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The effect of high pressure homogenization on the activity of a commercial β-galactosidase

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Abstract High pressure homogenization (HPH) has been proposed as a promising method for changing the activity and stability of enzymes. Therefore, this research studied the activity of β-galactosidase before and after HPH. The enzyme solution at pH values of 6.4, 7.0, and 8.0 was processed at pressures of up to 150 MPa, and the effects of HPH were determined from the residual enzyme activity measured at 5, 30, and 45 °C immediately after homogenization and after 1 day of refrigerated storage. The results indicated that at neutral pH the enzyme remained active at 30 °C (optimum temperature) even after homogenization at pressures of up to 150 MPa. On the contrary, when the β-galactosidase was homogenized at pH 6.4 and 8.0, a gradual loss of activity was observed, reaching a minimum activity (around 30 %) after HPH at 150 MPa and pH 8.0. After storage, only β-galactosidase that underwent HPH at pH 7.0 retained similar activity to the native sample. Thus, HPH did not affect the activity and stability of β-galactosidase only when the process was carried out at neutral pH; for the other conditions, HPH resulted in partial inactivation of the enzyme. Considering the use of β-galactosidase to produce low lactose milk, it was concluded that HPH can be applied with no deleterious effects on enzyme activity.

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Introduction

High pressure homogenization (HPH) is an emerging technology developed to process food with minimal sensory and nutritional damage [7] when compared to the traditional thermal process. HPH is based on the homogenization process widely used in the dairy industry for breaking up fat globules [23], but applying pressures 10 times higher. Using high pressures, this process inactivates vegetative bacterial [4, 23, 28], yeast, and molds cells [1, 29, 30]. Thus, HPH was proposed to improve the safety and microbiological quality of milk, being similar to pasteurization [9, 10].

The effects of HPH on milk are not limited to microbial reduction and also include changes in the milk constituents, such as modifications in the ratio of the nitrogen fractions [11], changes in the soluble forms of calcium and phosphate [11], aggregation of whey proteins with the casein [6], fat globule size reduction [10], and greater dissolution of the α- and κ-caseins [26]. These physical changes improve the sensory characteristics of HPH milk, such as its mouth feel and aeration capacity [2]. Also, HPH is of interest to prepare milk for the manufacture of fermented dairy products owing to (1) enhancement of proteolytic and lipolytic activity during cheese ripening [9]; (2) maximization of starter growth during fermentation and also reduction of losses in viability during refrigerated storage [15, 22]; (3) enhancement of hydrophobic peptides during fermentation, which have potential biological activities [27]; (4) reduction of syneresis and increase in firmness of fermented milk [22, 27]; (5) improvement of the aromatic

profile of the fermented products [22]; (6) improvement of the water binding capacity of cheese proteins with less whey separation [9].

Milk and dairy products are recognized as good sources of high-quality protein and calcium [5, 19]. However, lactose intolerance, which affects 3–70 % of people from different population groups, limits the digestion of these foods [12]. Moreover, lactose reduction improves the technological and sensory properties of dairy products [8, 12, 25]. Therefore, the production of lactose-free or low-lactose dairy products is desirable [12] and these could be obtained by the addition of β-galactosidase (EC 3.2.1.23) to the milk, because this enzyme catalyzes the hydrolysis of lactose into glucose and galactose [12, 13, 19].

HPH was previously described as a process capable [14, 16–18, 31, 32, 34] or otherwise [33] of changing enzyme activity and stability, the effects normally being associated with the individual enzyme being evaluated and with the homogenization pressure applied [16, 17].

Data about pectin methyl esterase indicated that homogenization was just able to inactivate the enzyme [14, 34], whereas results obtained for polyphenol oxidase showed that HPH between 120 and 160 MPa causes an increase of enzyme activity [16, 17]. Data obtained for α-amylase [33] and trypsin [18] showed no changes on enzyme activity upon HPH; however, an increase on trypsin thermal stability was observed [18]. Data on neutral protease [32] and amyloglucosidase [31] revealed that these enzymes can be activated or inactivated depending on the homogenization pressure applied, pH of enzyme solution, and the temperature of activity measurement [31, 32]. When passing through the homogenizer, the sample is submitted to a shear stress whose mechanical energy results in conformational change of the enzyme molecule. When an enzyme undergoes conformational changes, either activation or inactivation may be expected. Conformational change may expose the active site and increase its activity, or it may prevent its contact with the substrate, thus reducing enzyme activity. It is therefore not possible to establish a fixed rule about the effects of homogenization on enzymes.

The effect of HPH on β-galactosidase has not yet been evaluated. Considering the growing importance of the HPH process in the production of dairy products, the objective of this work was to evaluate the stability of this enzyme in HPH processing.

Materials and methods

β-Galactosidase and enzyme activity

The β-galactosidase used in these experiments was a commercial enzyme from Prozyn Biosolutions® (São Paulo, Brazil; batch number 368592910). The enzyme is a yellow, viscous liquid obtained as a fermentation product from *Kluyveromyces lactis*. It is a dimeric enzyme composed of two identical subunits, with an expected molecular weight of 200 kDa.

The enzyme was evaluated at a concentration of 0.1 % (w/v) and the enzyme activity was determined following the method previously described [12] with a few modifications. The enzyme solution was prepared in a 0.1 M phosphate buffer at pH 7.0, and 300 μL of this solution was added to 3 mL of a 2 % lactose solution (w/v) (Synth, Brazil). The reaction was carried out at 30 °C for 15 min and stopped by immersing the tubes in boiling water for 5 min. The tubes were then cooled in an ice bath.

Lactose hydrolysis was determined from the release of glucose, as measured using a glucose oxidase enzyme kit (Laborlab, Guarulhos, SP, Brazil) involving a colorimetric reaction. Sample absorbance was measured at 510 nm using a DU 800 UV–VIS spectrophotometer (Beckman Coulter®, Brea, CA, USA). One unit of enzyme was defined as the amount of enzyme able to produce one micromole of glucose per minute of reaction and per gram of enzyme. Tubes containing only lactose and only enzyme were used as the controls. The galactose and lactose present in the medium had no influence on the glucose determination.

Optimum pH and temperature

β-Galactosidase activity was evaluated at pH values of 5.7, 6.4, 7.0, 7.5, and 8.0. The assays were carried out in 0.1 M acetate buffer (pH 5.7) and 0.1 M phosphate buffer (pH 6.4–8.0). The effect of temperature on enzyme activity was evaluated at 4, 15, 30, 45, 60, and 75 °C. β-Galactosidase activity was determined by the glucose oxidase method, modifying the pH and incubation temperature. A high concentration of β-galactosidase (0.5 %) was evaluated with the aim of determining the activity even under extreme conditions. The conditions for maximum activity (pH and temperature) were considered as the optimum conditions, denominated as 100 % of enzymatic activity. The residual activity was calculated according to Eq. 1.

$$\text{Residual activity } (\%) = (\text{Activity under non ideal conditions}/\text{optimum activity}) \times 100 \quad (1)$$

High pressure homogenization of β-galactosidase at an inlet temperature of 8.5 °C

A Panda Plus high pressure homogenizer was used (GEA-Niro-Soavi, Parma, Italy). The equipment contains a single

acting intensifier pump that amplifies the hydraulic pressure up to 150 MPa. The pressure at the second stage valve was set at 0 MPa (gauge pressure). The equipment flow rate is 9 L h^{-1}. Two liters of the β-galactosidase solution at 8.5 °C (pH 6.4, 7.0, and 8.0) was homogenized at pressures of 0 (obtained by pumping the enzyme solution through the homogenizer with no pressure applied), 50, 100, and 150 MPa. Samples (50 mL) were collected as previously described [31–33], and non-processed β-galactosidase (native) solution was used as the control sample.

Enzyme activity was determined at 5, 30, and 45 °C using the glucose oxidase method. The UV absorption spectrum of the enzyme was obtained and evaluated following the method described elsewhere [32]. Both assays were performed immediately after HPH and after 24 h of refrigerated storage for the native β-galactosidase and that homogenized at 50 and 150 MPa.

High pressure homogenization of β-galactosidase with an inlet temperature of 20 °C

HPH of the β-galactosidase solution at pH 7.0 was carried out with an inlet temperature of 20 °C, using the same procedure described for the enzyme at 8.5 °C. As previously described [31, 32], the HPH process promotes intense shear and friction and involves the dissipation of mechanical energy as thermal energy, increasing the product temperature [24]. Therefore, the highest inlet temperature was set at 20 °C, because higher temperatures would thermally denature the enzyme during processing. The enzyme activity was determined at 5, 30, and 45 °C, immediately after homogenization and after 1 day of storage.

Statistical analysis

The analysis of variance (ANOVA) was carried out to compare the effects of the different treatments, and the Tukey test was used to determine the differences between them at the 95 % confidence level. The statistical analyses were carried out using the STATISTICA 5.0 software (StatiSoft, Inc., Tulsa, USA). All the processes and the determination of β-galactosidase activity were carried out in triplicate. The results were represented as the mean ± SD.

Results and discussion

Enzyme characterization

The effects of pH and temperature on β-galactosidase activity are shown in Fig. 1. The optimum conditions for enzyme activity were determined as pH 7.0 and 30 °C. Under these conditions the activity was 60,894 U/g, which was considered as 100 % of residual activity. The variation in pH resulted in significant loss of enzyme activity, reducing the activity by up to 50 % at pH 6.4 and 8.0. Variation in the temperature also affected β-galactosidase activity, with a reduction of around 40 and 80 % at 15 and 45 °C, respectively. The results also demonstrated that the enzyme had low thermal stability, being completely inactivated at 60 °C. On the other hand, it remained active at 5 °C (around 20 % of residual activity), which could be useful when the milk is stored cold for a period before processing. The optimum conditions observed (neutral pH and low temperature) were to be expected for the β-galactosidase produced by yeasts [8, 12, 13].

Considering the results shown in Fig. 1, pH 7.0 and 30 °C were chosen as the ideal conditions to measure the activity of β-galactosidase before and after HPH.

High pressure homogenization of β-galactosidase with an inlet temperature of 8.5 °C

The HPH processes were carried out at pressures of up to 150 MPa. The fast decompression during HPH promotes intense shear and friction, with consequent heating of the product. Since enzymes can be affected by heating, the temperature reached under each set of process conditions was also evaluated. The residence time at those temperatures was ±10 s, and Table 1 shows the temperatures reached after homogenization.

The increase in pressure promoted a linear increase in temperature of the enzyme solution of around 1.3 °C/10 MPa, the maximum temperature (32.6 °C) being reached at 150 MPa. This temperature was too low to promote thermal denaturation of the enzyme during the process residence time, and consequently, all the effects observed after HPH can be attributed exclusively to the homogenization process.

The enzyme changes due to HPH were evaluated by analysis of the UV absorption spectra of the homogenized β-galactosidase at pH 6.4, 7.0, and 8.0, at 8.5° C. The UV absorption results are shown in Fig. 2.

The results showed that the pH of the solution significantly affected the UV absorption peaks, the enzyme peak being lower at pH 6.4 than at pH 7.0. At pH 8.0, no statistically significant differences were observed compared with the peaks at pH 6.4 and 7.0. Therefore, to determine the effects of HPH, each pH value was evaluated separately.

At pH 6.4, significant differences were observed between the native enzyme and the sample homogenized at 150 MPa. At pH 8.0, all the homogenized samples (50, 100, and 150 MPa) were different from the native sample.

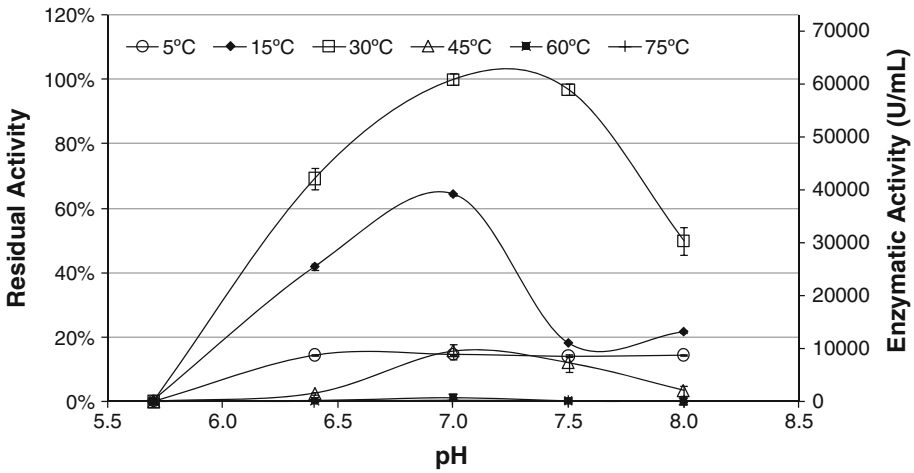

Fig. 1 Effect of pH (6.4, 7.0, and 8.0) and temperature (5, 30, and 45 °C) on β-galactosidase activity

Table 1 Increase in temperature during HPH

Pressure (MPa)	Final temperature (°C)	Temperature increment (°C)
0	13.0	4.5
50	18.1	9.6
100	25.6	17.1
150	32.6	24.1

Inlet temperature = 8.5 °C

On the contrary, at pH 7.0, no significant differences were observed between the native and homogenized samples. This demonstrates that homogenization at different pH values promotes specific changes in the enzyme, and that the enzyme was more stable at its optimum pH value. Also, the differences observed showed a tendency for the UV absorption of the enzyme to increase after homogenization.

The increase in UV absorption was associated with the gradual exposure of tyrosine and tryptophan hydrophobic residues after HPH processing [17, 18]. Therefore, the results obtained indicated that the hydrophobic residues of β-galactosidase were stable to HPH at pH 7.0 and highly unstable at pH 8.0.

The reversibility of the HPH changes was evaluated from the enzyme UV absorption peak after 1 day of storage at 8 °C at the different pH values (Fig. 2). The peaks measured immediately after homogenization and after 1 day of storage were different for all the conditions of pH and homogenization, indicating that the enzyme changes its configuration after 1 day in a buffer solution.

Comparing the results for the native and HPH enzymes after the storage period, no differences were observed between samples at pH 7.0. At pH 6.4 and pH 8.0, only the enzymes homogenized at 150 MPa were different from the native sample. This may indicate that the changes caused by homogenization at 50 and 100 MPa at a pH value of 8.0 were reversible, whereas the changes occurring at 150 MPa at pH values of 6.4 and 8.0 appeared to be permanent.

Figure 3 shows the results for the effects of HPH on β-galactosidase activity at 5, 30, and 45 °C, measured immediately after homogenization at pH 6.4, 7.0, and 8.0.

An evaluation of the results showed no differences between the triplicates for each sample evaluated, indicating good repeatability of the process and analysis methodology. The native enzyme activity was affected by the pH of homogenization and by the temperature at which the activity was measured. Therefore, the effect of HPH on β-galactosidase activity was evaluated for each temperature and pH, comparing the results obtained for the native and homogenized samples.

Homogenization of β-galactosidase (up to 150 MPa) at pH 7.0 did not change the enzyme activity measured at 30 °C. When the activity was measured at 5 and 45 °C and the β-galactosidase homogenized at pH 6.4 or 8.0, the enzyme presented a slight activity loss after homogenization at pressures of up to 100 MPa, and a significant, intense loss of activity after treatment at 150 MPa, reducing the β-galactosidase activity by up to 70 %. Therefore, the stability of β-galactosidase to homogenization is dependent on pH, which can be attributed to the fact that the effects of HPH are dependent on the enzyme conformation, which changes as a function of the positive and negative charge equilibrium of the molecule.

Previous results indicated that HPH may improve [16–18, 31, 34], have no effect [33], or reduce [14, 34] the activity of enzymes, depending on the type of enzyme and the level of pressure applied [16, 17].

The results obtained for β-galactosidase at neutral pH (close to the pH of milk) highlight that it is possible to produce high pressure homogenized milk with low lactose content by adding the enzyme to the refrigerated raw milk prior to homogenization.

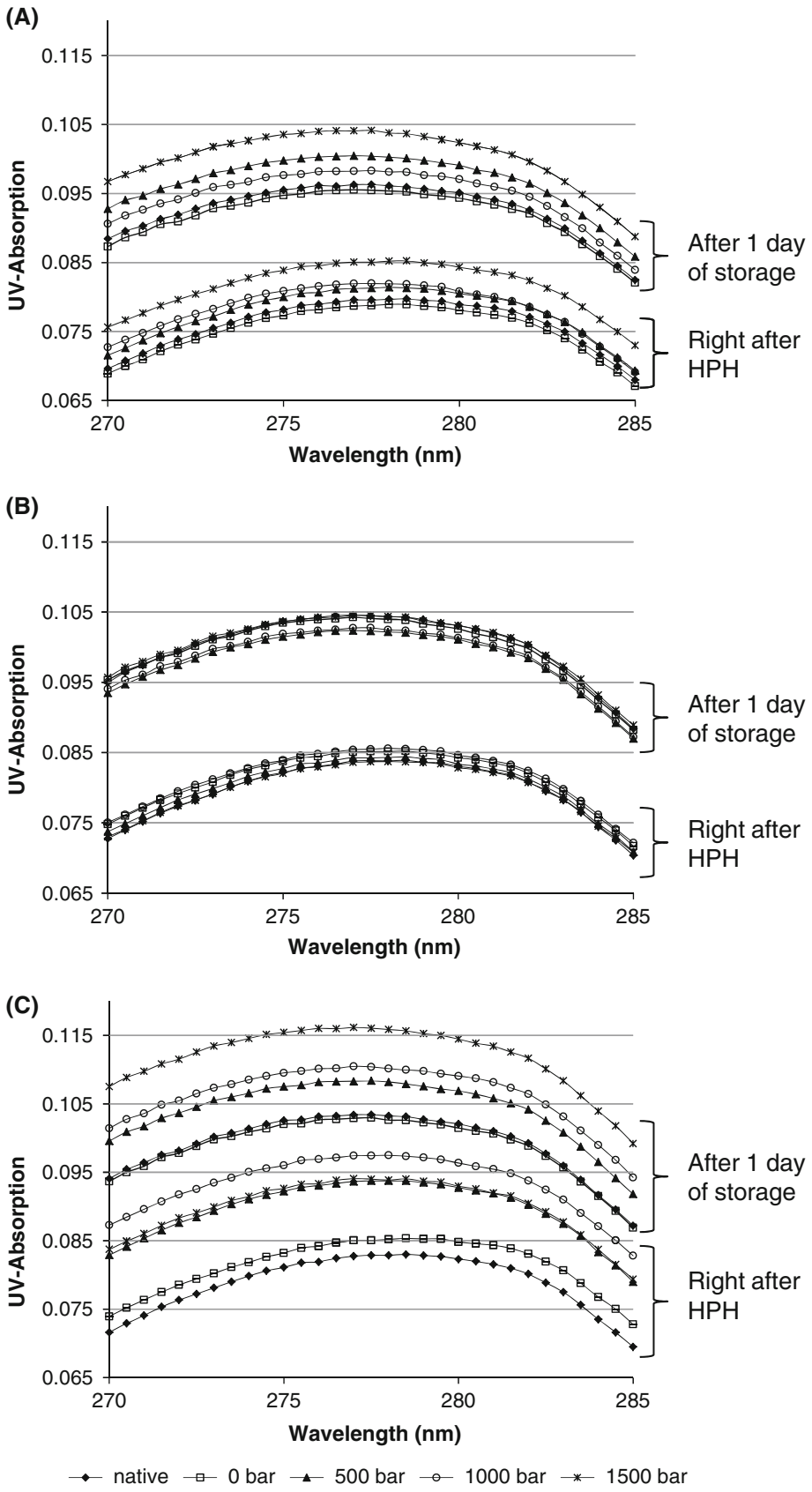

Fig. 2 UV absorption spectra of β-galactosidase at pH 6.4 (**a**), pH 7.0 (**b**), and pH 8.0 (**c**), immediately after homogenization and after 1 day of storage at 8 °C

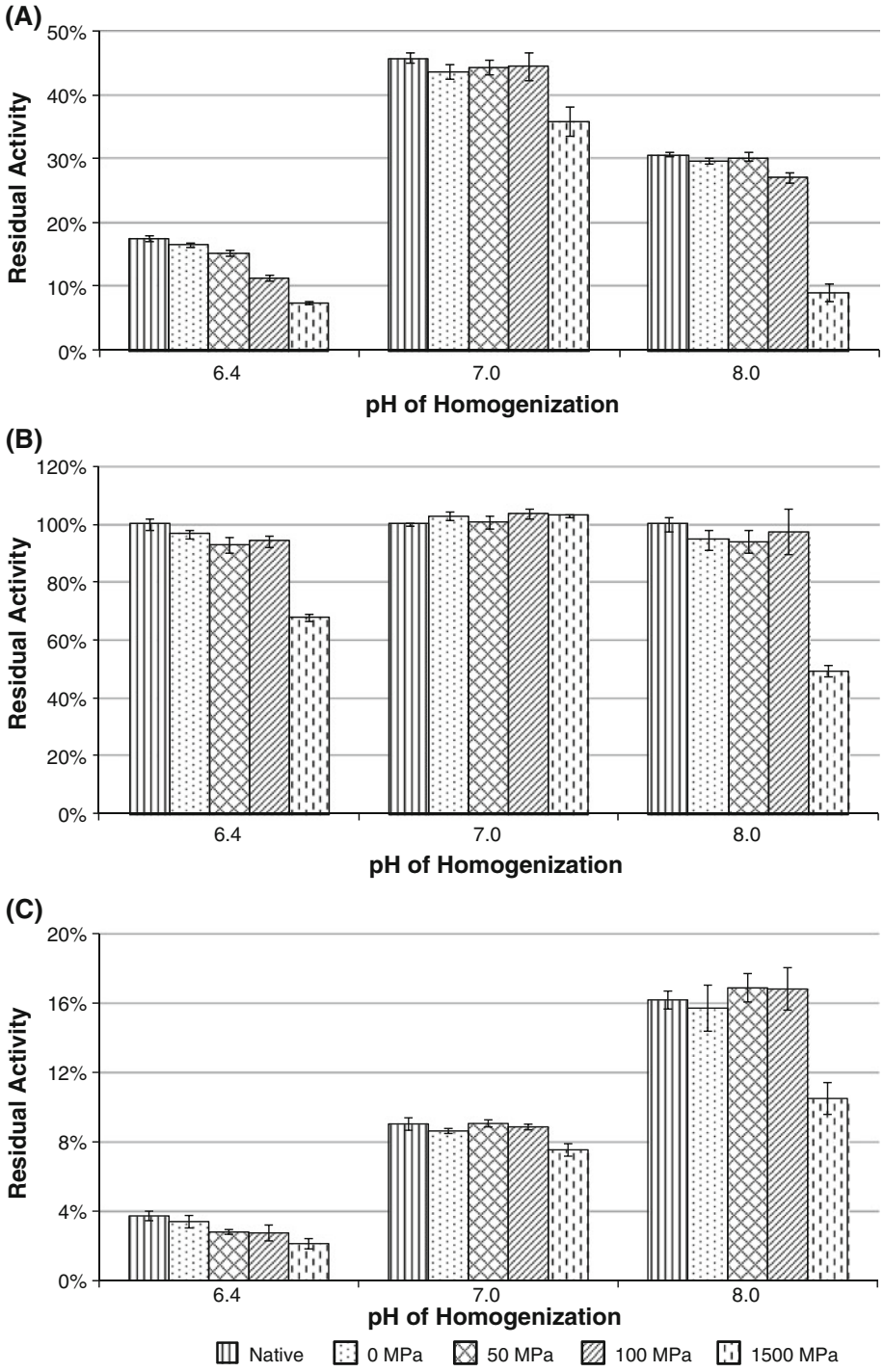

Fig. 3 Effects of HPH between 0 and 150 MPa on the β-galactosidase activity measured at 5 °C (**a**), 30 °C (**b**), and 45 °C (**c**)

The results obtained in the present work (i.e., a buffer model system) can be extended to milk systems, because the main factor that stabilizes β-galactosidase activity in milk is the milk's pH and its buffering capacity [21]. The presence of Mn^{2+} in milk could also contribute to an improved enzyme activity [20]; however, Mn^{2+} was not added to the buffer solution to simulate milk systems in the present study. Also, it is important to consider the possible protective effect of the milk constituents (e.g., proteins and fat) on the maintenance of β-galactosidase configuration. Although some research had studied the protective effect of milk on microorganisms during the homogenization process [3], no data describing milk's protective effect on enzymes were published. However, as HPH did not affect the β-galactosidase activity in phosphate buffer as demonstrated in this study, it is unlikely that with the additional

Table 2 Residual β-galactosidase activity at 5, 30, and 45 °C after 1 day of storage (8 °C) at pH 6.4, 7.0, and 8.0

Temperature of activity (°C)	Sample	pH 6.4		pH 7.0		pH 8.0	
		0 day (%)	1 day (%)	0 day (%)	1 day (%)	0 day (%)	1 day (%)
5	Native	17.7 ± 0.5^{a}	10.5 ± 0.1^{d}	45.9 ± 0.8^{a}	26.7 ± 0.5^{c}	30.7 ± 0.3^{a}	26.5 ± 0.1^{c}
	50 MPa	15.3 ± 0.4^{b}	8.6 ± 0.6^{d}	44.3 ± 1.1^{a}	24.7 ± 0.7^{c}	30.2 ± 0.9^{a}	25.2 ± 1.3^{c}
	150 MPa	7.5 ± 0.1^{c}	$7.6 \pm 0.5^{c,d}$	35.9 ± 2.4^{b}	23.8 ± 2.4^{c}	9.1 ± 1.3^{b}	14.2 ± 0.9^{d}
30	Native	100 ± 1.8^{a}	65.2 ± 0.3^{b}	100 ± 0.5^{a}	104.3 ± 1.2^{a}	100 ± 2.7^{a}	92.7 ± 2.1^{c}
	50 MPa	92.8 ± 2.6^{a}	51.6 ± 0.6^{c}	100.7 ± 2.1^{a}	96.4 ± 2.8^{a}	$93.9 \pm 3.9^{a,c}$	90.6 ± 0.5^{c}
	150 MPa	67.9 ± 2.6^{b}	47.1 ± 2.3^{c}	103.1 ± 1.8^{a}	95.1 ± 4.3^{a}	49.3 ± 1.8^{b}	53.5 ± 1.2^{d}
45	Native	3.7 ± 0.3^{a}	1.9 ± 0.1^{b}	9.0 ± 0.4^{a}	7.2 ± 0.4^{b}	16.2 ± 0.5^{a}	$8.8 \pm 0.6^{b,c}$
	50 MPa	2.8 ± 0.1^{a}	1.7 ± 0.1^{b}	9.1 ± 0.2^{a}	6.0 ± 0.1^{c}	16.9 ± 0.8^{a}	7.3 ± 0.5^{c}
	150 MPa	2.1 ± 0.3^{b}	1.8 ± 0.1^{b}	7.5 ± 0.4^{b}	5.8 ± 0.2^{c}	10.5 ± 0.9^{b}	5.7 ± 0.3^{d}

Temperature of activity indicates the temperature at which activity was determined. Different superscript letters mean significant differences in the results ($p < 0.05$); the data were evaluated individually for each pH and temperature of activity

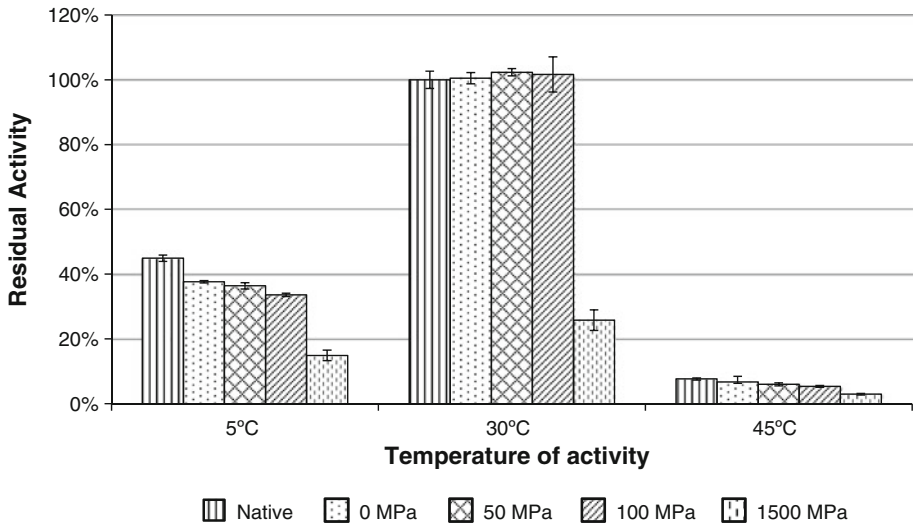

Fig. 4 Residual activity of β-galactosidase homogenized at pH 7.0 and at room temperature (20 °C)

Table 3 Residual β-galactosidase activity after homogenization at an inlet temperature of room temperature and 1 day of storage

Temperature of activity (°C)	Sample	Time of storage at 8 °C	
		0 day (%)	1 day (%)
5.0	Native	44.9 ± 1.0^{a}	27.1 ± 1.8^{d}
	50 MPa	36.4 ± 1.0^{b}	22.1 ± 0.4^{e}
	150 MPa	14.9 ± 1.7^{c}	14.8 ± 0.7^{c}
30.0	Native	100.0 ± 2.7^{a}	89.9 ± 1.9^{c}
	50 MPa	102.3 ± 1.1^{a}	86.1 ± 0.6^{c}
	150 MPa	25.8 ± 3.2^{b}	53.2 ± 1.0^{d}
45.0	Native	7.6 ± 0.3^{a}	$6.8 \pm 0.5^{b,c}$
	50 MPa	6.0 ± 0.5^{b}	5.4 ± 0.3^{b}
	150 MPa	2.9 ± 0.3^{c}	3.4 ± 0.5^{c}

Temperature of activity indicates the temperature at which activity was determined. Different superscript letters mean significant differences in the results ($p < 0.05$); the data were evaluated individually for each temperature of activity

potential protective effects of milk, the enzyme activity would be further reduced after the homogenization process.

The results of β-galactosidase activity after HPH can be especially interesting when the milk is to be used to produce fermented dairy products with low lactose content, exploiting the advantages of HPH [2, 16, 25, 30] in the preparation of milk for fermentation in response to the demand for low-lactose dairy products as a result of lactose intolerance disorders. It should be emphasized that fermentation alone does not guarantee lactose-free or low lactose content dairy [6].

A correlation between the UV absorption peak and β-galactosidase activity was observed, because no changes in UV absorption were observed at pH 7.0 (for samples homogenized up to 150 MPa), and higher UV absorption was observed for samples homogenized at 150 MPa when the buffer solution was pH 6.4. These results may indicate that the active site of the β-galactosidase was highly

affected by the changes in hydrophobic groups (tryptophan and tyrosine residues at pH 6.4). On the other hand, at pH 8.0, although higher UV absorption was observed at pressures above 50 MPa, the enzyme activity only reduced after homogenization at 150 MPa. This may be related to changes in the spatial configuration of the enzyme caused by the alkaline pH, changing the effects of HPH on the β-galactosidase configuration and active sites.

β-Galactosidase activity was measured after 1 day at 8 °C with the aim of evaluating the enzyme stability under this condition. The native and homogenized enzyme at 50 and 150 MPa (pressures that promoted minimum and maximum changes in the β-galactosidase activity) were evaluated. The results are shown in Table 2.

The native β-galactosidase activity was affected by refrigerated storage at almost all the pH values evaluated, with significant reduction in activity. The enzyme only remained active when stored at pH 7.0 and the activity measured at the optimum temperature, indicating that β-galactosidase has high stability and activity at pH 7.0. On the contrary, the native enzyme has low stability during storage in buffer at pH values of 6.4 and 8.0.

The pH of the solution, the pressure applied, and the temperature at which the activity was measured affected the activity of β-galactosidase after 1 day of storage. However, for any of the conditions tested, after 1 day of storage the activity of the homogenized enzyme was higher than the activity of the native one stored under the same conditions, showing that homogenization did not improve the enzyme storage stability.

After 1 day at pH 8.0, the 150 MPa homogenized enzyme and the native enzyme retained the same activity for the three temperatures evaluated, being proportional to the results obtained just after homogenization. This indicates that, at this pH, the loss in activity caused by HPH at 150 MPa was permanent. On the other hand, the activity at 5 °C (enzyme at pH 6.4 and 7.0), 30 °C (enzyme at pH 7.0), and 45 °C (enzyme at pH 6.4) was similar for the homogenized (50 and 150 MPa) and native enzymes after 1 day of storage. Therefore, the effects of homogenization may be reversible under these conditions.

No correlation could be made with the results for residual activity and UV absorption after 1 day of storage. As previously described, just diluting the enzyme and storing were sufficient to considerably change the UV absorption of the native enzymes, which may have overlapped with the different effects of homogenization.

High pressure homogenization of β-galactosidase with an inlet temperature of 20 °C

The effect of an inlet temperature of the homogenizer at room temperature (20 °C) on the activity of the β-galactosidase (pH 7.0) was evaluated. An inlet temperature of 20 °C was chosen considering the low thermal stability of the β-galactosidase studied and the expected heating promoted by the homogenization process. The evaluation of the temperature during processing showed that the maximum temperature reached was 40.1 °C at 150 MPa.

The results obtained for enzyme activity after HPH are shown in Fig. 4. The results obtained at 5 and 45 °C showed a significant reduction in β-galactosidase activity for each increment of pressure. At 30 °C, no significant differences were observed between the native and homogenized enzymes up to 100 MPa, but a reduction of around 80 % was observed after HPH at 150 MPa.

Comparing these results with those obtained for β-galactosidase homogenized at a refrigerated temperature (Fig. 3), it can be seen that the activity of the enzymes homogenized at room temperature was lower than that of those homogenized at refrigerated temperatures, for all the pressures evaluated (activity at 5 and 45 °C) and for the samples homogenized at 150 MPa (activity at 30 °C). These results indicated that the process at 20 °C negatively affected the activity of β-galactosidase, which could be associated with the sum of the effects of HPH with those of the heating caused by shear, especially during homogenization at 150 MPa, because the enzyme had low thermal stability. Therefore, it was concluded that homogenization at room temperature was deleterious for the enzyme, and that no advantages were found in homogenizing the enzyme under this condition.

The residual activity was measured after 1 day of refrigerated storage to evaluate if the activity loss caused by HPH at 20 °C was reversible and the results are shown in Table 3.

The results indicated that the native and homogenized β-galactosidases at 50 MPa showed an additional loss of activity after 1 day of storage. On the contrary, samples homogenized at 150 MPa showed no change in the activities measured at 5 and 45 °C after the storage period and, when the activity was measured at 30 °C, an increase in residual activity was observed, which may indicate that the inactivation caused by HPH at room temperature was partially reversible. On the other hand, a comparison of the results obtained for the residual activity of the native and homogenized β-galactosidases at pH 8.5 and 20 °C after 1 day of storage only indicated no differences between the activities when it was measured at 5 °C, whereas under all the other conditions evaluated, the enzymes homogenized at 20 °C presented lower enzyme activity.

Conclusion

The stability of the β-galactosidase during HPH was dependent on the pH and homogenization pressure, being

highly stable at pH 7.0, with no changes in the enzyme activity at 30 °C after homogenization at pressures up to 150 MPa. Considering that milk buffering ability is the main factor that affects β-galactosidase activity, the results observed in this present work may indicate that HPH can be used to process milk with added β-galactosidase with the aim of producing milk or dairy products with low lactose content.

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