Influence of High Hydrostatic Pressure and pH on the Rate of Maillard Browning in a Glucose−Lysine System

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Glucose−lysine solutions, initial pH 5.1, 6.5, 8.0, and 10.1, were incubated at temperatures in the range 40−60 °C under atmospheric pressure and 600 MPa. The rate of Maillard browning at 50 °C was shown to be retarded by pressure in solutions of initial pH 5.1 and 6.5 but significantly enhanced in solutions of initial pH 8.0 and 10.1, while the effect of pressure was negligible at pH 7.0−7.5. At pH 10.1 the activation energies for the two systems (high pressure and atmospheric pressure) were not significantly different. The rates of reaction of these systems carried out in phosphate and bicarbonate buffer, at 600 MPa and alkaline pH, were slower than expected. It is proposed that pressure-induced ionization of the carboxylate group on the lysine at low pH, or of the phosphate and bicarbonate groups at high pH, causes a decrease in pH and subsequent reduction in the rate of reaction. In systems in which the pH is unaffected by pressure, *i.e.*, those buffered by the amino groups of lysine, pressure accelerates the rate of reaction. Preliminary HPLC and UV data suggest there is no difference between the chemical pathways followed with and without the application of elevated pressure.

Keywords: *High pressure; Maillard browning; glucose; lysine; xylose; color*

INTRODUCTION

High-pressure processing is a technique that has been available for many years, but only recently has its potential for the preservation and processing of food been commercially exploited (Galazka and Ledward, 1995). The application of high pressure as a sterilization process has achieved recognition due to the superior color, flavor, and nutrient retention in the product compared to thermal processing. A major drawback of high-pressure technology, in addition to the capital cost, is that spores are moderately resistant to pressure inactivation (Gould, 1995). However, moderate temperatures, such as 40−60 °C, alongside pressurization, will sterilize products more rapidly than pressure alone and may also be the most efficient means of destroying such spores (Hoover, 1993).

Although it is well established that, under most circumstances, pressure has little effect on flavor and color since covalent bond rupture will not occur, flavor changes may prevail if enzyme action is catalyzed (Knorr, 1995) or lipid material subsequently oxidizes (Cheah and Ledward, 1995). In addition, the introduction of moderate temperatures may influence chemical reactions inherent in the system, and it is the Maillard reaction which is of interest in this study.

The Maillard reaction, which is manifested by the development of color in many processed foods, is known to be highly pH and temperature dependent (Ames, 1990), but few studies have been carried out on the effect of high pressure on the Maillard reaction. In the only published report of which we are aware, Tamaoka *et al.* (1991) stated that the development of brown color was greatly suppressed when pressures of 200−400 MPa were applied to a xylose−lysine system, at 50 °C, in a $NaHCO_3$ solution at pH 8.2.

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It has been well documented that solutions of glucose and lysine, incubated at 50 °C under atmospheric pressure, develop a brown color, due to the formation of colored compounds, as a result of the Maillard reaction (Ledl and Schleicher, 1990). The reaction is, however, known to be strongly influenced by reaction conditions and these variables can include temperature and time of incubation, water activity, the sugar and amino compounds involved, and pH. In general, an increase in the pH of a system has been shown to cause a subsequent increase in the rate of color development (Ames, 1990). Lee *et al.* (1984) demonstrated that, for a glucose−lysine system at 100 °C, a pH rise from 4 to 8 caused a subsequent increase in color development. Ashoor and Zent (1984) found maximum color development around pH 10 for a glucose−lysine system, although at higher values of pH the rate of color development declined. The study reported here was designed to compare the rate of browning of glucose−lysine systems at 50 °C, over a range of pH values, with and without the application of high hydrostatic pressure.

EXPERIMENTAL PROCEDURES

Materials. L-Lysine (97%), L-lysine monohydrochloride (99+%), D-glucose (ACS reagent), and D-xylose (99+%) were obtained from Aldrich Chemical Co. (Gillingham, Dorset, U.K.). Potassium dihydrogen orthophosphate (AnalaR), disodium hydrogen orthophosphate 2-hydrate (AnalaR), and sodium hydrogen carbonate (AnalaR) were obtained from BDH Laboratory Supplies (Poole, Dorset, U.K.).

Instruments. Samples were pressurized at 600 MPa at the set temperature, in heat-sealed polyethylene bags, in a prototype Stansted "Food-Lab" high-pressure rig (Stansted Fluid Power Ltd., Stansted Essex, U.K.) as described by De Faye *et al.* (1995). The degree of browning of the amino acid−sugar solutions was assayed by measurements at 420 nm using a Perkin-Elmer (Beaconsfield, Bucks, U.K.) 552 spectrophotometer. Absorbance spectra were recorded between 200 and 450 nm using a Perkin-Elmer Lambda 5 UV−vis spectrophotometer. HPLC data were collected using a Hewlett-Packard (Bracknell, Berks, U.K.) 1050 quaternary pump, autosampler,

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and diode array detector. Data analysis was carried out with Hewlett-Packard HP Chemstation software.

Sample Preparation. One molal aqueous solutions, with respect to both the amino acid and glucose, were prepared. Solutions with initial pH values of 5.1, 6.5, 8.0, and 10.1 were obtained by mixing appropriate volumes of the glucose−lysine solution (initial pH 10.1) and glucose−lysine monohydrochloride solution (initial pH 5.1). One molal glucose−lysine monohydrochloride solution was prepared using 0.2 M Sørensen's phosphate buffer (pH 8.0), resulting in a solution of pH 6.9. This was then adjusted to pH 8.0 using 1 molal aqueous glucose−lysine.

One molar xylose−lysine monohydrochloride solution was prepared using 0.1 M sodium bicarbonate solution (pH 8.3), resulting in a solution of pH 6.3. This solution was divided, and half was adjusted to pH 8.0 using 1 M aqueous xylose−lysine.

All solutions were incubated at the set temperature, either in covered test tubes in a water bath at atmospheric pressure (±0.1 °C) or in sealed polyethylene bags in the high-pressure rig at 600 MPa (±2 °C). During pressurization the temperature rose by approximately 15 °C but settled back to the experimental temperature within 5 min.

Samples were taken from both reaction systems at regular time intervals; sampling from the high-pressure rig was on a continuous basis and involved depressurization, sample removal, and subsequent repressurization of the remaining samples. Application of pressure to solutions for each time interval without de/repressurization was carried out at pH 10.1, but results were similar, *i.e.*, within the range found for solutions subjected to de/repressurization; hence, it was considered to be acceptable to follow the described method.

Absorbance Measurements. After sampling, the pH was adjusted to 7.0 using sodium hydroxide (0.1 and 1 M) solution or hydrochloric acid (0.1 and 1 M). Less than 0.5 mL of alkali or acid was required for 10 mL of glucose−lysine solution. The absorbance of the solutions was measured at 420 nm against distilled water. Absorbance spectra were also recorded between 200 and 450 nm. All solutions were cooled in ice, and absorbance measurements obtained within 5 min. For the systems with initial pH 6.5, 8.0, and 10.1, experiments were carried out in duplicate, using freshly prepared amino acid−glucose solutions. The experiment at pH 5.1 and 600 MPa was, however, run only once as the time to reach a significant degree of browning at this pH was more than 4 days. The experiments in phosphate buffer and sodium bicarbonate were not replicated.

HPLC. HPLC was carried out using a 25 cm, 0.49 cm i.d., 5 μm Spherisorb ODS2 column (Hichrom Ltd., Theale, Reading, Berks, U.K.). The method followed was that of Bailey *et al.* (1995). Duplicate samples of glucose−lysine solution (pH 10.1) incubated at 50 °C for 4 h (atmospheric pressure sample) and 1 h (600 MPa) were injected onto the column. These samples gave approximately the same absorbance readings at 420 nm.

RESULTS AND DISCUSSION

Figure 1 shows the browning curves of 1 molal glucose−lysine solutions of initial pH 8.0, incubated at 50 °C, both with and without the application of pressure. Replicate experiments are plotted separately. A rate-enhancing effect of 600 MPa on browning at this pH is clearly shown. As expected (Labuza, 1994), there is an induction period before the absorbance increases rapidly, and apparently linearly, with time. Similar curves were plotted at the other pH values, and the linear portions of the browning curves were used to determine the pseudo-zero-order rate constants. However, at pH 5.1, the rate under pressure was so slow that a clearly defined zero-order phase was not seen. Thus, to allow a comparison across the full pH range, the time to achieve an absorbance of 0.15 was plotted against pH (Figure 2). This plot shows that, at an initial pH of 8.0

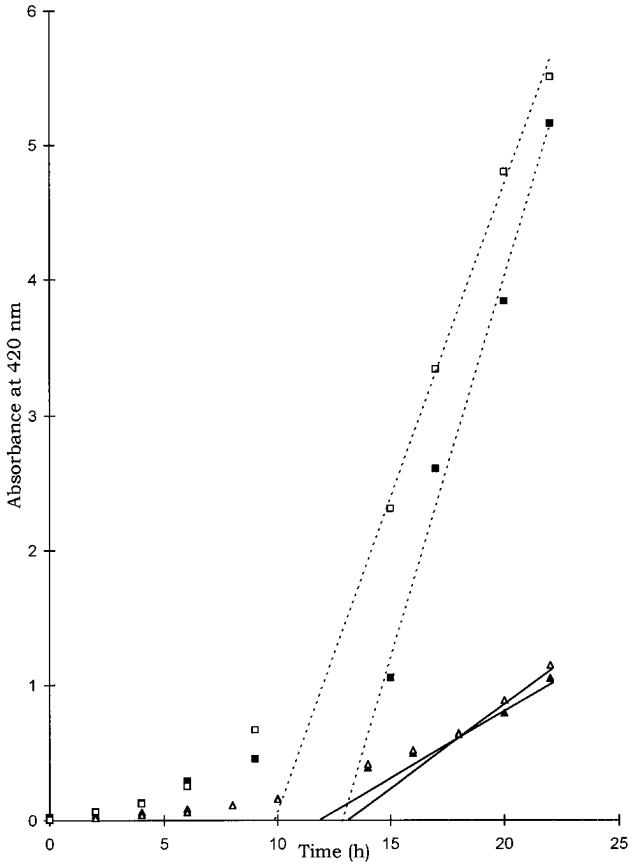

Figure 1. Increase in absorbance at 420 nm with time for 1 molal glucose−lysine solutions, initial pH 8.0, incubated at 50 °C under atmospheric pressure (▲, △) and 600 MPa (■, □).

or 10.1, pressure enhances browning, while at pH 6.5 and 5.1 the effect is reversed and application of pressure significantly depresses the rate of browning.

These results were confirmed from the plots of zero-order rate constant against pH (Figure 3), from which it can be seen that the rate constant increases markedly with pH, for both the pressurized and unpressurized systems. At atmospheric pressure, the rate increases approximately 40 times for each unit increase in pH, while at 600 MPa it increases around 1000 times for each unit increase in pH. Figure 3 also demonstrates that, at pH 6.5, the rate of browning is approximately 3 times faster under atmospheric pressure, while at pH 8.0 browning is accelerated around 4 times by pressure and at pH 10.1 this rate-enhancing effect is magnified to give browning approximately 9 times faster under pressure. Hence, Figures 2 and 3 show that the application of pressure to glucose−lysine systems with an initial pH of 5.1 and 6.5 suppresses the rate of color development but that the effect is reversed at a pH of between 7.0 and 7.5, so, at an initial pH of 8.0 or 10.1, pressure greatly accelerates the rate of browning.

Throughout the experiments the pH of the solutions was monitored, for both the atmospheric and high-pressure systems. Table 1 shows the rate of pH decline, for the glucose−lysine solutions of initial pH 5.1, 6.5, 8.0, and 10.1, during the initial phase of the reaction when the drop in pH was linear with time. It can be seen that the solutions of initial pH 6.5 and 8.0 gave initial rates of pH decline that were almost identical in the pressurized and unpressurized systems, while at pH 5.1 and 10.1 the application of pressure appears to have slightly enhanced the rate of pH decline. Hence, it is tentatively suggested that high pressure has increased

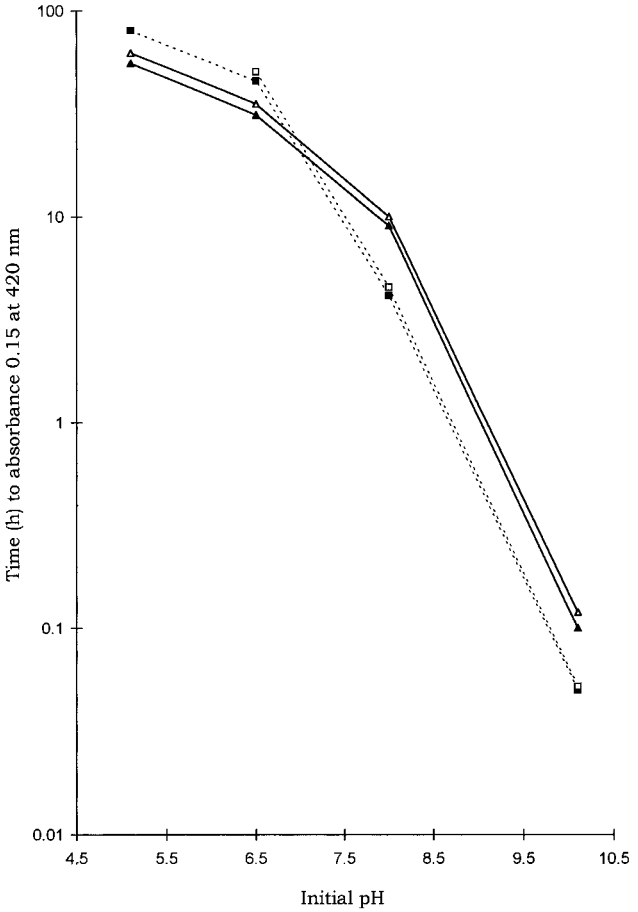

Figure 2. Time to reach absorbance of 0.15 at 420 nm for 1 molal glucose−lysine solutions, initial pH 5.1, 6.5, 8.0, and 10.1, incubated at 50 °C under atmospheric pressure (▲, △) and 600 MPa (■, □).

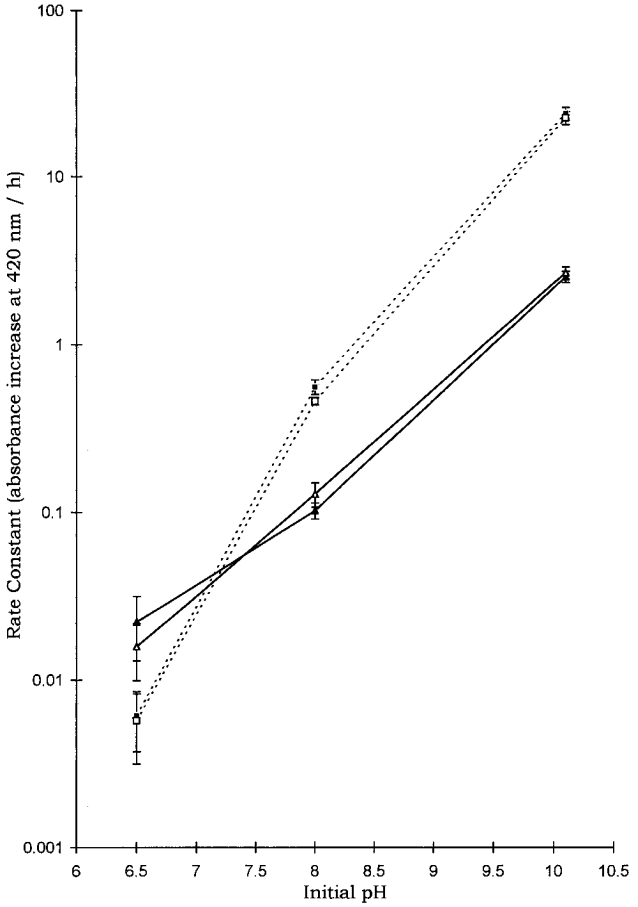

Figure 3. Rate constants (absorbance increase at 420 nm/h) for 1 molal glucose−lysine solutions, initial pH 6.5, 8.0, and 10.1, incubated at 50 °C under atmospheric pressure (▲, △) and 600 MPa (■, □). Errors shown are estimated from the slopes of absorbance against time.

Table 1. Rate of Initial Linear pH Decline for 1 Molal Glucose−Lysine Solutions, Initial pH 5.1, 6.5, 8.0, and 10.1, Incubated at 50 °C under Atmospheric Pressure and 600 MPa

initial pH	rate of pH decline (pH units/h) at atmospheric pressure[a]	rate of pH decline (pH units/h) at 600 MPa[a]
5.1	(1) 0.006 (2) 0.006	0.013
6.5	(1) 0.21 (2) 0.25	(1) 0.20 (2) 0.20
8.0	(1) 0.51 (2) 0.52	(1) 0.54 (2) 0.41
10.1	(1) 0.15 (2) 0.33	(1) 0.30 (2) 0.50

[a] (1) and (2) denote replicate experiments.

the rate of disappearance of the amino groups at the early stage of the Maillard reaction, in glucose−lysine solutions of initial pH 5.1 and 10.1. Heremans (1995) reports data on the effect of pressure on the changes in pH of amino and carboxylic acid groups and suggests that, under pressure, the equilibrium $CH_3COOH \rightleftharpoons CH_3COO^- + H^+$ favors the ionic species, causing a resultant drop of about 0.2 pH unit per 100 MPa of pressure applied. This decrease in pH may explain the inhibitory effect of pressure on browning at low pH since, at these pH values, buffering is principally by the carboxylic acid group on the amino acid, and thus a decrease of about 1.2 pH units may occur. It is well documented that a decrease in pH causes a decrease in the rate of Maillard browning.

However, at high pH values, buffering is by the alpha and epsilon amino groups of lysine and the application of pressure should not cause any significant effect on the pH of the system since the $NH_3^+ \rightleftharpoons NH_2 + H^+$ equilibrium is largely independent of pressure (Heremans, 1995). Therefore, we may postulate that, at pH values above approximately 7.0, pressure enhances the rate of color development. This enhancement may also operate at lower pH but is overridden by the pressure-induced pH decrease of the system.

It is possible that the observed pressure effects are due to differences in the composition of the reaction products. However, initial HPLC analysis carried out on pressurized and unpressurized glucose−lysine solutions (initial pH 10.1) at the same intensity of browning indicated there were no significant qualitative differences in the chromatograms at four detection wavelengths (254, 280, 360, and 460 nm), and, in addition, UV spectra of pressurized and unpressurized solutions were similar. Thus, this explanation seems unlikely.

Further confirmation that the mechanisms at high and normal pressure are very similar is suggested by the temperature dependence of the reaction at pH 10.1, at which it was found that the activation energies were not significantly different, 110.6 ± 18.6 kJ mol^{-1} at atmospheric pressure and 113.0 ± 25.4 kJ mol^{-1} at 600 MPa (Figure 4).

Experiments were also carried out using 1 molal glucose−lysine in 0.2 M Sørensen's phosphate buffer

Table 2. Rate of pH Decline and Rate Constants for Glucose− and Xylose−Lysine Solutions at Initial pH Values of 8.0 and 6.3 Incubated at 50 °C under Atmospheric Pressure and 600 MPa

	1 molal glucose−lysine, initial pH 8.0[a]	1 molal glucose−lysine in phosphate buffer, initial pH 8.0	1 M xylose−lysine in sodium bicarbonate, initial pH 8.0	1 M xylose−lysine in sodium bicarbonate, initial pH 6.3
rate of pH decline (pH units/h) at atmospheric pressure	0.52*	0.45	0.57	0.12
rate of pH decline (pH units/h) at 600 MPa	0.48*	0.38	0.79	0.16
rate constant (absorbance increase at 420 nm/h) at atmospheric pressure	0.12 ± 0.02*	0.84 ± 0.22	11.84 ± 1.47	4.18 ± 0.96
rate constant (absorbance increase at 420 nm/h) at 600 MPa	0.51 ± 0.04*	1.60 ± 0.39	14.48 ± 1.54	1.56 ± 0.60

[a] * denotes mean value of duplicate experiments. Other figures quoted are from single experiments.

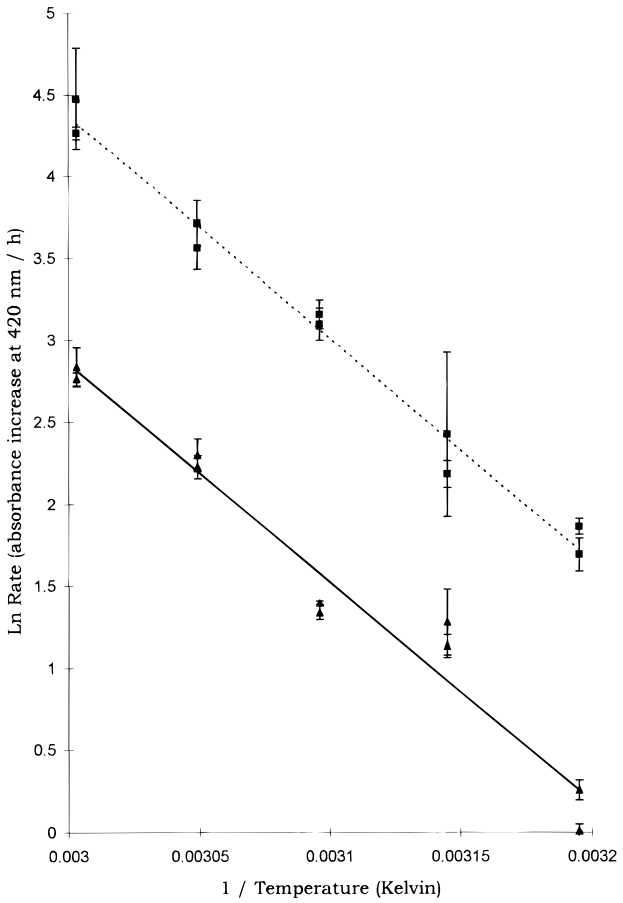

Figure 4. Arrhenius plot (ln rate of absorbance increase at 420 nm/h versus 1/temperature) for 1 molal glucose−lysine solutions, initial pH 10.1, incubated at 40−60 °C under atmospheric pressure (▲, △) and 600 MPa (■, □). Errors shown are estimated from the slopes of the absorbance against time plots.

(pH 8.0), at an initial pH of 8.0, and 1 M xylose−lysine in 0.1 M sodium bicarbonate (pH 8.3), initial pH 6.3 and 8.0. Rate constants and rate of pH decline were calculated and are shown in Table 2. The glucose−lysine−phosphate buffer solution, initial pH 8.0, gave a very similar rate of pH decline under atmospheric pressure and 600 MPa, but a comparison of the increase in browning shows that, under pressure, the solution browns at approximately twice the rate of that incubated under atmospheric conditions. However, glucose−lysine without the addition of phosphate browns about 4 times faster under pressure (Table 2). Thus the pressure-induced enhancement of browning in the phosphate system is less than expected. This may be explained by pressure shifting the equilibrium $H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$ to the right-hand side, causing a drop of 0.4 pH unit per 100 MPa of pressure applied, *i.e.*, 2.4 units at 600 MPa (Heremans, 1995). Hence, under pressure, the solutions will be at a lower pH than at normal pressure, with a concomitant reduction in Maillard browning. The buffering in these systems will be by both the alpha amino of lysine and phosphate groups and, thus, the decrease in pH will not be as great as in phosphate alone. The rate of color development in the phosphate systems, under atmospheric pressure, is considerably faster than in the aqueous systems due to the catalytic effect of phosphate on the Maillard reaction (de Kok and Rosing, 1994).

The experiment using a xylose−lysine solution in sodium bicarbonate was carried out in an attempt to duplicate the work of Tamaoka *et al.* (1991), who found that in a similar system browning was inhibited by the application of pressures from 200 to 400 MPa. In our experiment, a xylose−lysine solution prepared in sodium bicarbonate, as outlined by Tamaoka *et al.* (1991), resulted in an initial pH of 6.3 (not 8.2). Under these conditions it is seen that, under pressure, the rate of browning is approximately half that observed under atmospheric pressure (Table 2). This agrees well with the results of Tamaoka *et al.* (1991). The experiment was also carried out with the pH adjusted to 8.0, and here browning was slightly enhanced by pressure (Table 2). Once again it can be suggested that the application of pressure caused ionic dissociation of the sodium bicarbonate, resulting in a decrease in pH. Cheftel (1992) quotes the results of Kunugi (1991), which indicate a 0.49 decrease in pH for every 100 MPa of pressure applied to H_2CO_3. This pH decrease will be diminished due to the buffering of the alpha amino group of lysine but will explain the fact that the rate of browning at 600 MPa is similar to that under atmospheric pressure, compared to the bicarbonate-free system, in which the rate of browning is about 4 times faster under pressure (Table 2 and Figure 3).

In conclusion, the results obtained suggest that pressure exerts a rate-enhancing effect on Maillard browning at pH values above 7.0−7.5, while at lower pH the reverse is seen and color development is retarded by the application of pressure.

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