

Effect of High Pressure on Isomerization and Degradation of Lactose in Alkaline Media

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The combined effects of temperature (60 °C) and high-pressure treatments (400 MPa for 3 h) on the isomerization–degradation of lactose (10%) in basic media were studied. The formation of isomeric disaccharides (lactulose and epilactose) and galactose decreased by the application of high pressure in aqueous sodium hydroxide 4 mM (pH 10.2) and 8 mM (pH 10.6), and sodium carbonate–bicarbonate buffer (pH 10.0). In addition, no substantial color development was observed in the sodium hydroxide systems for high and atmospheric pressure, whereas the application of high pressure led to a noticeable decrease of color development in the carbonate–bicarbonate system.

KEYWORDS: High pressure; lactose; isomerization; browning

INTRODUCTION

The application of high pressure in processing of foods has been widely studied in the past few years. High-pressure sterilization process has achieved recognition due to the superior color, flavor, and nutrient retention in the product compared to thermal processing. High pressure processing successfully inactivates microorganisms but is less effective at inactivating bacterial spores and some enzymes. Processing at high pressure (100–1000 MPa) combined with moderate temperatures (40–60 °C) is recommended for effective elimination of spores (1, 2). The effect of the combined use of high pressure and temperature on chemical–physical modifications, that may occur on food constituents during processing, needs to be known for a realistic evaluation of the resulting food and to develop new processes for manufacturing products with improved properties.

Nonenzymatic browning (caramelization and Maillard reactions) is a complex sequence of reactions responsible for color development during heat treatment and storage of foods. Nonenzymatic browning during heating of foods has been widely studied, but very little research is available on the effects of high-pressure treatment on browning. Previous reported studies were limited to the effect of pressure on the Maillard reaction (2–7). However, during nonenzymatic browning of foods, various degradation products are formed from carbohydrates via caramelization making a significant contribution to the brown color development and may therefore lead to overestimation of the extent of the Maillard reaction in foods (8).

The fundamental reactions of carbohydrate caramelization include isomerization aldose to ketose, fragmentation reactions, and browning. During heat treatment of milk or lactose solutions in basic media, lactose is first converted into lactulose followed by the degradation of lactulose to isosaccharinic acids and

galactose, which in turn are converted into precursors of brown products (9–11). The purpose of the present study was to investigate the combined effect of high pressure and temperature on the initial stage of caramelization and the browning reaction in the advanced stage in basic solutions of lactose.

MATERIALS AND METHODS

Chemicals. Lactose monohydrate was obtained from Scharlau Chemie (Barcelona, Spain). Lactulose, epilactose, galactose, phenyl- β -glucoside, and trimethyl-silylimidazol were provided by Sigma (St. Louis, MO). Sodium hydroxide, sodium hydrogen carbonate, and anhydrous sodium carbonate were purchased from Merck (Darmstadt, Germany).

Sample Preparation and Treatments at High and Atmospheric Pressure. Samples were prepared by dissolving lactose in aqueous solutions of 4 and 8 mM sodium hydroxide (initial pH values of 10.2 and 10.6, respectively) or 0.2 M sodium carbonate–bicarbonate buffer (pH 10.0) to give solutions of 10% of lactose. Samples submitted to pressurization were placed into Eppendorf vials (750 μ L) avoiding headspace, vacuum-sealed in polyethylene bags, and pressurized using a 900 HP equipment (Eurotherm Automation, Lyon, France). The pressure was raised to 400 MPa at a rate of 2.5 MPa s^{-1} , maintained for 1 and 3 h at 60 °C, and released at the same rate. Samples heated at atmospheric pressure were also placed into Eppendorf vials and incubated in a water bath at 60 °C for the same periods of time. After treatments, all samples were cooled immediately in an ice–water bath. Duplicates of each model system were prepared.

Gas–Liquid Chromatography (GLC) Analysis. Carbohydrate concentrations were determined by means of gas chromatography of the trimethylsilyl derivatives of the free carbohydrate fraction using a Varian 3380 gas chromatograph (Varian Associates, Madrid, Spain) equipped with a 3 m \times 1.0 mm i.d. stainless steel column (Chrompack, Middelburg, The Nether-

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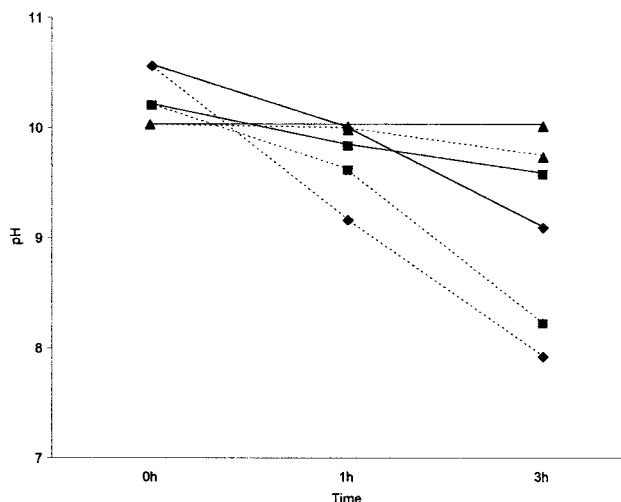


Figure 1. pH measurements before (0 h) and after (1 and 3 h) atmospheric (···) and high (—) pressure treatments at 60 °C in sodium carbonate–bicarbonate (▲) and sodium hydroxide solutions (◆, 8 mM; ■, 4 mM).

lands) packed with 2% OV-17 on nonsilanized 120/140 Volaspher A-2 (E. Merck AG, Darmstadt, Germany). Samples (0.5 mL) were mixed with 0.5 mL of 1% phenyl- β -D-glucoside (internal standard) in 70% methanol. The mixture was diluted to 5 mL with methanol and kept for 1 h at room temperature. One milliliter of the filtrate was evaporated under vacuum at 30 °C and converted to trimethylsilyl derivatives using *N*-trimethylsilylimidazole (12).

Color and pH Measurements. Browning was monitored by measuring the absorbance of the samples at 420 nm using a Shimadzu model UV-120–01 spectrophotometer (Shimadzu Instruments, Columbia, USA). pH measurement was carried out with a pH meter MicropH2001 (Crison Instruments, Barcelona, Spain). Both measurements were performed at 20 °C before and after the treatments.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of pH during heat treatment of lactose dissolved in sodium hydroxide solutions and carbonate–bicarbonate buffer. A noticeable decrease of pH was observed in the sodium hydroxide solutions, probably due to the formation of acidic compounds, such as acetic, formic, and isosaccharinic acids, derived from disaccharide degradation (13). As can be observed, higher pH values were found in samples subjected to high pressure than in samples treated at atmospheric pressure. However, no changes of pH in lactose solutions in carbonate–bicarbonate buffer were observed except for a decrease of 0.3 pH units in the sample heated for 3 h at atmospheric pressure. These results are probably due to the buffer capacity of the carbonate–bicarbonate system, which maintained a higher pH during the course of the treatment.

Table 1 shows the formation of lactulose, epilactose, and galactose in heat-treated lactose solutions incubated at either atmospheric pressure or 400 MPa. As expected, the concentrations of the three carbohydrates formed increased with time. The highest isomerization and degradation of lactose was attained in the samples of carbonate–bicarbonate buffer, heated at atmospheric pressure for 3 h.

At atmospheric pressure, both isomerization and degradation of lactose were higher in sodium carbonate–bicarbonate buffer than in sodium hydroxide solutions. Significant differences among samples were particularly noticeable after 3 h of treatment. Previous studies on isomerization of lactose in

Table 1. Formation of Lactulose, Epilactose, and Galactose during Heat Treatment at 60 °C of 10 g/100 mL Lactose Solutions in Basic Media under Atmospheric and High Pressure Conditions^a

basic media	pressure	carbohydrate (mg/100 mL)					
		lactulose		epilactose		galactose	
		1 h	3 h	1 h	3 h	1 h	3 h
carbonate–bicarbonate buffer	atmospheric	1294.8	1875.9	32.9	80.2	158.5	633.5
	pressure	(23.9)	(187.1)	(0.6)	(8.1)	(5.3)	(24.3)
	400 MPa	499.1	779.6	14.0	21.9	85.1	90.0
		(26.8)	(4.1)	(1.8)	(1.0)	(5.2)	(4.2)
8 mM sodium hydroxide	atmospheric	1056.7	1329.4	37.7	45.7	74.9	104.4
	pressure	(36.2)	(93.5)	(3.5)	(3.8)	(1.2)	(3.6)
	400 MPa	788.3	1046.5	16.5	21.4	43.9	86.7
		(17.2)	(16.5)	(1.1)	(1.1)	(5.5)	(2.8)
4 mM sodium hydroxide	atmospheric	687.5	822.8	16.7	18.6	21.8	30.3
	pressure	(64.2)	(52.6)	(0.9)	(0.2)	(0.1)	(0.0)
	400 MPa	378.2	633.0	6.2	10.9	16.4	26.6
		(33.8)	(13.0)	(2.0)	(1.2)	(0.1)	(1.6)

^a Data are the mean of duplicate and standard deviation is in brackets.

different buffer solutions showed that the type of buffer seemed to have no influence, and only pH was the source of variation. With increasing pH the formation of lactulose, epilactose, and galactose increased (11). The higher formation of these carbohydrates found in the sodium carbonate–bicarbonate buffer solution should be mainly attributed to the higher pH maintained during the heat treatment process. Regarding sodium hydroxide solutions, the highest rates of lactose isomerization and degradation were observed in the 8 mM system, which showed the highest initial pH.

At high pressure, the highest rates of isomerization and degradation of lactose were observed in the 8 mM sodium hydroxide system, whereas no statistical differences ($P > 0.01$) were found between the values of lactulose formed in 4 mM sodium hydroxide and sodium carbonate–bicarbonate systems.

In all cases studied, high pressure caused a considerable inhibition of isomerization and degradation of lactose as compared to the samples treated at atmospheric pressure. These effects were more noticeable in the carbonate–bicarbonate system, which achieved 7.8% of lactose conversion into lactulose and 1.8% of lactose degradation into galactose after 3 h under high pressure, as opposed to 18.8 and 12.6% for lactulose and galactose, respectively, under atmospheric pressure treatments. In sodium hydroxide solutions heated at 400 MPa for 3 h, the formation of lactulose and galactose was similar to that observed after 1 h heating under atmospheric pressure. These data agree with the lower decrease of pH observed in pressurized samples (**Figure 1**), which suggested a lower rate of lactose degradation to acidic compounds. It is generally admitted that high pressure, which favors processes characterized by volume decrease, favors the ionized form and, therefore, induces dissociation of weak acids causing a reversible drop of pH (14–16). Kunugi (17) reported a 0.49 decrease in pH for every 100 MPa of pressure applied to H_2CO_3 . This suggests that pressurization of the carbonate–bicarbonate buffer solutions probably caused a decrease of pH, resulting in lower formation of lactose and galactose.

In relation to a strong base, to our knowledge, no studies on pH variations under pressure have been performed. However, one of the most studied examples is the dissociation of hydronium and hydroxyl ions from water, which is accompanied by a volume decrease of 21.3 mL/mol at 25 °C (14, 18). Therefore, pressure favors the dissociation of water, and the pH of distilled water has been reported to decrease between

Table 2. Color Development during Lactose Isomerization at 60 °C in Alkaline Media at Atmospheric and High Pressure

basic media	pressure	1 h	3 h
carbonate buffer	atmospheric pressure	0.067	0.769
	400 MPa	0	0.047
sodium hydroxide (8 mM)	atmospheric pressure	0.044	0.077
	400 MPa	0.032	0.077
sodium hydroxide (4 mM)	atmospheric pressure	0	0.005
	400 MPa	0	0.009

−0.16 to −0.73 at 100 MPa and 25 °C (14–16, 19–21). In aqueous solutions of sodium hydroxide, the decrease in pH is not probably as pronounced as in pure water due to the elevated concentration of hydroxyl ions that could decrease the ionic dissociation of water. This could explain that the inhibition of isomerization and degradation of lactose produced by pressure was less noticeable in sodium hydroxide media than in the carbonate–bicarbonate medium as a result of a lower decrease of pH during the treatment in the former.

Browning measured at 420 nm is shown in **Table 2**. The most pronounced browning development was observed in the carbonate–bicarbonate system under atmospheric pressure, and the application of high pressure gave rise to a considerable decrease of browning. In the sodium hydroxide systems, a slight color development was observed and no differences between both high and atmospheric pressure treatments were found. Both color and galactose are formed as a consequence of carbohydrate degradation, and then the galactose formed in the carbonate–bicarbonate buffer at atmospheric pressure was greatly higher than those formed under pressure, whereas the levels found in the sodium hydroxide systems were more similar between atmospheric and high pressure.

On the basis of the results obtained we can conclude that an important reduction of caramelization of lactose can be achieved under pressure (400 MPa) at 60 °C in alkaline pH. Since degradation products of carbohydrates can be involved as starting materials in Maillard reactions pathways, the effect of pressure on carbohydrate caramelization should be considered in the studies of Maillard reaction under high pressure.

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