Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev Ciênc Farm Básica Apl., 2015;36(1):153-157
ISSN 1808-4532

Enzyme activity of *β*-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae* on simulated conditions of human gastrointestinal system

Alessandra Bosso[1]; Cínthia Hoch Batista de Souza[1*]; Hélio Hiroshi Suguimoto[1]

[1]Universidade Norte do Paraná, UNOPAR, Programa de Pós-Graduação em Ciência e Tecnologia de Leite e Derivados, Londrina, PR, Brasil.

ABSTRACT

An alternative to relieve the symptoms of lactose intolerance is the intake of the enzyme β-galactosidase in pharmaceutical dosage forms. The ability of β-galactosidase produced by *Kluyveromyces lactis* **and** *Aspergillus oryzae* **to hydrolyze lactose in simulated conditions of the human gastrointestinal tract was investigated. The experiment was carried out in the optimum temperature for each enzyme activity, 40 and 55°C, respectively, and at the normal human body temperature (37°C) at concentrations of 1.5, 3.0, and 5.0 g/L (enzyme from** *A. oryzae*) **or mL/L (enzyme from** *K. lactis*). **Both enzymes were completely inactivated under simulated gastric conditions (pH 2). When the enzymes were subjected to simulated small intestine conditions (pH 7.4), lactose hydrolysis has occurred, but at 37°C the percentage was lower than that under the optimal temperatures. At concentrations of 1.5, 3.0, and 5.0 mL/L the enzyme from** *K. lactis* **hydrolyzed 76.63%, 88.91% and 94.80% of lactose at 40°C, and 55.99%, 80.91% and 81.53% at 37°C, respectively. In contrast, the enzyme from** *A. oryzae* **hydrolyzed 7.11%, 16.18% and 21.29% at 55°C, and 8.4%, 11.85% and 16.43% at 37°C. It was observed that under simulated intestinal conditions, the enzyme from** *K. lactis* **was more effective on lactose hydrolysis as compared to the enzyme from** *A. oryzae*. **Considering the findings of this study, it is extremely necessary to use an enteric coating on β-galactosidase capsules so that this enzyme is released only in the small intestine, which is its site of action, thus not suffering the action of the stomach pH.**

Keywords: Lactase. Hydrolysis. Lactose intolerance. Gastrointestinal tract.

Autor correspondente: Cínthia Hoch Batista de Souza, Universidade Norte do Paraná, UNOPAR, Programa de Pós-Graduação em Ciência e Tecnologia de Leite e Derivados, Londrina, PR, Brasil. E-mail: cinthiahoch@yahoo.com.br

INTRODUCTION

Glucose and galactose are lactose digestion products almost completely absorbed in the small intestine (Alliet & Lebenthal, 1989). The digestion of this carbohydrate depends on the activity of the enzyme β-galactosidase, also called lactase, present in the intestine (Ingram & Swallow, 2009). However, approximately 80% of the world's population is lactase-deficient individuals (total or partial absence). This clinical syndrome is characterized by the inability of individuals to hydrolyze lactose into its monosaccharide, being considered one of the most common human genetic disorders (During *et al*., 1998; Téo, 2002; Fernandes & Cabral, 2006; Uggioni & Fagundes, 2006).

In Brazil, about 58 million people have troubles digesting lactose (Cunha *et al*., 2007). To deal with lactose intolerance is excluding this sugar from the diet. However, the total and definitive exclusion of dairy products aimed at eliminating lactose from diet should be avoided, since the low calcium intake as well as phosphorus and vitamins may decreased bone mineral density leading to bone fractures (Di Stefano *et al*., 2002). Thus, an alternative to this problem is to adopt pharmacological measures including the oral ingestion of β-galactosidase whenever the individual with lactase deficiency consume lactose-containing products (Turner *et al*., 2011). Drugs that need to be released in intestine, such as the enzyme β-galactosidase necessarily have to pass intact through the stomach and should therefore be resistant to the acidic pH of this region (pH 1.0 to 3.0) (Sandra *et al*., 2008).

However, the use of supplemental digestive enzymes has often been the subject of controversy with respect to its efficacy, because not all commercial enzymes has proven effective in resisting the harsh environmental of the stomach (Mamadou *et al*., 2005). Thus, the gastro-resistant coating is a technique that can be used to prepare enteric-coated dosage forms (Ferreira & Holandino, 2008), allowing enzymes to resist the action of gastric juice without changing its activity, rapidly disintegrating in the intestinal juice (Santos *et al*., 2007).

The lactose digestion occurs in the small intestine, where the enzyme ingested by intolerant individuals should act. Thus, it is important to elucidate the behavior of β-galactosidase in the human intestine, so that its use as a drug is effective. Therefore, the aim of this study was to determine the enzyme activity of both β-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae* when exposed to simulated conditions of the human gastrointestinal system.

MATERIAL AND METHODS

Enzymes

The enzymes used in lactose hydrolysis were commercial β-galactosidase from microbial origin, in liquid and lyophilized forms. According to the manufacturers, the liquid enzyme (Maxilact® LX5000, DSM Food Specialties, Seclin Cedex, France) was obtained from the yeast *Kluyveromyces lactis*, and the lyophilized enzyme (Bio-Cat Inc., USA) from the filamentous fungus *Aspergillus oryzae*. Both enzymes had lactase activity of 5000 ALU (acid lactase units).

Simulating the human gastrointestinal tract conditions

Two experiments were performed to assess the enzyme activity of β-galactosidase obtained from *K. lactis* and *A. oryzae*, on simulated conditions of the human gastrointestinal tract. In the first experiment (A), the resistance of enzymes to conditions simulated human stomach and small intestine was evaluated. In experiment B, the enzymes were subjected only to simulated intestinal conditions. In both experiments, the enzyme concentrations were 1.5, 3.0, and 5.0 mL/L (for liquid enzyme) and 1.5, 3.0, and 5.0 g/L (for lyophilized enzyme).

Both experiments followed the methodology described by Rao *et al*. (1989) and Thantsha *et al*. (2008). The enzymes from *K. lactis* and *A. oryzae* were added to Erlenmeyer flasks containing 10 mL of acidic solution pH 2.0 (0.08 M HCl + 0.2% NaCl), simulating the condition of the stomach for 15 minutes. Then, 90 mL of a solution pH 7.4 (0.05 M KH_2PO_4 + 0.6% bile salts) were added to simulate the conditions of the small intestine. In this stage, 5% lactose PA (Synth, Diadema, Brazil) was added to the flasks. The solutions were kept in a water bath for 60 minutes. Experiment B followed the methodology described above, except the gastric phase. Both experiments A and B were also carried out at 37°C to evaluate the enzyme activity in the temperature of the human body.

The solutions containing the enzymes from *K. lactis* and *A. oryzae* were tested at 40°C and 55°C, respectively, since these temperatures are considered optimal for the action of these enzymes.

The experiments were performed in a static way, with two replicates for each test (A and B). All analyses were performed in triplicate. The enzyme activity was determined according to percentage of lactose hydrolysis by the enzymes from *K. lactis* and *A. oryzae*.

Determining lactose hydrolysis

To determine the lactose hydrolysis, 2 mL sample were collected at the end of each experiment. The samples were kept in a boiling water bath for 5 minutes and then on ice for 3 minutes to stop hydrolysis reaction (Campos *et al*., 2009). The glucose concentration was determined by glucose oxidase method using Glucose Kit PP (Analisa, Belo Horizonte, Brazil). The initial lactose concentration and the concentration of glucose released were used to calculate the percentage of hydrolysis.

Statistical analysis

The results were analyzed by analysis of variance (ANOVA) and Tukey's test for comparison of means, considering a significance level of 5% ($p<0.05$) (Callegari-Jacques, 2003). Statistical analyses were carried out using the Statistica software, version 10.0(Statsoft Inc, Tulsa, OK, USA).

RESULTS

In experiment A, after simulating the passage of the enzymes through the stomach (pH 2.0), both enzymes were completely inactivated, since no lactose hydrolysis was observed in the following stage (pH 7.4).

Table 1. Lactose hydrolysis by the enzymes from *K. lactis* and *A. oryzae* (mean ± standard deviation) at different concentrations and temperatures, after exposure to simulated conditions of the human small intestine (pH 7.4).

Enzyme/ Temperature	Hydrolysis (%)*		
	1.5 (g/L or mL/L)**	3.0 (g/L or mL/L)	5.0 (g/L or mL/L)
A. oryzae / 37 °C	8.403 ± 0.259i	11.856 ± 0.450h	16.432 ± 0.259g
K. lactis / 37 °C	55.995 ± 0.401e	80.914 ± 0.314c	81.538 ± 0.190c
K. lactis / 40 °C	76.635 ± 1.087d	88.917 ± 0.596b	94.806 ± 0.317a
A. oryzae / 55 °C	7.114 ± 0.147j	16.187 ± 0.448g	21.299 ± 0.448f

* Means followed by the same lowercase letter in the line, between treatments, do not differ significantly ($p<0.05$) from each other by Tukey's test.

** g/L: Enzyme from *A. oryzae*. mL/L: Enzyme from *K. lactis*.

Table 1 shows the results of lactose hydrolysis by the enzymes from *K. lactis* and *A. oryzae* in experiment B (simulated conditions of the human small intestine, pH 7.4). When the intestinal condition (pH 7.4) was simulated alone (experiment B), the enzyme from *K. lactis* exhibited higher hydrolysis rate at 40°C than it did at 37°C for all concentrations tested. The same behavior was observed for the enzyme from *A. oryzae* except for the concentration of 1.5 g/L at 37°C, since the hydrolysis was slightly higher than that at 55°C. Although the temperature of 37°C is lower than the optimum temperatures of these enzymes, the lactose hydrolysis was observed for both enzymes, and the enzyme from *K. lactis* exhibited the best performance for all temperatures and concentrations studied.

At concentrations of 1.5, 3.0, and 5.0 mL/L, the enzyme from *K. lactis* hydrolyzed 55.99%, 80.91% and 81.53% of lactose at 37°C, respectively. In contrast, when the experiments were carried out at 40°C, these results were higher (76.63%, 88.91% and 94.80%, respectively) (Table 1). At 37°C, there was no significant difference in the enzymatic activity of *K. lactis* at concentrations of 3 and 5 mL/L. The results showed that lowering the temperature from 40°C to 37°C resulted in a significant difference in the enzyme activity ($p<0.0001$). Reductions of 20.6%, 8.0%, and 13.27% were observed for the enzyme activity at concentrations of 1.5, 3.0, and 5.0 mL/L, respectively. At 40°C, there was a significant difference between all enzyme concentrations ($F = 1653.62$, $p<0.0001$).

The enzyme activity of *A. oryzae* at 37°C showed a percentage of lactose hydrolysis of 8.4%, 11.85% and 16.43% at concentrations of 1.5, 3.0 and 5.0 g/L, respectively. When the experiments were performed at 55°C, the percentages of lactose hydrolysis were 7.11%, 16.18% and 21.29%. Thus, significant differences were detected between the temperatures studied ($F = 690.11$, $p<0.0001$).

DISCUSSION

Despite the same enzyme specificity, the optimum temperature and pH of β-galactosidase activity may vary according to its source. The commercial β-galactosidase is classified into two groups, depending on whether it is active under acid or neutral conditions. The acid galactosidase presents optimum pH ranging from 3 to 5, and temperatures from 46 to 55°C, while for neutral galactosidase these values vary from 6.5 to 7.3 and 35 to 40°C, respectively. Generally, the enzymes produced by yeast are considered to be neutral, and those obtained by filamentous fungi are acidic (Mlichová & Rosenberg, 2006; Rodriguez *et al*., 2008).

Although several authors reported that the enzyme from *A. oryzae* exhibited maximum activity at acidic pH (Holsinger & Kligerman, 1981; Gekas & López-Leiva, 1985; Robinson, 1991; Vitolo, 2001) (optimum condition), this did not occur in the present study, at pH 2.0 (gastric stage). Mamadou *et al*. (2005) demonstrated that some digestive enzymes may have reduced their activity when passing through the gastric fluid, but by reversible inactivation they restore its activity in colon. However, this characteristic was not observed for the enzymes from *K. lactis* and *A. oryzae* under the conditions of this study.

The enzyme from *A. oryzae* in lyophilized form is the most commonly prescribed by nutritionists and doctors for patients. However, even at the highest concentration evaluated in this study (5 g/L), a low enzyme activity was observed, since only 16.4% and 21.3% of lactose was hydrolyzed at 37°C and 55°C, respectively. For lactase-deficient individuals, this percentage of lactose hydrolysis is lower than required, thus these individuals may present typical lactose intolerance symptoms even after the ingestion of the enzyme at this concentration.

The enzyme from *K. lactis* in liquid form is used by dairy industries to produce lactose-free or lactose-reduced products (Rodriguez *et al*., 2008). At a concentration of 5 mL/L, this enzyme hydrolyzed 94.8% lactose at 40°C. By comparison with the lyophilized enzyme from *A. oryzae*, the lactose hydrolysis was 4.5 times higher. Therefore, if the enzyme from *K. lactis* were used, lactose intolerance symptoms would be eliminated or drastically reduced.

However, it is important to emphasize that the enteric coating of capsules containing β-galactosidase is required, allowing the capsules to pass intact through the stomach, releasing the enzyme only in the small intestine.

Solomons & Barillas (1986) compared the activity of β-galactosidase from different sources and found total lactose hydrolysis in milk using the enzyme from *K. lactis*. In the same study, when the enzyme from A. niger was investigated, the same performance was not observed even with an increase in enzyme concentration.

Kwak (2001) evaluated the enzyme activity of microencapsulated β-galactosidase from *K. lactis* in simulated human intestinal system (pH 7-8), and found percentage of hydrolysis from 60.8 to 68.8% after 60 minutes of testing. These percentages are lower than the results found in the present study, since lactose hydrolysis up to 81% was obtained for *K. lactis* at 37°C even with non-encapsulated enzyme, indicating a stronger resistance and high activity of β-galactosidase from *K. lactis*.

Although gastric and intestinal fluids exhibit some pH variations due to several factors including nutrition and health of the individuals (Kong & Singh, 2008), for healthy individuals those values are similar to the values found in the present study. Thus, the inactivation of both enzymes occurred at pH 2. However, at pH 7.4 the lactose hydrolysis occurred at different levels, depending on the type of enzyme, temperature and enzyme concentration.

Although the specificity of β-galactosidase from different sources is similar, there are discrepancies in their actions. Thus, the degree of lactose hydrolysis should be evaluated so that the enzyme activity is maximized and the expected results are obtained. Both enzymes of the present study were inactivated in the experiments simulating the human stomach conditions. The enzyme from *A. oryzae* was less effective on lactose hydrolysis than the enzyme from *K. lactis* under simulated intestinal conditions, once the lactose hydrolysis was over 90% when the highest concentration of the enzyme from *K. lactis* was evaluated at 40°C. However, at 37°C satisfactory results were obtained, especially for enzyme concentrations of 3 and 5 mL/L. Therefore, in addition to its use in dairy industries, this enzyme is recommended to relieve symptoms of lactose intolerance (therapeutic use). For this, studies on enteric coating are needed to improve its resistance to stomach pH in order to ensure suitable activity in the human small intestine.

RESUMO

Atividade de β-galactosidase de Kluyveromyces lactis e Aspergillus oryzae, em condições simuladas do sistema gastrintestinal humano

Uma das alternativas para amenizar os sintomas da intolerância à lactose é a ingestão de β-galactosidase em formas farmacêuticas. Neste trabalho avaliou-se a capacidade de hidrólise de β-galactosidase produzida por *Kluyveromyces lactis* **e** *Aspergillus oryzae* **simulando as condições do trato gastrintestinal humano. O teste foi realizado nas temperaturas ótimas de ação para cada enzima, 40 e 55°C, respectivamente, e na temperatura corpórea humana (37°C), nas concentrações de 1,5; 3,0 e 5,0 g/L para a enzima de** *Aspergillus oryzae* **ou mL/L para a de** *Kluyveromyces lactis***. Na simulação da condição estomacal humana (pH 2), ambas enzimas foram totalmente inativadas. Quando as enzimas foram submetidas às condições simuladas do intestino delgado (pH 7,4), observou-se hidrólise da lactose, porém, a 37°C, a porcentagem foi menor do que a observada nas temperaturas ótimas de cada enzima. A enzima de** *K. lactis* **nas concentrações de 1,5; 3,0 e 5,0 mL/L apresentou hidrólise de 76,63%, 88,91% e 94,80% a 40°C e 55,99%, 80,91% e 81,53%, a 37°C, respectivamente. Nas concentrações 1,5; 3,0 e 5,0 g/L, a porcentagem de hidrólise pela enzima de** *A. oryzae* **a 55°C foi de 7,11%, 16,18% e 21,29%. Para esta enzima, nessas concentrações, a hidrólise obtida a 37°C foi 8,4%, 11,85% e de 16,43%. Sob condições intestinais simuladas, a enzima de** *K. lactis* **apresentou maior eficiência na hidrólise da lactose quando comparada à enzima de** *A. oryzae***. Considerando-se as etapas avaliadas neste estudo, observa-se que é extremamente necessário o uso de um revestimento entérico em cápsulas de β-galactosidase, para que esta enzima seja liberada somente no intestino delgado, seu local de ação, não sofrendo, portanto, a ação do pH estomacal.**

Palavras-chave: Lactase. Hidrólise. Intolerância à lactose. Trato gastrintestinal.

REFERENCES

Alliet KN, Lebenthal E. Lactase deficiency, lactase malabsortion and lactase intolerance. In: Lebenthal E (editor). Text book of gastroenterology and nutrition in infancy. New York: Raven Press; 1989. p.459-69.

Callegari-Jacques SM. Bioestatística: princípios e aplicações. São Paulo: Artmed; 2003, 255p.

Campos TCAS, D'Almeida WK, Aragon-Alegro LC, Roig SM, Suguimoto HH. Utilização da β-galactosidase na hidrólise da lactose do leite em baixa temperatura UNOPAR Cient Ciênc Biol Saúde. 2009;11(4):51-4.

Cunha LR, Soares NFF, Assis FCC, Melo NR, Pereira AF, Silva CB. Desenvolvimento e avaliação de embalagem ativa com incorporação de lactase. Ciênc Tecnol Aliment. 2007;27:23-6.

Di Stefano M, Veneto G, Malservisi S, Cecchetti L, Minguzzi L, Strocchi A, *et al*. Lactose malabsorption and intolerance and peak bone mass. Gastroenterology. 2002;122(7):1793-9.

During MJ, Xu R, Young D, Kaplitt MG, Sherwin RS, Leone P. Peroral gene therapy of lactose intolerance using an adeno-associated virus vector. Nat Med. 1998;4(10):1131-5.

Fernandes P, Cabral JMS. Biotransformation. In: Ratledge B, Kristiansen, B (editor). Basic biotechnology. Cambridge: University; 2006. p.579-626.

Ferreira AO, Holandino C. Pharmaceutical development of enteric-release hard gelatin capsules in the compounding setting. Int J Pharm Compd. 2008;12(2):163-9.

Gekas V, López-Leiva M. Hydrolysis of lactose: a literature review. Process Biochem. 1985;20(1):2-12.

Holsinger VH, Kligerman AE. Applications of lactase in dairy foods and other foods containing lactose. Food Technol. 1991;45(1):92-5.

Ingram CJE, Swallow, DM. Lactose malabsorption. In: McSweeney PLH, Fox PF (editor). Advanced dairy chemistry. New York: Springer; 2009. p.203-29.

Kong F, Singh RP. Disintegration of solid foods in human stomach. J Food Sci. 2008;73(5):67-80.

Kwak HM. Microencapsulation of β-galactosidase with fatty acid esters. J Dairy Sci. 2001;84(7):1576-82.

Mamadou M, Marr S, Paydon K, Medhektar R. Stability and activity of supplemental digestive enzymes in a simulated gastric fluid environment. 2005 Jan. Available from http://www.transformationenzymes.com.

Mlichová Z, Rosenberg M. Current trends of β-galactosidase application in food technology. J Food Nutr Res. 2006;45(2):47-54.

Rao AV, Shiwnarain N, Maharaj L. Survival of microencapsulate Bifidobacterium pseudolongum in simulated gastric and intestinal juices. Can Inst Food Sci Tech J. 1989;22(4):345-9.

Robinson DS. Bioquímica y valor nutritivo de los alimentos. Zaragoza: Acribia; 1991.

Rodriguez VA, Cravero, BF, Alonso A. Proceso de elaboración de yogur deslactosado de leche de cabra. Ciênc Tecnol Aliment. 2008;28:109-15.

Sandra S, Decker, EA, McClements, DJ. Effect of interfacial protein cross-linking on the *in vitro* digestibility of emulsified corn oil by pancreatic lipase. J Agric Food Chem. 2008;56(16):7488-94.

Santos L, Guterres SS, Bergold AM. Preparação e avaliação de cápsulas gatro-resistentes de diclofenaco de sódio. Lat Am J Pharm. 2007;26(3):355-61.

Solomons NW, Barillas C. The cut-off criterion for a positive hydrogen breath test in children: a reappraisal. J Pediatr Gastroenterol Nutr. 1986;5(6):920-5.

Statsoft Inc., Statistica for Windows, Version 10.0, 2300 East 14th Street, Tulsa, OK, 74104, USA.

Téo CRP. A intolerância à lactose: uma breve revisão para o cuidado nutricional. Arq Ciênc Saúde Unipar. 2002;6(3):135-40.

Thantsha MS, Cloete TE, Moolman FS, Labuschagne PW. Supercritical carbon dioxide interpolymer complexes improve survival of B. longum Bb-46 in simulated gastrointestinal fluids. Int J Food Microbiol. 2008;129(1):88-92.

Turner KM, Pasut G, Veronese FM, Boyce A, Walsh G. Stabilization of a supplemental digestive enzyme by post-translational engineering using chemically-activated polyethylene glycol. Biotechnol Lett. 2011;33(3): 617-21.

Uggioni PL, Fagundes RLM. Tratamento dietético da intolerância à lactose infantil: teor de lactose em alimentos. Hig Aliment. 2006;21(140): 24-9.

Vitolo M. Aplicações de enzimas na tecnologia de alimentos. In: Aquarone E (editor). Biotecnologia industrial: biotecnologia na produção de alimentos. São Paulo: Edgard Blücher; 2001. p.387-420.

Received on February 26th 2014

Accepted on May 5th 2014