Accepted March 29, 1999. We thnak the European Union for financial support (TMR Program ERB4001GT970501).

JF981191G