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High-Pressure Effects on Maillard Reaction between Glucose and Lysine

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Glucose−lysine model systems prepared over a range of pH values (5−10) in unbuffered and buffered media were incubated at 60 °C either under atmospheric pressure or at 400 MPa. The results obtained showed that high pressure affected in different ways the different stages of the Maillard reaction and that such effects were strongly influenced by pressure-induced changes in the pH of the systems. In unbuffered media, at an initial pH ≤8.0, the formation of Amadori rearrangement products (ARP) was not considerably affected by pressure, whereas the intermediate and advanced stages of the Maillard reaction were suppressed, suggesting a retardation of the degradation of the ARP. In buffered media, at pH values ≤8.0, pressure slowed the Maillard reaction from the initial stages. These effects are attributed to the pH drop caused by the pressure-induced dissociation of the acid groups. In unbuffered and buffered media at initial pH = 10.2, high pressure accelerated the formation and subsequent degradation of ARP, leading to increased levels of intermediate and advanced reaction products.

KEYWORDS: Maillard reaction; high pressure; pH; Amadori compounds; browning

INTRODUCTION

The Maillard reaction is one of the most important and complex processes in food chemistry due to the large number of components able to participate through different pathways that give rise to the complex mixture of products (*1*). Briefly, it is initiated by a condensation between the carbonyl group of a reducing sugar and an amino compound, which then cyclizes to the N-substituted glycosylamine, and it is reorganized to form the Amadori rearrangement product (ARP). Further reactions, the nature of which depends on different factors such as oxygen, water, temperature, and pH of the system, give rise to different compounds that include reductones, furfurals, and a variety of other cyclic substances. Brown polymers, so-called melanoidins, are the final products of the reaction (*2*).

High pressure (100−1000 MPa), which is gaining increasing importance as a food-processing technology (*3*), and, in particular, its combination with moderate temperatures (30−60 °C), may influence the Maillard reaction with important consequences to the flavor, color, and nutritional value of foods. However, only a few studies have evaluated the effects of pressure on chemical reactions between proteins and carbohydrates and, with the information available, it is difficult to build a full picture of the mechanism of the Maillard reaction under pressure. Concerning the initial condensation reactions, estimated by measurements of the activation volumes, Tamaoka et al. (*4*) indicated that the early stages of the Maillard reaction are little affected by pressure, whereas Isaacs and Coulson (*5*) stated that pressure accelerates the initial condensation processes. With regard to the advanced stages, it was described that the formation of final heterocyclic products, melanoidins, and volatiles is greatly suppressed under pressure because of a retardation of the degradation of the ARP (*4*−*6*). However, Hill et al. (*7*) postulated an overall promoting effect of pressure on the Maillard reaction, which is overridden at low pH, because of the rate-reducing effect of the pH drop caused by the pressure-induced ionization of the acid groups present. At higher pH, because the amino group of lysine is responsible for buffering, the pH of the system is independent of pressure and the promoting effect predominates. Therefore, the lowest yields of all the identified volatile compounds, found in systems incubated under pressure at pH 10.1, were attributed to an acceleration of subsequent aldol condensation reactions (*8*). Similarly, the formation of pentosidine, a marker for the advanced Maillard reaction, was found to increase with increasing pressure at neutral pH (*9*).

In this paper we have studied the effects of pressure (400 MPa applied at 60 °C for 1−3 h) on the Maillard reaction in model lysine−glucose systems. To this end, we have looked at the initial, intermediate, and advanced stages of the reaction over a range of pH (5−10), in unbuffered and buffered media, to gain a better understanding of the influence of high pressure on this important food process.

MATERIALS AND METHODS

Chemicals. L-Lysine (98+%), L-lysine monohydrochloride (98+%), and D-glucose (99.5+%) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dihydrogen phosphate monohydrate, disodium

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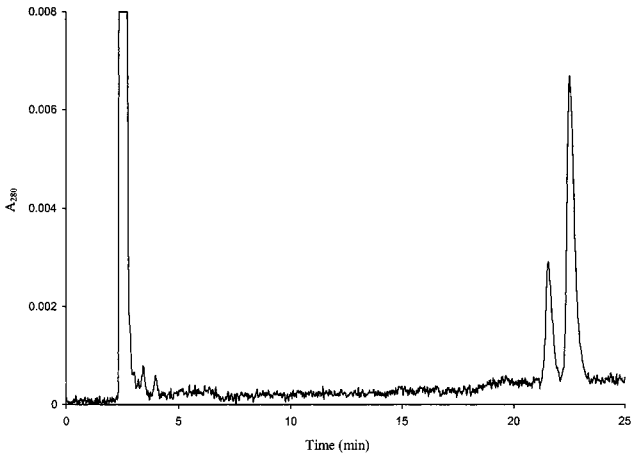

Figure 1. Reverse phase HPLC chromatogram with detection at 280 nm obtained after acid hydrolysis of the glucose–lysine unbuffered solution incubated at 60 °C and 400 MPa for 2 h, initial pH 8.0.

hydrogen phosphate dihydrate, sodium hydrogen carbonate, anhydrous sodium carbonate, and potassium chloride were obtained from Merck (Darmstadt, Germany), and hydrochloric acid was from Panreac (Barcelona, Spain). HPLC grade acetic acid was from Scharlau Chemie (Barcelona, Spain). The commercial pure standard of furosine (ϵ-2-furoylmethyl-lysine) was from Neosystem Laboratories (Strasbourg, France).

Preparation of Model Systems. Equimolar mixtures of glucose and lysine in unbuffered and buffered media (1 M concentration of each reactant) were prepared. Unbuffered solutions with initial pH values of 7.0, 8.0, and 9.2 were obtained by mixing appropriate volumes of the glucose−lysine solution (initial pH 10.2) and glucose−lysine monohydrochloride solution (initial pH 5.2), as described in ref *7*.

Sodium phosphate buffered samples at pH 7.0 and 8.0 were prepared by dissolving glucose and lysine monohydrochloride in 0.2 M sodium phosphate buffer (pH 8.0), resulting in a final pH of 7.0. Aliquots were adjusted to pH 8 by adding appropriate volumes of the glucose−lysine unbuffered solution. Sodium carbonate−bicarbonate buffered systems at pH 10.2 were prepared by dissolving glucose and lysine in 0.2 M sodium carbonate−bicarbonate buffer (pH 10.2).

In addition, 1 M solutions of glucose in the absence of lysine were prepared separately. Glucose was dissolved in distilled water, sodium phosphate buffer, and sodium carbonate−bicarbonate buffer, giving pH values of 6.4, 8.0, and 10.0, respectively. These solutions were treated in the same way as the mixtures of glucose and lysine at atmospheric and high pressure.

Heat Treatments at High and Atmospheric Pressure. Model systems were poured into Eppendorf vials (750 μL) avoiding headspace and vacuum-sealed in polyethylene bags. Pressure treatments were carried out at 400 MPa and 60 °C using a 900 HP apparatus (Eurotherm Automation, Lyon, France). The pressure was raised to 400 MPa at a rate of 2.5 MPa/s, maintained for 1−3 h at 60 °C, and released at the same rate. Control samples were heated for the same periods at 60 °C in a water bath. Model systems were cooled immediately after the treatments. Treatments were performed in duplicate, and all of the analytical determinations were performed at least in duplicate.

2-Furoylmethyl-lysine Analysis. Samples (0.5 mL) were diluted to 3.5 mL with distilled water, and 1 mL was hydrolyzed at 110 °C for 23 h under inert conditions (helium) with 2.4 mL of 11.4 N HCl, resulting in a final concentration of 8 N HCl. After filtering through Whatman 40 filter paper, 500 μL of the hydrolysate was applied to a previously activated Sep-Pak C_{18} cartridge (Millipore Corp., Bedford, MA). 2-Furoylmethyl-lysine was eluted with 3 mL of 3 N HCl, and 50 μL was used for injection. Analysis of 2-furoylmethyl-lysine was by an ion-pair RP-HPLC method using a C_8 (Alltech furosine-dedicated; Alltech, Nicolasville, KY) column (250 × 4.6 mm i.d.) and a variable wavelength detector at 280 nm (LDC Analytical, SM 4000; LDC Analytical, Salem, NH). Operating conditions were as follows: column temperature, 35 °C; flow rate, 1.2 mL/min; solvent A, 0.4% HPLC grade acetic acid in double-distilled water; solvent B, 0.3% KCl in solvent A (*10*). Calibration was performed by using known concentrations of a commercial pure standard of ϵ-2-furoylmethyl-lysine, from 0.52 to 5.2 mg/L. The presence of 2-furoylmethyl-lysine in heated samples was confirmed by RP-HPLC coupled with electrospray ionization mass spectrometry (ESI-MS) using the previously mentioned C_8 Alltech column, maintained at 37 °C and with 2% acetic acid as mobile phase. The flow rate was 0.6 mL/min, and the ES parameters were as follows: fragmentor voltage, 40 V; needle potential, 4 kV; gas temperature, 320 °C; drying gas, 10.0 L/min; nebulizer pressure, 0.28 MPa (*11*).

Absorbance Measurements. Absorbance was measured at room temperature and 294 nm, as an indication of the formation of intermediate products of nonenzymatic browning, and at 420 nm, as an index of the brown polymers formed in more advanced stages (*12*, *13*). Appropriate dilutions were made when required.

RESULTS

Effects of High Pressure on the Maillard Reaction on Glucose—Lysine Unbuffered Media. The RP-HPLC chro-

Table 1. Contents of 2-Furoylmethyl-lysine (Milligrams per 100 mg of Lys ± SD) Obtained after Acid Hydrolysis of Glucose–Lysine Unbuffered Solutions Incubated under Atmospheric (0.1 MPa) and High Pressure (400 MPa) at 60 °C at Different pH Values

pH	time (h)	α-2-furoylmethyl-lysine[a]		ε-2-furoylmethyl-lysine[a]		2-furoylmethyl-lysine total[a,b]	
		0.1 MPa	400 MPa	0.1 MPa	400 MPa	0.1 MPa	400 MPa
5.2	1					0.03 ± 0.003	0.05 ± 0.001
	2	0.03 ± 0.002 (30.0%)[c]	0.02 ± 0.002 (28.6%)	0.07 ± 0.002 (70.0%)	0.05 ± 0.001 (71.4%)	0.10 ± 0.004	0.07 ± 0.002
	3	0.04 ± 0.003 (26.7%)	0.02 ± 0.002 (22.2%)	0.11 ± 0.003 (73.3%)	0.07 ± 0.005 (77.8%)	0.15 ± 0.005	0.09 ± 0.007
7.0	1	0.19 ± 0.02 (27.5%)	0.24 ± 0.01 (27.0%)	0.50 ± 0.05 (72.5%)	0.65 ± 0.04 (73.0%)	0.69 ± 0.06	0.89 ± 0.05
	2	0.30 ± 0.02 (25.4%)	0.28 ± 0.01 (24.3%)	0.88 ± 0.08 (74.6%)	0.87 ± 0.01 (75.7%)	1.18 ± 0.08	1.15 ± 0.01
	3	0.33 ± 0.02 (24.4%)	0.37 ± 0.01 (25.0%)	1.02 ± 0.07 (75.6%)	1.11 ± 0.07 (75.0%)	1.35 ± 0.08	1.48 ± 0.08
8.0	1	0.74 ± 0.04 (25.2%)	0.78 ± 0.04 (25.7%)	2.20 ± 0.12 (74.8%)	2.26 ± 0.09 (74.3%)	2.94 ± 0.13	3.04 ± 0.11
	2	0.97 ± 0.06 (24.9%)	1.07 ± 0.08 (25.1%)	2.93 ± 0.15 (75.1%)	3.20 ± 0.15 (74.9%)	3.90 ± 0.18	4.27 ± 0.21
	3	0.96 ± 0.06 (24.4%)	0.92 ± 0.06 (23.5%)	2.98 ± 0.17 (75.6%)	3.00 ± 0.04 (76.5%)	3.94 ± 0.23	3.92 ± 0.10
9.2	1	2.45 ± 0.15 (24.1%)	2.43 ± 0.08 (23.0%)	7.71 ± 0.44 (75.9%)	8.14 ± 0.08 (77.0%)	10.16 ± 0.57	10.57 ± 0.16
	2	2.87 ± 0.22 (23.1%)	3.04 ± 0.02 (23.4%)	9.54 ±0.44 (76.9%)	9.96 ±0.15 (76.6%)	12.41 ± 0.65	13.00 ± 0.16
	3	nd[d]	nd	nd	nd	nd	nd
10.2	1	2.75 ± 0.17 (17.6%)	3.28 ± 0.30 (17.8%)	12.88 ± 0.37 (82.4%)	15.13 ±0.80 (82.2%)	15.63 ± 0.53	18.41 ± 1.10
	2	2.98 ± 0.25 (18.3%)	2.77 ± 0.06 (19.3%)	13.28 ± 0.68 (81.7%)	11.55 ± 0.18 (80.7%)	16.26 ± 0.91	14.32 ± 0.22
	3	3.14 ± 0.26 (18.8%)	2.72 ± 0.22 (19.2%)	13.52 ± 0.96 (81.2%)	11.46 ± 1.14 (80.8%)	16.66 ± 1.22	14.18 ± 0.95

[a] Treatments were carried out in duplicate, performing two separate chromatographic analyses of each sample. [b] Peaks corresponding to α- and ε-2-furoylmethyl-lysine were integrated together. [c] Percentage distribution of both 2-furoylmethyl-lysine derivatives is shown in parentheses. [d] Not determined.

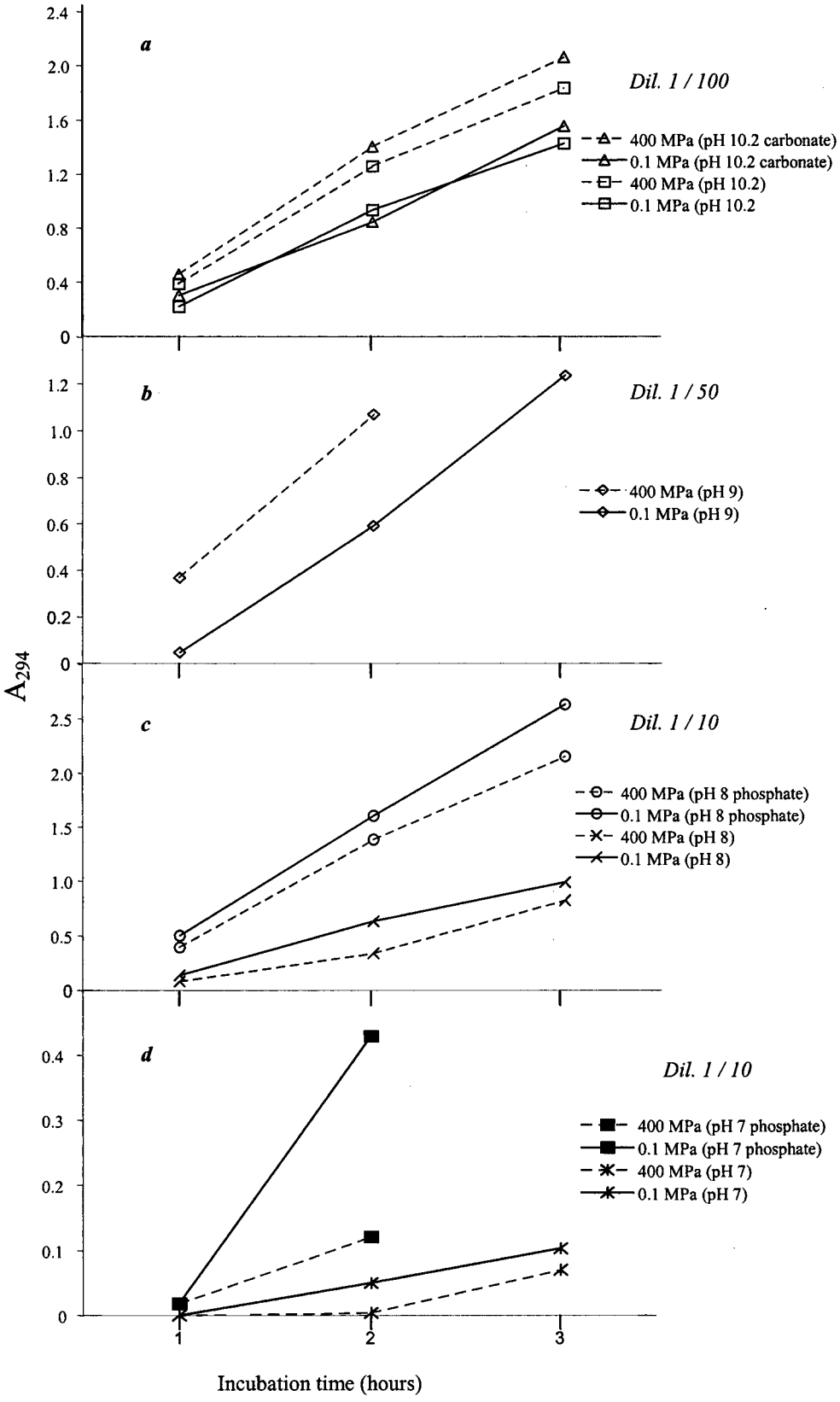

Figure 2. UV absorbance at 294 nm of glucose–lysine model systems under 0.1 MPa (solid line) and 400 MPa (dotted line) at pH 10.2 (a), 9.2 (b), 8 (c), and 7 (d) in unbuffered and buffered solutions. Treatments were carried out in duplicate, performing two separate analyses of each sample (SD < 10%). Missing data were not determined.

matograms obtained after hydrolysis of the glucose−lysine mixtures incubated at 60 °C revealed the presence of two peaks, which eluted between 21 and 23 min (**Figure 1**). The mass spectra of both peaks were characterized mainly by the presence of the ion $(M + H)^+$ at 255.2, which allowed the identification of 2-furoylmethyl-lysine. The retention time of the ϵ-2-furoyl-

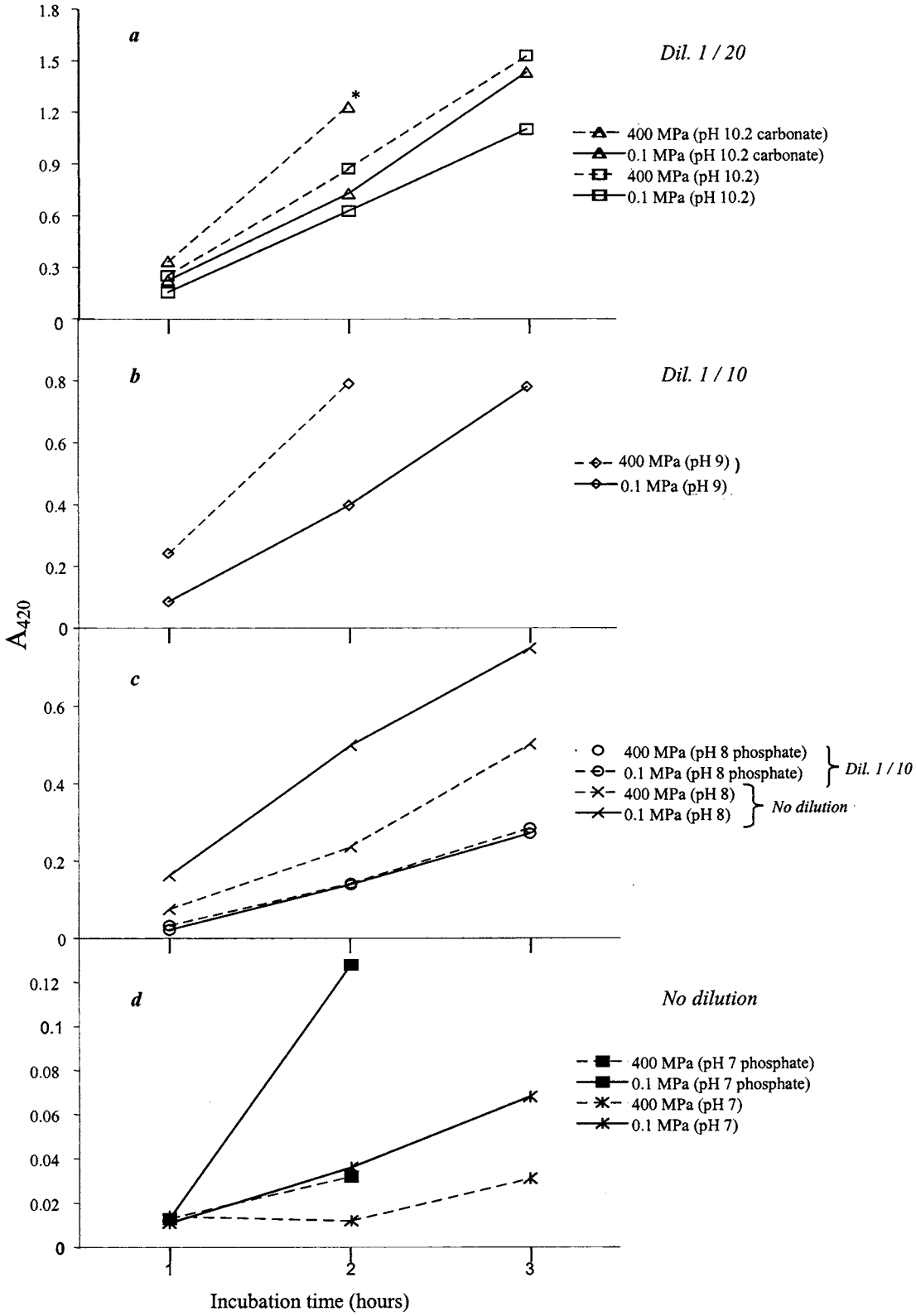

Figure 3. Browning at 420 nm of glucose–lysine model systems under 0.1 MPa (solid line) and 400 MPa (dotted line) at pH 10.2 (a), 9.2 (b), 8 (b, c), and 7 (d) in unbuffered and buffered solutions. Treatments were carried out in duplicate, performing two separate analyses of each sample (SD < 10%). *The carbonate–bicarbonate systems incubated at 400 MPa for 3 h gave a saturated absorbance value. Missing data were not determined.

methyl-lysine standard coincided with that of main, more retained compound, suggesting that the less retained peak could correspond to α-2-furoylmethyl-lysine. Thus, ARP derived from N^{α}- and N^{ϵ}-amino groups of lysine could be tentatively separated and quantified.

The levels of both 2-furoylmethyl-lysine derivatives in samples heated for 1, 2, and 3 h at atmospheric and high pressure are shown in **Table 1**. The formation of ARP rose considerably with increasing pH. N^{ϵ}-Amino derivatives were more abundant than N^{α}-amino derivatives, and these differences increased with

Table 2. Contents of 2-Furoylmethyl-lysine (Milligrams per 100 mg of Lys ± SD) Obtained after Acid Hydrolysis of Glucose–Lysine Buffered Solutions Incubated under Atmospheric and High Pressure at 60 °C at pH 7.0 and 8.0 (Sodium Phosphate Buffer) and at pH 10.2 (Sodium Carbonate–Bicarbonate Buffer)

	time	α-2-furoylmethyl-lysine[a]		ε-2-furoylmethyl-lysine[a]		2-furoylmethyl-lysine total[a,b]	
pH	(h)	0.1 MPa	400 MPa	0.1 MPa	400 MPa	0.1 MPa	400 MPa
7.0	1	0.51 ± 0.04 (34.9%)[c]	0.43 ± 0.03 (34.4%)	0.95 ± 0.09 (65.1%)	0.82 ± 0.06 (65.6%)	1.46 ± 0.13	1.25 ± 0.08
	2	1.35 ± 0.06 (33.3%)	0.71 ± 0.08 (35.0%)	2.70 ± 0.15 (66.7%)	1.32 ± 0.05 (65.0%)	4.05 ± 0.17	2.03 ± 0.13
	3	nd[d]	nd	nd	nd	nd	nd
8.0	1	1.60 ± 0.15 (28.5%)	1.43 ± 0.08 (27.4%)	4.02 ± 0.28 (71.5%)	3.78 ± 0.11 (72.6%)	5.62 ± 0.43	5.21 ± 0.18
	2	2.16 ± 0.03 (27.9%)	2.07 ± 0.10 (27.9%)	5.57 ± 0.20 (72.1%)	5.36 ± 0.25 (72.1%)	7.73 ± 0.18	7.43 ± 0.34
	3	3.01 ± 0.05 (29.5%)	2.26 ± 0.05 (27.7%)	7.20 ± 0.08 (70.5%)	5.89 ± 0.09 (72.3%)	10.21 ± 0.11	8.15 ± 0.13
10.2	1	2.46 ± 0.12 (17.6%)	2.40 ± 0.08 (17.7%)	11.55 ± 0.29 (82.4%)	11.14 ± 0.51 (82.3%)	14.01 ± 0.40	13.54 ± 0.58
	2	2.76 ± 0.13 (18.5%)	2.36 ± 0.15 (19.1%)	12.18 ± 0.33 (81.5%)	10.01 ± 0.36 (80.9%)	14.94 ± 0.45	12.37 ± 0.50
	3	2.45 ± 0.16 (18.7%)	2.39 ± 0.07 (21.0%)	10.66 ± 0.69 (81.3%)	9.00 ± 0.29 (79.0%)	13.11 ± 0.84	11.39 ± 0.36

[a] Treatments were carried out in duplicate, performing two separate chromatographic analyses of each sample. [b] Peaks corresponding to α- and ε-2-furoylmethyl-lysine were integrated together. [c] Percentage distribution of both 2-furoylmethyl-lysine derivatives is shown in parentheses. [d] Not determined.

the pH of the glucose−lysine solutions. At pH 5.2, ε-2-furoylmethyl-lysine represented ∼70% of the total, whereas at pH 10.2 it reached values ∼80%.

Regarding the effects of pressure, our results indicate that the formation of ARP at acid, neutral, and slightly basic pH was not considerably influenced by treatments at 400 MPa. However, the highest amount of 2-furoylmethyl-lysine was observed after 1 h in pressurized systems at pH 10.2. This was then followed by a decrease, while in unpressurized systems 2-furoylmethyl-lysine increased continuously during the incubation period. This trend suggested that at pH 10.2 the initial stages of the Maillard reaction, as well as the degradation of ARP, proceeded at a faster rate at 400 MPa than at 0.1 MPa.

Values of absorbance at 294 and 420 nm, attributed to intermediate products and subsequent brown polymers, respectively, were also measured. Results illustrated in **Figures 2** and **3** show that the higher the pH of the system, the higher was the absorbance at both wavelengths. The 294/420 nm absorbance ratio increased considerably at pH 9.2 and 10.2, over lower pH values, indicating that the polymerization extent depended on the pH of the reaction media (*13*). Similar A_{294}/A_{420} ratios were obtained in model systems incubated at 0.1 and 400 MPa under the same pH. As compared with atmospheric pressure, high pressure depressed the advanced stages of the Maillard reaction at pH 7.0 and 8.0, whereas it enhanced browning at pH 9.2 and 10.2. The high A_{294} and A_{420} values of pressurized systems at pH 10.2 agree with the fast degradation of ARP mentioned above.

Effects of High Pressure on the Maillard Reaction on Glucose—Lysine Buffered Media. As shown in **Table 2**, sodium phosphate buffer enhanced the rate of 2-furoylmethyl-lysine formation over nonbuffered systems (**Table 1**) at pH 7.0 and 8.0, due to the catalytic effect of the phosphate anion on the Maillard reaction (*14*, *15*). The initial stages of the Maillard reaction proceeded more quickly under atmospheric pressure at pH 7. At pH 8, the rates of formation of ARP were similar under atmospheric and high pressure during the first 2 h of incubation, but the formation then slowed in the systems incubated at 400 MPa. At pH 10.2, levels of 2-furoylmethyl-lysine were lower than in the corresponding unbuffered systems, probably because the Maillard reaction was accelerated in the buffered systems, leading to a faster degradation of the ARP. As illustrated in **Table 2**, degradation of ARP at pH 10.2 appeared to be enhanced under pressure.

Sodium phosphate buffer also enhanced the rate of formation of intermediate Maillard reaction products and browning over nonbuffered systems (**Figures 2** and **3**). At pH 7, this promoting effect was much more noticeable at atmospheric pressure than at high pressure, whereas at pH 8, the advanced stages of the reaction proceeded at very similar rates in phosphate buffer at 0.1 and 400 MPa. At pH 10.2, sodium carbonate−bicarbonate buffer also increased the rate of the reaction as compared with unbuffered systems, particularly under pressure. The highest browning development took place under pressure at pH 10.2 in buffered systems. This was in agreement with their lower levels of 2-furoylmethyl-lysine, stressing that an accelerated decomposition of ARP had occurred as compared with the model systems incubated under atmospheric pressure.

Table 3. Absorbance at 294 nm (Dilution 1/10) and 420 nm of Glucose Solutions (1 M) Incubated under Atmospheric and High Pressure at 60 °C and pH 10.0 (Dissolved in Sodium Carbonate–Bicarbonate Buffer)[a]

		A_{294}		A_{420}	
pH	time (h)	0.1 MPa	400 MPa	0.1 MPa	400 MPa
10.0	1	0.001	ND[b]	0.003	ND
	2	0.202	ND	0.077	ND
	3	0.950	0.002	0.305	0.008

[a] Treatments were carried out in triplicate. [b] Not detected.

Effects of High-Pressure Glucose Caramelization. The contribution of sugar caramelization to the nonenzymatic browning was also considered. It has been clearly established that the fragmentation of sugars increases considerably at high pH values and temperatures, yielding colored *N*-free polymers (*13*, *16*, *17*), but very few data have been found on the effect of high pressure on these reactions (*18*). Thus, samples of glucose in the absence of lysine were treated simultaneously with the mixtures of glucose−lysine at atmospheric and high pressure. At pH values <10.0, neither A_{294} nor A_{420} increased as a result of incubation at 60 °C at 0.1 or 400 MPa. However, at pH 10.0, high values of A_{294} and A_{420} were obtained after 2 and 3 h of incubation at atmospheric pressure, whereas sugar caramelization was completely inhibited under high pressure (**Table 3**). This indicates that the promoting effect of high pressure on the intermediate and advanced stages of the Maillard reaction at pH 10.2 was not overestimated in the assayed conditions, as glucose caramelization did not contribute to the browning observed.

DISCUSSION

The results presented in this work support previous papers stating that the effect of pressure on the Maillard reaction is very sensitive to the pH and nature of the medium (*7*). The Maillard reaction was strongly promoted by alkaline pH values at both 0.1 and 400 MPa. The formation of ARP underwent a large pH-dependent acceleration that can be explained by an increased reactivity of the amino groups of lysine toward the carbonyl group of glucose (*19*) and by an enhanced rate of sugar mutarotation at high-pH conditions (*20*). The promoting effect of pH on browning has also been widely documented (*21*, *22*). It is well-known that degradation of the ARP via 2,3-enolization is favored by increased pH values, and this route possesses a greater browning potential than 1,2-enolization, which is favored at acid pH (*23*).

Analyses of 2-furoylmethyl-lysine were conducted as an indication of the formation of ARP during the initial stages of Maillard reaction. ϵ-Furoylmethyl-lysine was the most abundant derivative, particularly at high pH, which might be attributed to its higher pK_a value (*24*). Another possible explanation for this may be that the N^{ϵ}-group reacts with less efficiency with α-dicarbonyl compounds than the N^{α}-amino group (*25*, *26*). Because the Maillard reaction is favored at high pH values, the degradation of ARP prevailed over their formation, as indicated by the decrease in 2-furoylmethyl-lysine found in some of our model systems incubated at pH 10.2. This may suggest that lysine, and especially its N^{α}-amino group, could react with α-dicarbonyl intermediates via several pathways, such as Strecker degradation (*27*), decreasing its participation in the formation of ARP.

At pH values $\leq$8.0, in unbuffered media, pressure did not significantly affect the initial stages of the Maillard reaction, but it retarded the intermediate and advanced stages, as measured by the absorbance at 294 and 420 nm. It has been reported that high pressure inhibits the rate of Maillard browning because the decomposition of the ARP has a positive volume of activation (*4*−*6*). However, at pH >8.0 in unbuffered solutions, the trend followed by the levels of 2-furoylmethyl-lysine, together with an increased rate of formation of intermediate and advanced products, suggested that pressure was accelerating the Maillard reaction from the initial stages.

Hill et al. (*7*), in similar glucose−lysine model systems, observed that at an initial pH of 5.1 and 6.5 pressure depressed browning; its effect was negligible at pH 7.0 and 7.5, and at pH 8.0 and 10.1 pressure significantly enhanced browning. The slight discrepancy with our results at pH 7.0 and 8.0 could be attributed to the different pressures and temperature−time combinations used (600 MPa and 50 °C from 10 to 22 h versus 400 MPa and 60 °C from 1 to 3 h in our study). According to these authors, the most likely explanation is that pressure exerts a promoting effect on the rate of the Maillard reaction that is overridden at low pH because it also induces the ionization of the carboxylic groups of lysine. This causes a decrease in pH that, as mentioned above, is responsible for a subsequent reduction in the rate of reaction. At higher pH values, buffering is by the amino group of lysine; its dissociation equilibrium is independent of pressure and, therefore, the accelerating effect of pressure predominates (*7*). Considering the present results, it should be emphasized that, at low pH, the formation of ARP did not diminish in unbuffered systems under pressure, whereas the formation of intermediate and brown polymers was suppressed. This suggests that the pressure-induced pH decrease mainly affected the formation of intermediate and advanced products through the inhibition of the degradation of the ARP via 1,2-enolization and supports previous findings on the different effect of pressure on the initial and subsequent stages of the Maillard reaction (*4*−*6*, *28*).

The influence of pH changes under pressure was confirmed by the study of the course of the Maillard reaction in buffered systems. Sodium phosphate buffer enhanced the rate of the reaction over nonbuffered systems (*14*, *15*). However, this catalytic effect was less marked under high pressure, probably because the dissociation of $H_2PO_4^-$ to HPO_4^{2-} and H^+ is promoted, causing a drop of pH (*7*, *29*). Therefore, phosphate systems with an initial pH of 7.0 were, at 400 MPa, at a lower pH than under atmospheric pressure, with a concomitant reduction in the rate of the Maillard reaction. At pH 8, the pressure-induced pH drop had less significance in the rate of the Maillard reaction, because the decrease in pH in glucose−lysine systems is partially counterbalanced by the buffering effect exerted by the N^{α}- and N^{ϵ}-amino groups of lysine (*7*, *29*). Finally, the promoting effect of high pressure on the Maillard reaction was very noticeable in carbonate buffer at pH 10.2. Carbonate buffer also increased the rate of the reaction as compared with unbuffered systems.

It should be noted that A_{294} and A_{420}, developed in systems incubated under pressure at high pH, were due to the course of only the Maillard reaction, whereas glucose caramelization contributed to the overall browning effect at atmospheric pressure. This stresses the promoting effect of high pressure on Maillard browning at high pH.

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