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Production of low-dosage lactose milk using lactase immobilised in hydrogel

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ABSTRACT

A hydrogel based on chitosan was employed for the immobilisation of lactase with the aim of hydrolysing lactose and producing low-dosage lactose milk. The degree of swelling of the hydrogel was affected by the type of aqueous solution, pH and temperature. The lactase immobilisation capacities at pH 4.0 and pH 7.0 were 257.12 ± 3.18 and 157.87 ± 1.96 mg enzyme per g dried hydrogel, respectively, after 1440 min at room temperature. The activity of immobilised lactase ranged from 97.91 to 56.04 and 97.91 to 71.80% from the first to the tenth cycle of hydrolysis of standard lactose and lactose contained in UHT milk, respectively. Immobilised lactase in hydrogel could be applied for the production of low-dosage lactose milk for at least ten successive hydrolysis cycles. Moreover, hydrogels containing immobilised lactase could also be useful for the enzyme release in individuals with lactose intolerance.

1. Introduction

Lactase is a glycoside hydrolase enzyme that catalyses the hydrolysis of lactose by breaking glycosidic bonds for the production of low-dosage lactose and lactose-free foods (Gekas & Lopez-Leiva, 1985). The hydrolysis of lactose has also been achieved in individuals with lactose intolerance after the ingestion of a controlled enzyme-release capsule (Corgneau et al., 2017; Vieira et al., 2013). Most existing lactose hydrolysis methods for the production of low-dosage lactose and lactose-free foods are applied with the free enzyme. It increases the cost of an industrial process as the free enzyme is easily denatured and it may not be reused (Wolf, Gasparin, & Paulino, 2018). To overcome this problem, the hydrolysis of lactose can be accomplished by applying lactase immobilised in solid supports, such as natural polysaccharide-based hydrogels (Liu et al., 2012).

Solid supports are capable of keeping lactase with highly ordered native structure, which can be applied for successive cycles of lactose hydrolysis without denaturing (Facin, Moret, Baretta, Belfiore, & Paulino, 2015; Wolf et al., 2018). Polysaccharide-based hydrogels containing immobilised lactase can be applied for the hydrolysis of lactose in the production of dairy foods and as capsules of controlled enzyme-release into individuals with lactose intolerance (Facin et al., 2015). In general, the immobilisation of enzymes in insoluble, inert supports preserves partially the enzymatic activity and stability (Husain, Ansari, Alam, & Azam, 2011). Hence, lactase can be applied in different hydrolysis cycles without significantly losing its enzymatic activity, which lowers the cost/benefit of the industrial process (Virgen-Ortiz et al., 2017a).

Hydrogels are cross-linked hydrophilic three-dimensional polymer networks capable of absorbing high amounts of either water or biological fluids (Paulino, Guilherme, Mattoso, & Tambourgi, 2010). Chitin is a natural polymer obtained from crustaceos, insects, silkworm chrysalides, and so forth. Chitosan (β-(1,4)-N-acetyl-D-glucosamine) is a natural polymer derivative of chitin after deacetylation, being biodegradable, biocompatible and non-toxic (Paulino, Simionato, Garcia, & Nozaki, 2006). Commonly, chitosan-based hydrogels are synthesised through the chemical crosslinking of chitosan, acrylic acid and N′,N′-methylenebisacrylamide, in presence of persulfate as initiator (Paulino et al., 2009).

Hydrogels based on chitosan are excellent insoluble, inert supports for the immobilisation of enzymes due to their biodegradability, biocompatibility and non-toxicity (Paulino et al., 2009). These hydrogels have been widely studied for biological,

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pharmaceutical, medical and biotechnological applications (Kim, An, Won, Kim, & Lee, 2012), including enzyme immobilisation and controlled-release processes (Facin et al., 2015). The immobilisation of lactase on chitosan-based hydrogels occurs preferably through intermolecular interactions between glucuronic groups in the hydrophilic three-dimensional polymer network and enzyme chemical structure (Kim et al., 2012; Virgen-Ortíz et al., 2017b). Probally, strong interactions occur during the immobilisation process of lactase on chitosan-based hydrogels as the immobilised enzyme can be efficiently applied for different successive cycles of lactose hydrolysis (Elnashar & Yassin, 2009; Facin et al., 2015). As intermolecular interactions do not alter the conformation of the biocatalyst, enzymatic activity and high lactose hydrolysis capacity are maintained (Rueda et al., 2016). Strong intermolecular interactions during the immobilisation process enable the use of immobilised lactase in the production of low-dosage lactose and lactose-free foods, since the enzyme does not easily diffuse out of the polymer hydrogel network, whereas weak intermolecular interactions enable the use of a hydrogel containing immobilised lactase as a controlled-release capsule for individuals with lactose intolerance (Fernandez-Lopez et al., 2016; Hartmeier, 1988; Panesar, Kumari, & Panesar, 2010).

The purpose of this work was to synthesise a hydrogel based on chitosan through the crosslinking of chitosan, acrylic acid and N′,N′-methylenebisacrylamide for the immobilisation of lactase with the aim of hydrolysing lactose and producing low-dosage lactose milk. Immobilised lactase was employed for the hydrolysis of lactose contained in aqueous solutions of standard lactose and UHT dairy milk. Finally, the lactose release from the hydrogel was monitored to simulate a controlled enzyme-release system with the aim of hydrolysing lactose into individuals with lactose intolerance.

2. Materials and methods

2.1. Reagents

Chitosan from shrimp (CS – deacetylation degree: 92.0 wt% and 1.0×10^6 Da molar mass), acrylic acid (AAc), N,N′-methylenebisacrylamide (MBA), potassium persulphate (PPS), Bradford reagent and bovine serum albumin (BSA) were all purchased from Sigma–Aldrich®. Lactase from *Kluyveromyces lactis* (EC 3.2.1.23) was supplied by Prozyn Brazil. Other reagents used were all of analytical grade and all solutions were prepared with Milli-Q® water.

2.2. Hydrogel synthesis

A hydrogel was synthesised by solubilising 0.30 g of chitosan in a three-neck glass flask containing 30.0 mL 2% (v/v) acetic acid. This solution was deaerated for 30 min with nitrogen gas. Next, a deaerated solution containing 15.0 mL Milli-Q® water, 0.5215 mmol of potassium persulphate, 3.40 mL acrylic acid and 0.150 g N′,N′-methylenebisacrylamide was added to the system. The resulting solution was kept under constant magnetic stirring for 3 h at 70.0 ± 1.0 °C for complete crosslinking. The formed hydrogel was washed with Milli-Q® water for 72 h (renewing the recipient water each 8 h), cut into cylindrical pieces and dried through lyophilisation (TFD5503, Ilshin Lab. Co. Ltd., Korea) at −60.0 ± 1.0 °C for 24 h.

2.3. Degree of swelling

The degree of swelling (DS) was determined by immerging 100.0 mg dried cylindrical hydrogel pieces in glass flasks containing 50.0 mL of one of (i) distilled water, (ii) drinking water, (iii) 0.10 mol L^{-1} acetate buffer solution at pH 4.0 or (iv) 0.10 mol L^{-1} phosphate buffer solution at pH 7.0, for different contact times, and at room temperature and 37.0 ± 1.0 °C. Room temperature was used with the aim of evaluating the hydrogel behaviour during an industrial application in the hydrolysis of lactose. The temperature of 37.0 ± 1.0 °C was used with the aim of evaluating the hydrogel behaviour during the hydrolysis of lactose into individuals. Distilled water was used as standard condition. Drinking water was used with the aim of simulating a real-world situation during the hydrogel application. Buffer solution at pH 4.0 was used with the aim of simulating an acid medium and buffer solution at pH 7.0 was used with the aim of simulating a real-world situation during the hydrolysis of lactose in the food industry. The DS was calculated using Equation (1):

$$DS = \frac{m_t - m_{t=0}}{m_{t=0}} \quad (1)$$

in which m_t and $m_{t=0}$ are the masses of the swollen hydrogel at time t and dried hydrogel, respectively.

2.4. Immobilisation of lactase

Dried hydrogel pieces of 100.0 mg were immersed in aqueous solutions containing 8.5 mL of either 0.10 mol L^{-1} acetate buffer, pH 4.0, or 0.10 mol L^{-1} phosphate buffer, pH 7.0, and 1.5 mL of free lactase, at room temperature. The lactase immobilisation was monitorated by collecting aliquots of solution from 0 to 1440 min. The remaining lactase concentration in solution after the immobilisation process was determined by UV-vis spectrophotometry (Femto Cirrus 80SA), using Bradford reagent and analytical calibration curve for bovine serum albumin (standard calibration curve). The immobilisation capacity (q_i) of enzyme was calculated using Equation (2) (Wolf et al., 2018):

$$q_i = \left(\frac{C_{(t=0)} - C_{equilibrium}}{m_{(t=0)}}\right).V_{solution} \quad (2)$$

in which $C_{(t=0)}$ and $C_{equilibrium}$ are the initial and equilibrium enzyme concentrations, respectively, $m_{t=0}$ is the dried hydrogel mass and $V_{solution}$ is the volume of the buffered enzyme solution.

Efficiency of immobilisation (EI) was calculated using Equation (3) (Wolf et al., 2018):

$$EI(\%) = \frac{Cenzyme_{(t=0)} - Cenzyme_{(t)}}{Cenzyme_{(t=0)}} \quad (3)$$

in which $C_{enzyme(t=0)}$ is the initial enzyme concentration in the aqueous solution without the hydrogel and $C_{enzyme(t)}$ is the remaining enzyme concentration after the immobilisation process at time t.

2.5. Hydrolysis of standard lactose

For the hydrolysis of standard lactose, 100.0 mg dried hydrogel cylindrical pieces containing immobilised lactase were immersed in glass flasks with 9.0 mL of 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 and 1.0 mL of a 5.0% (w/v) standard lactose solution at 37.0 ± 1.0 °C. Aliquots were collected at different time intervals for the determination of the glucose concentration formed during the hydrolysis of lactose. The glucose concentration was determined by UV-vis spectrophotometry at 504 nm using a Bioclin Kit, and confirmed by HPLC using a C-18 Zorbax ODS reverse phase column and 9.0 mmol L^{-1} H_2SO_4 as mobile phase at 50 °C, with flowrate of 0.7 mL min^{-1}.

2.6. Cycles of lactose hydrolysis with immobilised lactase

The hydrogel containing immobilised lactase was reutilised for ten successive hydrolysis cycles by analysing the efficiency of the hydrolysis process and enzymatic activity after each cycle. The activity of the immobilised lactase after each cycle was calculated using Equation (4):

$$\text{Relative enzyme activity} = \frac{\text{Enzyme activity in n cycle}}{\text{Enzyme activity in first cycle}} \quad (4)$$

2.7. Released enzyme fraction

The released enzyme fractions during cycles of lactose hydrolisys were confirmed by immersing dried hydrogel pieces containing immobilised lactase in glass dissolution flasks with 9.0 mL of 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 at 37.0 ± 1.0 °C. Aliquots of the buffer solution were collected at different time intervals for the determination of the free enzyme concentrations by UV-vis spectrophotometry using the Bradford reagent. The released enzyme fractions were calculated using Equation (5):

$$\text{Released Enzyme Fraction} = \frac{\text{amount of enzyme released}}{\text{amount of enzyme immobilised}} \quad (5)$$

2.8. Statistical analysis

All results were expressed as mean ± standard deviation and determined using the software statistica 7.0. All data were calculated for triplicate samples using ANOVA, considering the t-test with a 95% significance level ($p < 0.05$).

3. Results and discussion

3.1. Degree of swelling

Fig. 1 shows the degrees of swelling of the hydrogel based on chitosan in distilled water, drinking water, 0.10 mol L^{-1} acetate buffer solution at pH 4.0 and 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 at room temperature (a) and 37.0 ± 1.0 °C (b). The maximum swelling capacity of the hydrogel was reached after 2880 min at room temperature for all the aqueous media. Otherwise, the maximum swelling capacity of the hydrogel was reached only after 4320 min at 37.0 ± 1.0 °C for some aqueous media.

The maximum degrees of swelling in distilled water were 113.89 and 167.09 g water per g dried hydrogel at room temperature and 37.0 ± 1.0 °C, respectively. The maximum degrees of swelling in drinking water were 101.01 and 146.47 g water per g dried hydrogel, respectively. These values were, respectively, 45.46 and 60.27 in 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 and 9.58 and 21.68 g in 0.10 mol L^{-1} acetate buffer solution at pH 4.0. The degree of swelling increased with the increase in time, temperature and pH. The higher swelling degrees by increasing the temperatures of the aqueous solutions were associated to thermal expansion and destabilisation of the cross-linking points formed in the hydrogel. It expands the hidrophylic three-dimensional network after the absorption, diffusion of water and biological fluids through the matrix pores (Facin et al., 2015; Moura, Rubira, & Muniz, 2008; Wolf et al., 2018).

During the swelling of a hydrogel, anionic groups in the hydrophilic three-dimensional network interact with either metal cations or protons contained in the aqueous solution. It favours the deactivation of these groups and decreases the degree of swelling (Zonatto, Muniz, Tambourgi, & Paulino, 2017). Consequently, the degree of swelling decreased with the decrease in the pH of the solution due to strong electrostatic attraction forces between protons and anionic groups of the polymer network. High alkali metal concentrations in buffer solutions also decrease the degree of swelling due to electrostatic attraction forces between these cations and glucuronic groups in the hydrogel network (Guilherme et al., 2015). The degree of swelling increased with the increase in pH as result of the electrostatic repulsion interactions between anionic groups in the hydrogel network (Khare & Peppas, 1995; Peppas, Bures, Leobandung, & Ichikawa, 2000). The effect of hydrogen and cation ions in the absorption of water from aqueous solutions was also observed for swelling in drinking water. In this case, hydrogen, sodium, potassium, magnesium and calcium ions contained in drinking water can decrease the degree of swelling compared to distilled water due to the high mass transfer coefficient (Caetano et al., 2011). Thus, the hydrogel based on chitosan is pH-responsive and thermosensitive and could be efficiently applied for the diffusion of water and different solutes through its hydrophilic three-dimensional polymer network.

3.2. Immobilisation of lactase

Fig. 2 shows the capacities (a) and efficiencies (b) of immobilisation of lactase in hydrogel based on chitosan versus time at pH 4.0, pH 7.0 and room temperature. The immobilisation capacity in 0.10 mol L^{-1} acetate buffer solution at pH 4.0 and 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 was 257.12 ± 3.18 and 157.87 ± 1.96 mg enzyme per g dried hydrogel, respectively, after 1440 min. The efficiency of immobilisation was 27.91 ± 0.08 and

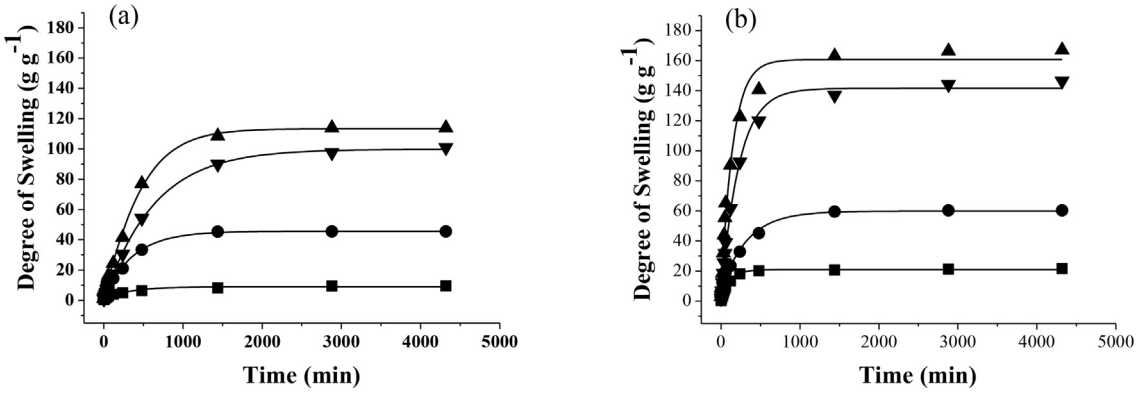

Fig. 1. Degrees of swelling of the hydrogel based on chitosan in (▼) distilled water, (▲) drinking water, (■) 0.10 mol L^{-1} acetate buffer solution at pH 4.0 and (●) 0.10 mol L^{-1} phosphate buffer solution at pH 7.0 at (a) room temperature and (b) 37.0 ± 1.0 °C.

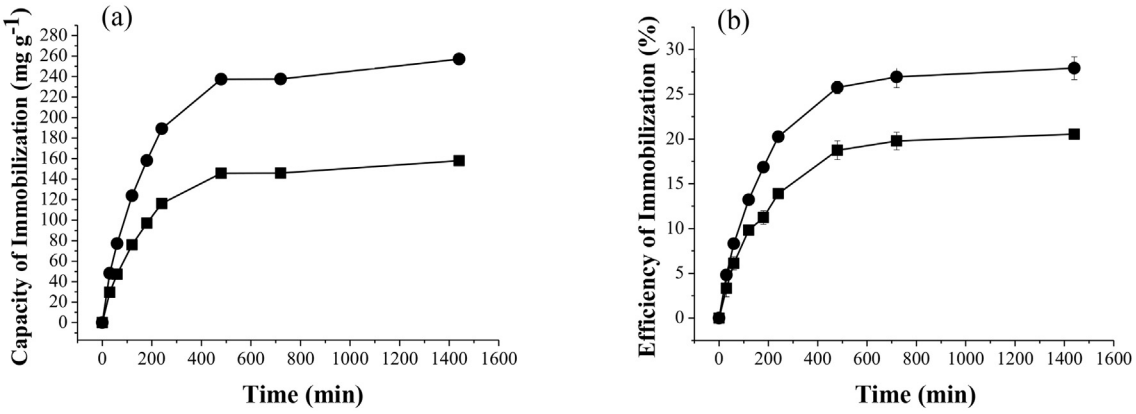

Fig. 2. Capacity (a) and efficiency (b) of immobilisation of lactase in the hydrogel based on chitosan versus time at (●) pH 4.0 and (■) pH 7.0 at room temperatures.

20.54 ± 0.55%, respectively, after 1440 min. The different immobilisation capacities in acid, neutral and alkaline media are related to the conformation changes of an enzyme and the ionisation/protonation of the glucuronic groups in polymer networks (Ciborowski & Silberring, 2016; Paulino et al., 2010). The chemical structure of lactase at pH lower than 4.8, which is the pH of the isoelectric point of this enzyme (Becerra, Cerdan, & Siso, 1998; Cavaille & Combes, 1995), is positively charged and interacts with anionic groups of the polymer network by electrostatic attraction forces, increasing the immobilisation capacity (Zhang et al., 2017; Zhang, Zhang, Chen, & McClements, 2016a). At pH higher than 4.8, however, electrostatic repulsion forces occur, since lactase is negatively charged, thereby decreasing the immobilisation capacity. Moreover, pH from 4.0 to 7.0 alters the molecular conformation of lactase and increases steric hindrance during immobilisation processes, decreasing the immobilisation capacity (Dong-Hao, Li-Xia, Chao, & Ya-Qiong, 2012). Although the hydrogel based on chitosan is pH-sensitive and temperature-sensitive, immobilisation capacity also depends on the isoelectric point of the lactase and degree of swelling of the hydrogel.

3.3. *Hydrolysis of standard lactose*

Fig. 3 shows the concentrations of glucose (a) and enzymatic activities (b) versus time during the hydrolysis of standard lactose using lactase immobilised in the hydrogel based on chitosan at pH 4.0 and pH 7.0. The concentrations of glucose increased with the increase of the lactose hydrolysis times at both pH values. Lower hydrolysis efficiencies were found for the immobilisation of lactase at pH 4.0 compared with pH 7.0 due to the partial denaturation of the enzyme, also resulting in lower enzymatic activities during the hydrolysis of lactose, as reported elsewhere (Panesar et al., 2010; Zhang, Zhang, Zou, & McClements, 2016b). Thus, the experimental conditions for the immobilisation of lactase can significantly influence enzymatic activity and decrease the efficiency of the hydrolysis of lactose.

The use of pH 4.0 was chosen with the aim of evaluating the effects in the immobilisation of lactase and hydrolysis of lactose in acid media. It could help future work related to the production of capsules containing immobilised lactase for the production of either low-dosage lactose or lactose-free foods. Moreover, it could also be useful for understanding the process of lactose hydrolysis in gastrointestinal tract. The findings show that the immobilisation process at pH 4.0 is more efficient than pH 7.0. However, the hydrolysis of lactose is more efficient at pH 7.0 and 37.0 ± 1.0 °C as the enzyme does not denature, keeping their enzymatic activity.

The hydrolysis of lactose using lactase immobilised in chitosan-based hydrogel was only studied at 37.0 ± 1.0 °C, which is the optimal operation temperature for application of lactase. Moreover, higher temperatures can deactivate the immobilised enzyme (Zhou & Chen, 2001). The decrease of the enzymatic activity after long periods of lactose hydrolysis (Fig. 3b) can be related with the enzyme release from the hydrogel. After being released, the lactase is immediately deactivated during the hydrolysis of lactose, since it acts as free enzyme. Finally, lactase dissolved in the hydrogel pores can also be deactivated during the hydrolysis of lactose, decreasing the enzymatic activity.

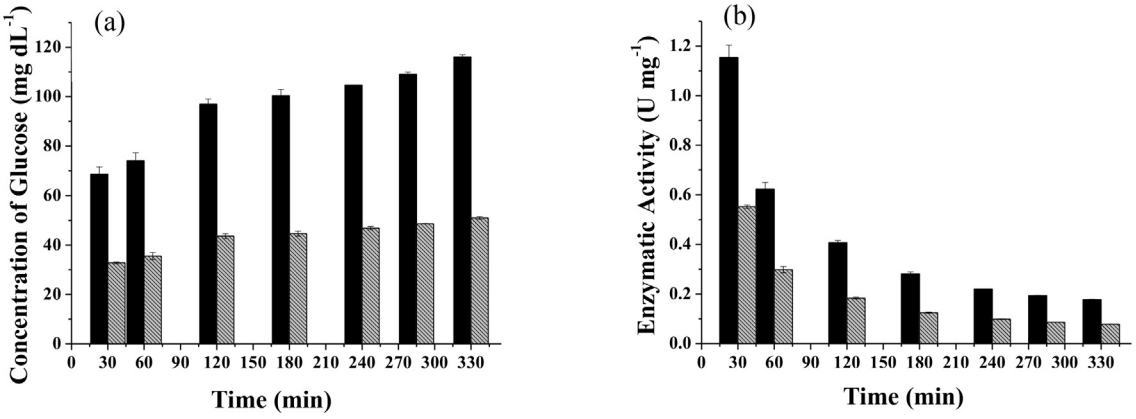

Fig. 3. Concentrations of glucose (a) and enzymatic activities (b) versus time during the hydrolysis of standard lactose using lactase immobilised in the hydrogel based on chitosan at (▨) pH 4.0 and (■) pH 7.0. Experimental conditions for the hydrolysis of standard lactose: pH 7.0 and temperature of 37.0 ± 1.0 °C.

3.4. Released enzyme fraction

Fig. 4 shows the lactase fractions released from the hydrogel based on chitosan after immobilisation at pH 4.0 and 7.0. Higher released enzyme fractions were observed after immobilisation at pH 7.0 than at pH 4.0 due to the weaker intermolecular interactions during the immobilisation process. The immobilisation process at pH 4.0, which is lower than the pH of the isoelectric point of lactase (pH 4.8), occurs mainly through strong electrostatic interactions between positive groups of the enzyme molecule and ionised negative groups of the hydrogel network. In contrast, the immobilisation process at pH 7.0, which is higher than the pH of the isoelectric point of lactase, occurs mainly through weak physical/hydrophobic interactions, since the enzyme and ionised groups of the hydrogel network are negatively charged. Thus, the enzyme remains more strongly immobilised at pH 4.0 than pH 7.0, making its release more difficult (Virgen-Ortíz et al., 2017a, b). The immobilisation mechanism at lower pH than that of the isoelectric point of the enzyme can sometimes also be a partially irreversible process (Fernandez-Lopez et al., 2016), also making its release more difficult. Moreover, electrostatic interactions occur more strongly with inactivated enzyme molecules, such as those commonly found in a more acidic medium. Inactive enzyme molecules are formed by the chemical degradation of active/native enzymes and the subsequent formation of different intermediates, increasing the electrostatic interaction forces between the enzyme and hydrogel groups, which is unfavourable to the release process (Virgen-Ortíz et al., 2017a, b; Zhang et al., 2016b). As release processes occur through the destabilisation of intermolecular interactions between enzyme and polymer segments, the mechanism of immobilisation at pH 7.0 with a large amount of active enzyme molecules and lower electrostatic interaction forces favours the release and diffusion of lactase to outside the hydrogel network, as observed experimentally (Paulino et al., 2010; Wolf et al., 2018; Zonatto et al., 2017). Osmotic pressure between the concentrated enzyme in the external aqueous solution and within the hydrophilic three-

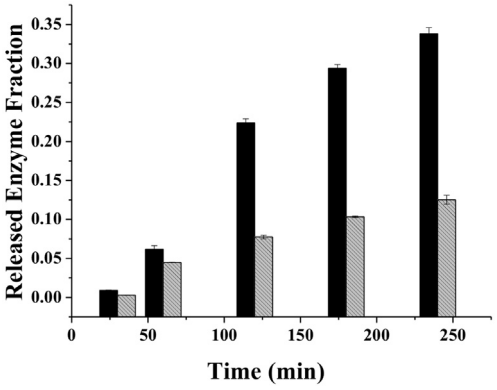

Fig. 4. Lactase fractions released from the hydrogel based on chitosan after immobilisation at (▨) pH 4.0 and (■) pH 7.0. Experimental conditions for the release process: pH 7.0 and temperature of 37.0 ± 1.0 °C.

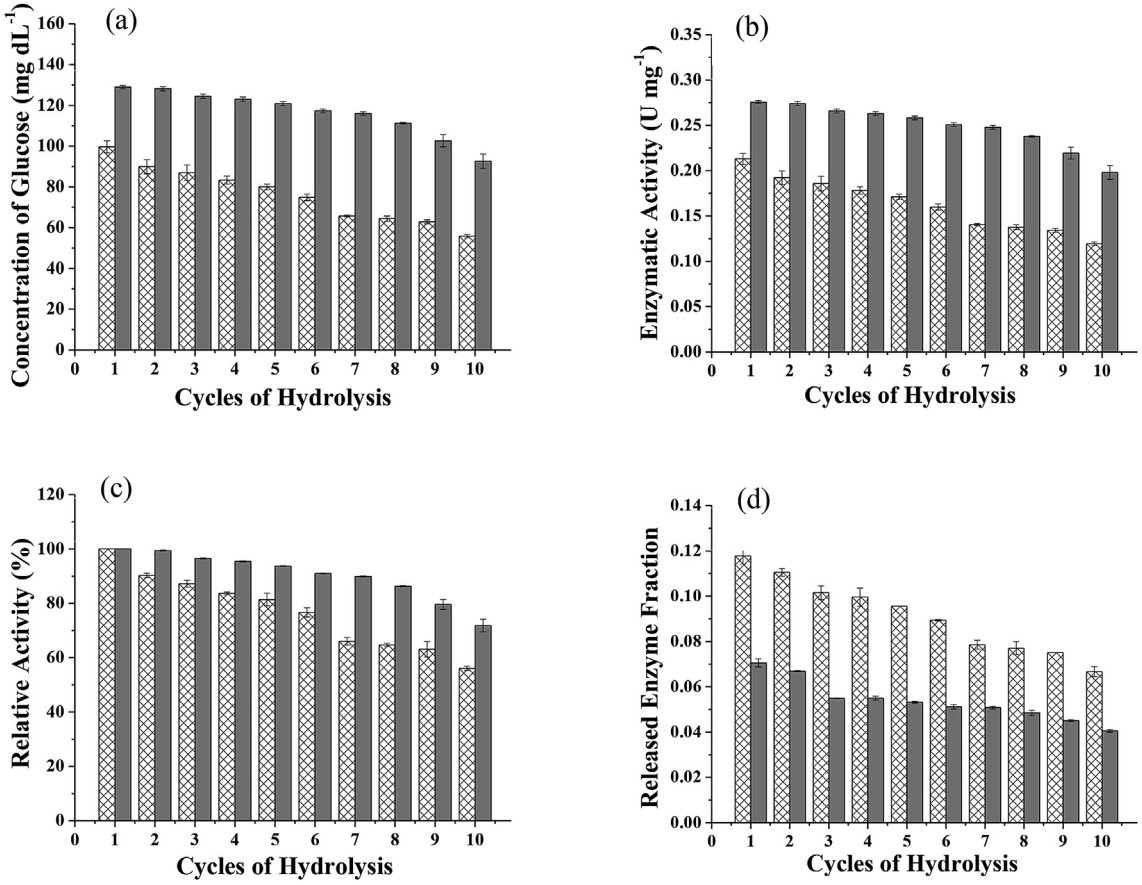

Fig. 5. Concentrations of glucose (a), enzymatic activities (b), relative activities (c), and released enzyme fractions (d) during ten successive lactose hydrolysis cycles at pH 7.0 and 37.0 ± 1.0 °C (▨, hydrolysis of standard lactose; ■, hydrolysis of lactose contained in UHT milk).

dimensional network of the hydrogel facilitates diffusion through the pores and release processes (Zhang et al., 2017).

3.5. Cycles of lactose hydrolysis with immobilised lactase

Fig. 5 shows the concentrations of glucose (a), enzymatic activities (b), relative activities (c), and released enzyme fractions (d) during ten successive lactose hydrolysis cycles at pH 7.0 and 37.0 ± 1.0 °C. The concentrations of glucose slightly decreased with the increase in the numbers of the hydrolysis cycles. Nevertheless, the immobilised enzyme was efficiently applied for ten successive cycles of hydrolysis of standard lactose and lactose contained in UHT milk. The relative enzymatic activities of lactase after ten successive cycles of hydrolysis of standard lactose and lactose contained in UHT milk were 56.04 ± 0.17 and 71.80 ± 0.89%, respectively, indicating that the immobilised enzyme could be efficiently applied in successive cycles of hydrolysis without completely losing its enzymatic activity. The better efficiency of the hydrolysis of lactose contained in UHT milk was related to the presence of enzymatic cofactors, such as Na^{+}, Mg^{2+} and Mn^{2+} ions, in the aqueous solutions. Enzymatic cofactors favour hydrolysis reactions in dairy foods as well as in individuals with lactose intolerance (Bovenhuis et al., 2016; Plou, Polaina, Sanz-Aparicio, & Fernández-Lobato, 2016; Vieira et al., 2013).

The cumulative fractions of lactase released after ten successive cycles of hydrolysis of standard lactose and lactose contained in UHT milk were 0.53 ± 0.02 and 0.30 ± 0.01, respectively. The higher enzymatic activities after each cycle of lactose hydrolysis in UHT milk compared with enzymatic activity of lactose hydrolysis in standard lactose solutions is result of the presence of enzymatic cofactors, fatty acids and proteins (Brasil, 2011) Moreover, the osmotic pressure through the three-dimensional polymer network facilitates the diffusion of the enzyme to outside the hydrogel in the initial cycles, decreasing after each successive cycle (Schroeder & Best, 2015). The release of lactase decreased gradually during the reutilisation cycles, indicating possible applications of the immobilised enzyme in industrial biocatalyst reactors (Guerrero, Vera, & Illanes, 2017).

4. Conclusion

The immobilisation of lactase in hydrogel based on chitosan is a feasible strategy for the hydrolysis of lactose and production of low-dosage lactose and lactose-free foods with a lower cost/benefit ratio and higher performance compared with the use of free enzyme. This technology is affected by the pH of the aqueous solution, temperature and isoelectric point of the enzyme. Finally, the hydrolysis of lactose is more efficient in the presence of enzymatic cofactors, such as Na^{+}, Mg^{2+} and Mn^{2+} ions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2018.12.004.

References

Becerra, M., Cerdan, E., & Siso, M. I. G. (1998). Micro-scale purification of beta-galactosidase from Kluyveromyces lactis reveals that dimeric and tetrameric forms are active. *Biotechnology Techniques, 12*, 253–256.
Bovenhuis, H., Visker, M. H. P. W., Poulsen, N. A., Sehested, J., Van Valenberg, H. J. F., Van Arendonk, J. A. M., et al. (2016). Effects of the diacylglycerol o-acyltransferase 1 (DGAT1) K232A polymorphism on fatty acid, protein, and mineral composition of dairy cattle milk. *Journal of Dairy Science, 99*, 3113–3123.
Brasil. (2011). *Instrução Normativa n.62, de 29 de dezembro de 2011*. Brasília, DF, Brasil: Diário Oficial da União.
Caetano, M. N. P., Costa, R. M. R., Alves, T. G. V., de Deus, D. M. V., Bezerra, S. S., & Santos Magalhães, N. S. (2011). A new transdermal drug delivery system containing hydroquinone. *Latin American Journal of Pharmacy, 30*, 1833–1842.
Cavaille, D., & Combes, D. (1995). Characterization of beta-galactosidase from Kluyveromyces lactis. *Biotechnology and Applied Biochemistry, 22*, 55–64.
Ciborowski, P., & Silberring, J. (2016). *Proteomic profiling and analytical chemistry* (2nd ed.). Amsterdam, The Netherlands: Elsevier.
Corgneau, M., Scher, J., Ritie-Pertusa, L., Le, D.t.l., Petit, J., Nikolova, Y., et al. (2017). Recent advances on lactose intolerance: Tolerance thresholds and currently available answers. *Critical Reviews in Food Science and Nutrition, 57*, 3344–3356.
Dong-Hao, Z., Li-Xia, Y., Chao, L., & Ya-Qiong, L. (2012). Effect of poly(vinyl acetate–acrylamide) microspheres properties and steric hindrance on the immobilization of Candida rugosa lipase. *Bioresource Technology, 124*, 233–236.
Elnashar, M. M. M., & Yassin, M. A. (2009). Lactose hydrolysis by β-galactosidase covalently immobilized to thermally stable biopolymers. *Applied Biochemistry and Biotechnology, 159*, 426–437.
Facin, B. R., Moret, B., Baretta, D., Belfiore, L. A., & Paulino, A. T. (2015). Immobilization and controlled release of lactase from chitosan-grafted hydrogels. *Food Chemistry, 179*, 44–51.
Fernandez-Lopez, L., Rueda, N., Bartolome-Cabrero, R., Rodriguez, M. D., Albuquerque, T. L., dos Santos, J. C., et al. (2016). Improved immobilization and stabilization of lipase from Rhizomucor miehei on octyl-glyoxyl agarose beads by using CaCl2. *Process Biochemistry, 51*, 48–52.
Gekas, V., & Lopez-Leiva, M. (1985). Hydrolysis of lactose: A literature review. *Process Biochemistry, 20*, 2–12.
Guerrero, C., Vera, C., & Illanes, A. (2017). Synthesis of lactulose in batch and repeated-batch operation with immobilized lactase in different agarose functionalized supports. *Bioresource Technology, 230*, 56–66.
Guilherme, M. R., Aouada, F. A., Fajardo, A. R., Martins, A. F., Paulino, A. T., Davi, M. F. T., et al. (2015). Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: A review. *European Polymer Journal, 72*, 365–385.
Hartmeier, W. (1998). *Immobilized biocatalysts: An introduction* (1st ed.). New York, NY, USA: Springer-Verlag.
Husain, Q., Ansari, S. A., Alam, F., & Azam, A. (2011). Immobilization of Aspergillus oryzae β-galactosidase on zinc oxide nanoparticles via simple adsorption mechanism. *International Journal of Biological Macromolecules, 49*, 37–43.
Khare, A. R., & Peppas, N. A. (1995). Swelling/deswelling of anionic copolymer gels. *Biomaterials, 16*, 559–567.
Kim, M. H., An, S., Won, K., Kim, H. J., & Lee, S. H. (2012). Entrapment of enzymes into cellulose–biopolymer composite hydrogel beads using biocompatible ionic liquid. *Journal of Molecular Catalysis B Enzymatic, 75*, 68–72.
Liu, H., Liu, J., Tan, B., Zhou, F., Qin, Y., & Yang, R. (2012). Covalent immobilization of Kluyveromyces fragilis β-galactosidase on magnetic nanosized epoxy support for synthesis of galacto-oligosaccharide. *Bioprocess and Biosystems Engineering, 35*, 1287–1295.
Moura, M. R., Rubira, A. F., & Muniz, E. C. (2008). Semi-IPN hydrogels based on alginate-Ca2+ network and PNIPAAm: Hydrophilic, morphological and mechanical properties. *Polímeros, 18*, 132–137.
Panesar, P. S., Kumari, S., & Panesar, R. (2010). Potential applications of immobilized lactase in food processing industries. *Enzyme Research, 16*. Article 473137.
Paulino, A. T., Guilherme, M. R., Almeida, E. A. M. S., Pereira, A. G. B., Muniz, E. C., & Tambourgi, E. B. (2009). One-pot synthesis of a chitosan-based hydrogel as a potential device for magnetic biomaterial. *Journal of Magnetism and Magnetic Materials, 321*, 2636–2642.
Paulino, A. T., Guilherme, M. R., Mattoso, L. H. C., & Tambourgi, E. B. (2010). Smart hydrogels based on modified gum Arabic as a potential device for magnetic biomaterial. *Macromolecular Chemistry and Physics, 211*, 1196–1205.
Paulino, A. T., Simionato, J. I., Garcia, J. C., & Nozaki, J. (2006). Characterization of chitosan and chitin produced from silkworm chrysalides. *Carbohydrate Polymers, 64*, 98–103.
Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics, 50*, 27–46.
Plou, F. J., Polaina, J., Sanz-Aparicio, J., & Fernández-Lobato, M. (2016). Microbial enzyme technology in food applications. In C. M. Rosell, & R. C. Ray (Eds.), *Lactases for lactose hydrolysis and galactooligosaccharide synthesis* (pp. 121–144). London, UK: CRC Press.
Rueda, N., Santos, J. C., Ortiz, C., Torres, R., Barbosa, O., Rodrigues, R. C., et al. (2016). Chemical modification in the design of immobilized enzyme biocatalysts: Drawbacks and opportunities. *Chemical Record, 16*, 1436–1455.

Schroeder, A. N., & Best, T. (2015). Is self myofascial release an effective preexercise and recovery strategy: A literature review. *Current Sports Medicine Reports, 14*, 200–208.

Vieira, D. C., Lima, L. N., Mendes, A. A., Adriano, W. S., Giordano, R. C., Giordano, R. L., et al. (2013). Hydrolysis of lactose in whole milk catalyzed by lactase from Kluyveromyces fragilis immobilized on chitosan-based matrix. *Biochemical Engineering Journal, 81*, 54–64.

Virgen-Ortiz, J. J., Santos, C. S., Berenguer-Murcia, A., Barbosa, O., Rodrigues, R. C., & Fernandez-Lafuente, R. (2017a). Polyethylenimine: A very useful ionic polymer in the design of immobilized enzyme biocatalysts. *Journal of Materials Chemistry B, 5*, 7461–7490.

Virgen-Ortíz, J. J., Tacias-Pascacio, V. G., Hirata, D. B., Torrestiana-Sanchez, B., Rosales-Quintero, A., & Fernandez-Lafuente, R. (2017b). Relevance of substrates and products on the desorption of lipases physically adsorbed on hydrophobic supports. *Enzyme and Microbial Technology, 96*, 30–35.

Wolf, M., Gasparin, B. C., & Paulino, A. T. (2018). Hydrolysis of lactose using β-d-galactosidase immobilized in a modified Arabic gum-based hydrogel for the production of lactose-free/low-lactose Milk. *International Journal of Biological Macromolecules, 115*, 157–164.

Zhang, W., Abbaspourrad, A., Chen, D., Campbell, E., Zhao, H., Li, Y., et al. (2017). Osmotic pressure triggered rapid release of encapsulated enzymes with enhanced activity. *Advanced Functional Materials, 27*, 1–7.

Zhang, Z., Zhang, R., Chen, L., & McClements, D. J. (2016a). Encapsulation of lactase (β galactosidase) into κ-carrageenan-based hydrogel beads: Impact of environmental conditions on enzyme activity. *Food Chemistry, 200*, 69–75.

Zhang, Z., Zhang, R., Zou, L., & McClements, D. J. (2016b). Protein encapsulation in alginate hydrogel beads: Effect of pH on microgel stability, protein retention and protein release. *Food Hydrocolloids, 58*, 308–315.

Zhou, Q. Z. K., & Chen, X. D. (2001). Effects of temperature and pH on the catalytic activity of the immobilized-galactosidase from Kluyveromyces lactis. *Biochemical Engineering Journal, 9*, 33–40.

Zonatto, F., Muniz, E. C., Tambourgi, E. B., & Paulino, A. T. (2017). Adsorption and controlled release of potassium, phosphate and ammonia from modified Arabic gum-based hydrogel. *International Journal of Biological Macromolecules, 105*, 363–369.