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Lactose hydrolysis in milk using a commercial recombinant β-galactosidase (lactase) from *Bifidobacterium bifidum*

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Abstract

We have identified the most relevant properties of a commercial recombinant lactase from *Bifidobacterium bifidum* (RBBL, Saphera 2600 L) for milk hydrolysis, with a yeast neutral lactase (GODO-YNL2) being used for comparison. Both products were characterized according to their lactase and invertase activities, protein profiles by sodium-dodecyl-sulfate-polyacrylamide-gel electrophoresis, and Kms for lactose. RBBL exhibited properties that permitted milk hydrolysis over a broader range of conditions than YNL: apparently, milk's ionic composition was not an activity-limiting property, an optimum temperature range between 45 and 50 °C and a considerable activity at pH 4.5, which would permit lactose hydrolysis in acidic dairy products. Like YNL, the bacterial lactase retains a considerable activity under refrigeration (3-7 °C). To describe the lactose-hydrolysis time course, an empirical model was used in which the glucose obtained was expressed as a function of the standard activity per unit volume and the reaction time. This model proved adequate to describe the glucose-formation kinetics at 7 °C and 45 °C, up to a 95% hydrolysis and to facilitate calculation of the volumen-based enzyme dosage to obtain a certain degree of hydrolysis – a fundamental consideration in evaluating the costs of applying the enzyme

Keywords: enzyme technology; low-lactose milk; bacterial recombinant lactase.

Practical Application: Use of novel recombinant lactase for milk hydrolysis.

1 Introduction

ß-galactosidase enzyme (E.C.3.2.1.23) – commonly known as lactase and widely used in the dairy industry (Husain, 2010; Harju et al., 2012) – catalyzes mainly the hydrolysis of lactose to glucose and galactose. Several galactooligosaccharides (GOSs) are also formed simultaneously with monosaccharides owing to the enzyme's transglycosidase activity (Zárate & López-Leiva, 1990). The greatest impact of lactase in the dairy industry is the production of food and beverages with reduced lactose levels for disaccharide-intolerant people (Nivetha & Mohanasrinivasan, 2017; Szilagyi & Ishayek, 2018). More recently, emphasis has been made on the production of prebiotics from GOS-enriched milk (Rodriguez-Colinas et al., 2014).

The main lactase commercial products in milk are generically called "yeast neutral lactases" (YNLs), which have been in use since the seventies (Dekker & Daamen, 2019). The enzyme source of these products comes from special strains of the yeast *Kluyveromyces lactis* (Coenen et al., 2000). The biochemical and kinetic properties of the ß-galactosidase of *K. lactis* have been widely investigated (Albuquerque et al., 2021). From a practical point of view, two properties of the YNL are the most relevant to lactose-specific hydrolysis: an optimum pH between 6.5 and 7.0, milk's normal pH, and an acceptable activity at low temperatures (3-7 °C). This last property is especially relevant since milk can then be hydrolyzed in the refrigerated facilities of the dairy industry (Horner et al., 2011; Dekker & Daamen, 2019). Low temperatures, moreover, reduce microbial contamination during processing and prevent the denaturation of the enzyme, which is intrinsically unstable at temperatures higher than 45 °C (Flores et al., 1995).

A recombinant ß-galactosidase from the *Bifidobacterium bifidum* (RBBL) expressed in *Bacillus licheniformis* has been recently introduced to the market (Novozymes, 2016). The effectiveness of RBBL for hydrolyzing lactose under *in-vitro*-simulated gastric conditions appears to be similar to that exhibited by YNL (Zolnere et al., 2018). RBBL has also been used for the selective synthesis of GOSs from highly concentrated lactose solutions (Füreder et al., 2020). To date, no studies have been reported on the properties of RBBL in relation to milk hydrolysis. In the present work, we analyzed the effects of different variables of industrial relevance to milk hydrolysis using RBBL. Certain other features of the enzymatic product – such as protein content, protein profile by sodium-dodecyl-sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE), invertase activity, and Km for lactose – were also determined. A commercial YNL was used as the reference for comparison.

2 Materials and methods

2.1 Reagents and solutions

Lactose monohydrate was provided by Research AG (Argentina). Lactic acid (L+, 85% pure) was provided by ADAMA

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Food Ingredients (Argentina). Other reagents were of the highest analytical grade possible.

The composition of the phosphate solution PPB was 25 mM potassium dibasic phosphate adjusted to pH 6.6 with 1.0 N HCl. The PPB-Mg-ethylenediaminetetraacetate (-EDTA) buffer PME was prepared by adding $MgSO_4.7H_2O$ and Na_2EDTA to PPB in order to obtain final concentrations of 2 mM and 0.05 mM respectively. Ultrapasteurized whole milk (La Serenisima, Argentina) was purchased from a local market. The milk pH was ~6.6 and lactose, calcium and sodium contents, as indicated in the product label, were 132, 26.0, and 21.3 mM, respectively. The whey permeate used was Variolac® 850 with lactose and protein contents of 88% (w/w) and 3% (w/w), respectively. The commercial lactase products used were: RBBL (Saphera 2600 L, Novozymes, Bagsvaerd, Denmark, batch OFN O4009 and OFF00010, 2600 LAU-B.g^{-1}, density 1.17 g.mL^{-1}) and YNL (GODO YNL2, Danisco-Dupont, USA, batch 27022, > 50,000 ONPGU.g^{-1}). Lactase products were kindly provided by Nutring S.A. (Argentina). Unless otherwise stated, the RBBL batch used throughout the work was OFN O4009. Molecular weight markers (10-170 kDa) for SDS-PAGE were provided by Thermo Fisher, and the albumin was from Sigma Chemical Company (St. Louis, MO).

2.2 Standard lactase assay

The units of the lactase activities of YNL and RBBL have been defined according to the substrate used in the enzyme assay: lactose or chromogen o-nitrophenyl-ß-D-galactopyranoside. For convenience in this work, the lactase activities were measured under the same assay conditions (standard activity). This standard lactase activity was accordingly determined by incubating 2.4 mL of lactose substrate (5.2% [w/v] lactose monohydrate in PME, pH 6.6) with 0.1 mL of enzyme for 10 min at 30 °C. The reaction was stopped by immersion in boiling water for 1 min. The glucose formed was determined with an enzymatic kit (Glicemia, Wiener lab, Argentina). The YNL and RBBL concentrations in the reaction mixture were ~ 60 µg mL^{-1} and 100 µg mL^{-1}, respectively. One unit of standard lactase activity (LAUst) was defined as the enzyme necessary to produce one µmol of glucose per min under assay conditions. To calculate the standard activity per volume unit (LAUst.mL^{-1}), the density on the lactase-containing product was taken into account.

2.3 Milk hydrolysis

Lactose hydrolysis in milk was performed by adding 50 µL of enzyme solution, appropriately diluted with PPB, to 5 mL of milk submerged in a thermostatic bath at the working temperature. A simple and reproducible method was developed to determine glucose during milk hydrolysis. The procedure stated in brief: At the selected reaction time, 0.5 mL of sample were taken from the milk, mixed immediately in a 13-mL round-bottom polypropylene centrifuge tube with 1.5 mL of 0.016 N H_2SO_4 (final pH of the mixture ~4.7) and placed in boiling water for 2 min for enzyme inactivation and casein precipitation; the tube was then kept in an ice bath before centrifugation. As established in preliminary trials, a two-min incubation was necessary for the complete inactivation of RBBL in the acidified coagulated milk. YNL, in contrast, was almost instantaneously inactivated by the same treatment. The centrifugation of the precipitate formed during the heating was at 5,000 *g* for 20 min in a Sorvall SM24 rotor, with the clarified supernatant finally being used for glucose determination. A control sample without enzyme and a standard milk sample containing 4 g.L^{-1} of glucose were also incubated and treated in the same way. The supernatants were frozen at –20 °C until the time of analysis. Trials adding glucose to milk indicated a 98-100% sugar recovery monitored by enzymatic glucose determination. Typical lactase concentrations during the milk-hydrolysis experiments (initial rates of lactose hydrolysis) varied between 100 and 300 µg.mL^{-1}.

2.4 Cation effects

The effects of cations on lactase activity in buffer and milk was determined in a reaction mixture containing 4.9 mL of substrate (5.1% [w/v] lactose.H_2O in PPB, pH 6.6 or milk), 100 µL of cation solution, and 50 µL of enzyme solution diluted in PPB. The cations tested and their concentration were selected according to their prevalence in milk (Fuente & Juárez, 2015). The cations tested in milk were (final concentration, cation source): Ca^{+2} (8 mM, $CaCl_2.2H_20$), Mg^{+2} (2 mM, $SO_4Mg.7H_2O$); Mn^{+2} (0.1 mM, $MnCl_2.4\ H_20$), and Na^+ (20 mM, NaCl). In PPB the same cations were tested with the exception of Ca^{+2} and K^+. PPB and milk without the addition of cations were used as controls.

2.5 Temperature effects

The effect of the temperature on enzymatic activity in milk was determined between 7 °C and 60 °C. The reaction times varied according to the reaction temperature: 90 min (3 °C), 20-60 min (7 °C-25 °C), 15 min (30 °C and 37 °C) and 10 min at temperatures > 35 °C.

2.6 Effect of pH on the activity in whey permeate

A pH-activity profile in the pH range 3.7-6.6 was performed with cheese-whey permeate. This substrate was used in order to prevent milk coagulation at an acid pH, provided that the permeate was totally soluble at a wide range of pH. The bulking agent Variolac™ was dissolved in deionized water and the pH adjusted to 6.6 with KOH, then readjusted to the set value with lactic acid. The final lactose concentration in the reconstituted whey permeate was 4.7% (w/v). The reaction mixture contained 5 mL of substrate solution and 50 µL of enzyme diluted in PPB. The reaction was stopped after 20 min incubation at 30 °C by placing the mixture in a boiling-water bath for 1 min. No precipitate was observed after the sample heating, therefore glucose was determined directly in the heat treated sample.

2.7 Lactose-hydrolysis empirical model

Modeling the enzymatic lactose hydrolysis involves a certain complexity due to GOS formation. However, with lactose concentrations similar to those found in milk, where GOS formation is relatively low – *i. e.*, lower than 10% (Zárate & López-Leiva, 1990; Rodriguez-Colinas et al., 2014) – the

lactose-hydrolysis time course can be satisfactory described by Michaelis-Menten kinetics with competitive inhibition by galactose or with the formation of an intermediate galactosyl-enzyme complex (Yang & Okos, 1989; Flores et al., 1996b; Jurado et al., 2002,). The experimental data are usually obtained by measuring the glucose formed over time, as this sugar is less compromised in transglycosidation reactions (Mahoney, 1998). If the enzyme is not inactivated during hydrolysis, the active enzyme concentration will remain constant throughout the experimental period. Under this condition kinetics equations can be linearized giving rise to an expression in which the glucose formed could be expressed as a function of the parameter Eot, where Eo is the concentration of the active enzyme and t is the reaction time. The quantity of glucose formed for a given Eot value will depend on the model's kinetic parameters. In order to describe lactose hydrolysis in a simple and practical way, in this work the following empirical model was used (Equation 1):

$$\text{Glucose}\left(gL^{-1}\right)=\frac{B \times Eot}{\left(K+Eot\right)} \quad (1)$$

where Eo is the quantity of enzyme added to the milk, expressed in LAUst.L^{-1} and t is the reaction time in h; B and K are the model parameters.

The degree of hydrolysis, expressed as a percent, can be calculated as: (glucose/theoretical maximum glucose) x 100. The theoretical maximum glucose produced at 100% hydrolysis was calculated from the lactose content in milk. According to the composition of the milk used, the value was 23.7 $g.L^{-1}$. The experimental data were obtained from at least 3 independent trials in which different volumetric doses of lactase-containing product were incubated for between 3 and 24 h at 7 °C or between 10 to 40 min at 45 °C. The maximum Eot values (LAUst L^{-1} h) tested, which correspond to approximately 95% hydrolysis, were 100,000 and 25,000 at 7 °C and 9,000 and 2,200 at 45 °C for YNL and RBBL, respectively.

2.8 Kinetic parameters

The kinetic Michaelis constant Km for lactose hydrolysis was determined in PME buffer at 30 °C by measuring the initial rates of glucose formation with lactose concentrations in the range of 4 to 200 mM. The protocol was the same as used for the standard assay.

2.9 SDS-PAGE

SDS-PAGE was performed by means of mini-PROTEAN Tetra-cell electrophoresis equipment (Bio-Rad) at a constant voltage of 150 mV, following Laemmli's procedure (Laemmli, 1970). The gel was prepared at 8% (w/v) with 5 to 11 µg of protein per lane. Commercial samples were diluted 1:25 with water and mixed with an equal volume of (2X) loading buffer containing 8% (w/v) SDS, 24% (v/v) glycerol, 1.22% (w/v) Tris-HCl, 10% (v/v) ß-mercaptoethanol, 2% (v/v) dithiothreitol, and 0.02% (w/v) Coomassie Brilliant Blue R 250 at pH 6.8; followed by an incubation at 90 °C for 5 min before loading onto the gel. The bands were stained with 2% (w/v) colloidal Coomassie G-250.

2.10 Analysis

The protein concentration was determined according to Bradford (1976) with albumin as a standard. Samples were diluted 1:50 in water. The density of YNL was determined with a 10 mL Gay-Lussac picnometer (± 0.005 g/mL). Invertase activity was determined at 30 °C under the same conditions used to measure the standard lactase activity, but replacing the lactose with 5% (w/v) sucrose. The concentration of the lactase-containing product in the reaction mixture for invertase determination was 1 $mg.mL^{-1}$. One unit of invertase activity was the same as defined for the lactase activity.

2.11 Statistical analysis

An analysis of variance (ANOVA) and Tukey´s mean at a 5% level of significance were performed with MINITAB® statistical software (Minitab, LCC, State College, Pennsylvania). Parameter estimation by nonlinear adjustment of experimental data and curve fitting to the model were performed with Sigma Plot (Systat Software Inc. San Jose, California).

3 Results and discussion

3.1 Product characterization

Table 1 summarizes the data of the two commercial lactases. The standard activity of YNL (~13,000 $LAU.g^{-1}$) was six times higher than that of the RBBL (2,025 LAU g^{-1}). As the density of the commercial products was similar, the same ratio applied when the standard activity was expressed per unit volume. The yeast product contained invertase activity and a protein concentration of 49.8 $mg.g^{-1}$ which was similar to the value reported by

Table 1. Characteristics of commercial lactase.

Enzyme product	Density g mL^{-1}	Standard activity** LAUst g^{-1}	Protein mg g^{-1}	Milk activity*** LAU g^{-1}	Invertase IAU g^{-1}	km lactose (mM)
RBBL, SAPHERA 2600 L*	1.16	2025 ± 31.5 (2350)	20.0 ± 1.13 (23)	2095 ± 88.4 R ~ 1.0	n.d	13.8 ± 0.9
GODO-YNL2	1.17	12720 ± 443 (15000)	49.8 ± 1.2 (58)	3598 ± 161.5 R ~ 0.28	10.2 ± 0.83	29.1 ± 1.2

Average standard and milk activity: average LAU g^{-1} ± standard deviation from 6 independent determinations; between parentheses: volumetric activity (LAUst mL^{-1}) and protein per unit volume (mg mL^{-1}); R: activity milk/activity PME; Km determined in PME, pH 6,6; n.d: not detected. All activities were determined at 30 °C. *No significant differences ($P < 0.05$) were found between two batches of the commercial product (OFN O4009, OFF00010). **Lactose at 139 mM in PME buffer, pH 6,6. ***Whole milk UP, pH 6.5-6.6, lactose at 132 mM, Ca^{+2} 26 mM, Na^{+} 21,3 mM.

Erich et al. (2015). Invertase activity was not detected in RBBL, and the protein concentration of that product was 20 mg.g^{-1}.

Figure 1 illustrates the protein analysis by SDS-PAGE. A main band with a molecular weight of 131 ± 1.1 kDa was observed in YNL, which analyte is consistent with the MW reported for the lactase subunits of *K. lactis* (Dickson et al., 1979; Pereira-Rodríguez et al., 2012). In the example of RBBL, both batches displayed a similar pattern, the main band corresponding to a MW of 140 kDa and at least three less intense bands were also visible with MWs of 120, 108, and 103 kDa, respectively; the 108-kDa band being the most intense of those in both lanes. The intensity of the 120-kDa band in batch OFNO4009 was higher than in batch OFF00010 whereas the inverse intensity was observed for the 108-kDa band. Unlike *K. lactis*, whose lactase is encoded by a unique gene (LAC4, UniProtKB- P00723; BGAL_KLULA), four genes (Møller et al., 2001; Goulas et al., 2007) encoding different ß-galactosidase isoenzymes had been identified in *B. bifidum*, all of which were expressed in *Escherichia coli*. The cloned isoenzymes were dimers or hexamers with subunit sizes ranging from 80 to 190 kDa, as determined by SDS-PAGE. As the *B. bifidum* gene from which RBBL is derived is unknown, the recombinant lactase cannot be assigned to any of the aforementioned isoenzymes.The Km value for lactose was more favorable for RBBL, with the Kms obtained in PME buffer at 30 °C being 14 ± 0.86 and 29 ± 2.72 mM for RBBL and YNL, respectively. The Km value for YNL was slightly higher than the Km reported by Erich et al. (2015), but close to published data for the Kms from other *K. lactis* preparations measured under similar reaction conditions (Voget, 2018). In contrast, the Km values obtained for different *B. bifidum* ß-galactosidases isoenzymes have been reported to be quite varied within the range of 1.5-47 mM (Garman et al., 1996; Goulas et al., 2009).

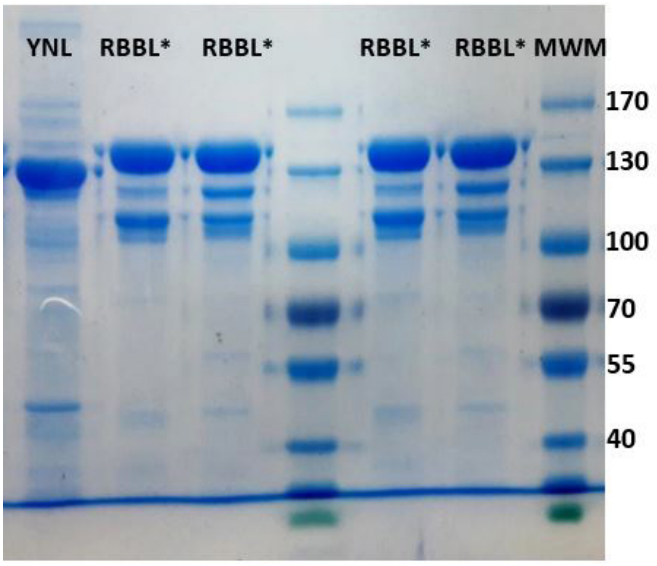

Figure 1. SDS-PAGEs of lactase products. Protein load: RBBL 4.6 µg; YNL 11.6 µg. YNL: Yeast Neutral Lactase; RBBL: Recombinant *Bifidobacterium bifidum* Lactase; MWM: molecular-weight markers. RBBL: *Batch OFF00010; **Batch OFN O4009.

3.2 Effect of cations on the enzymic activity

The ionic composition is the main element controlling yeast ß-galactosidase activity in milk (Mahoney & Adamchuk, 1980). Cations such as Na^+, K^+, Mg^{+2}, and Ca^{+2} affect the enzyme activity in concentrations normally found in the natural substrate. Furthermore, Garman et al. (1996) found that Na^+, K^+, and Mg^{+2} contributed to obtaining maximum rates of lactose hydrolysis by the *B. bifidum* ß-galactosidase in buffer solutions. To determine whether cations had any effect on RBBL activity, lactose hydrolysis was determined in PPB (containing 50 mM K^+) and milk with or without the addition of different cations. YNL was also included in the experiments as a reference. Figure 2 depicts the results. The addition of cations had no significant effect ($p < 0.05$) on RBBL activity, either in the buffer solution or in milk. The results with YNL are consistent with the properties reported for this enzyme. All the cations affected that enzyme activity in some way. In PPB, the addition of Mg^{+2} and Mn^{+2} increased lactose hydrolysis, while Na^+ was inhibitory. A similar response was obtained in milk where the enzyme activity increased with the addition of Mn^{+2} (18.5%), Mg^{+2} (22%) and K^+ (15%) and was reduced with Na^+ (–26%) and Ca^{+2} (–15%). The increase in activity that was observed when K^+ was added to milk can be explained by the fact that the enzyme activity depends on the

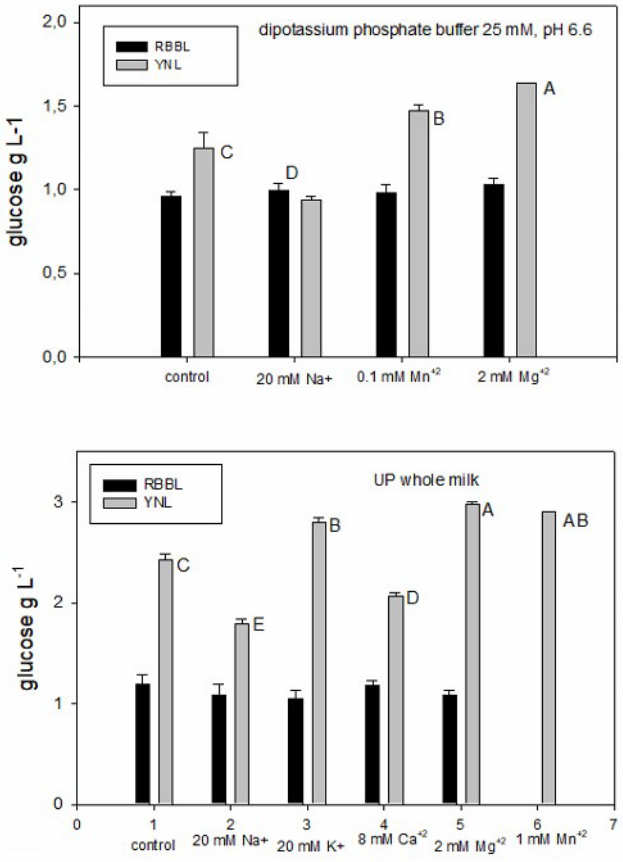

Figure 2. Cation effects on lactase activity in buffer solutions and milk at 30 °C. Buffer system: 25 mM K_2HPO_4-HCl, pH 6.6, lactose 139 mM. Ultrapasteurized (UP) whole milk (lactose 132 mM). The error bars represent the standard deviation of the mean from duplicate assays in two independent experiments. Different letters indicate significant differences for a given cation.

concentration ratio of these cations rather than on the absolute concentration of Na^+ (Flores et al., 1996a).

The ionic composition of milk clearly can limit YNL activity because of an insufficient cofactor concentration and/or an inhibition by Na^+ and Ca^{+2}. With RBBL, however, the assumption would be reasonable that the concentration of cations present in milk are adequate for optimal activity and that variations in the ionic composition of the different milk substrates may be expected to have little effect on the rate of lactose hydrolysis. This conclusion may also obtain upon comparing standard activities with certain activities in milk determined under similar conditions of lactose concentration and pH. The catalysis of RBBL was equivalent in either buffer or whole milk, whereas the YNL activity in milk was at 28% of the standard activity (Table 1). In another words, a standard RBBL unit equals to 0.28 standard YNL units with milk as substrate.

3.3 Temperature effects

Figure 3 illustrates the temperature dependency of lactase activity. The optimum temperature is 42-45 °C for YNL and 45-50 °C for RBBL. The most salient difference is that the bacterial lactase is active at temperatures higher than 50 °C, and even significantly at 60 °C. RBBL also shares the essential property of the yeast lactase of having some activity at low temperatures – even at as low as 3 °C. YNL at 7 °C catalyzes at a 10% of its maximum activity whereas the corresponding activity of RBBL is 12% of the maximum. From these data, our conclusion was that both lactases had the same capability of hydrolyzing milk in the cold relative to the corresponding activities at the optimum temperatures. For YNL, the Arrhenius plot within the temperature range of 3-45 °C evidenced a substantial linearity ($R^2 = 0.981$) with an activation energy of ~10.8 ± 0.45 kcal.mol^{-1}. The data obtained with RBBL seemed to follow a similar pattern, though with a linearity in the same temperature range that was not so evident. The possible cause of that difference was not investigated.

3.4 pH effects

Figure 4 depicts the dependance of enzyme activity on pH measured at 30 °C in whey permeate. The yeast lactase is clearly almost inactive at pHs lower than 6.0, the optimum range being 6.3-6.6. In similar manner, the pH optimum of RBBL is close to 6.1, whereas the recombinant enzyme maintains a considerable activity under more acidic conditions, even at pH levels of 4.5. For instance, at pH of 4.5 about 45% of the optimum activity is retained. This property makes RBBL a promising option to remove lactose from acidic dairy products (Dorau et al., 2021).

3.5 Kinetics of milk hydrolysis

Assays in milk or permeate related to ionic environment, temperature or pH were performed on the basis of initial-rate measurements. In order to analyze the kinetics of the hydrolysis up to a higher degree of hydrolysis, several assays were carried out combining different amounts of the commercial product and reaction times with an aim at obtaining a 90-95% máximum yield of glucose. Those trials were performed at 7 °C and 45 °C because of the need to analyze the hydrolysis both under refrigeration conditions and at optimum temperatures. An empirical model that related the glucose formed to the Eot product (Equation 1) was used to describe the enzyme action. Figure 5 contains hydrolysis curves with the corresponding model fitted to them. At both temperatures, the quantity of glucose formed was the same for a given Eot value, indicating that enzyme deactivation did not occur during the course of the reaction. The low values of the standard error of estimate (SEE) indicated that the fit of the model was statistically highly significant for both YNL and RBBL at the two reaction temperatures. Even though the model is nonlinear, in practice the formation of glucose occurs at a constant rate up to approximately 15-20% of the total hydrolysis (yielding a glucose concentration of 3.55 and 4.74 g.L^{-1}) regardless the nature of the enzyme or reaction temperature. At this stage, the model might be expressed as (Equation 2):

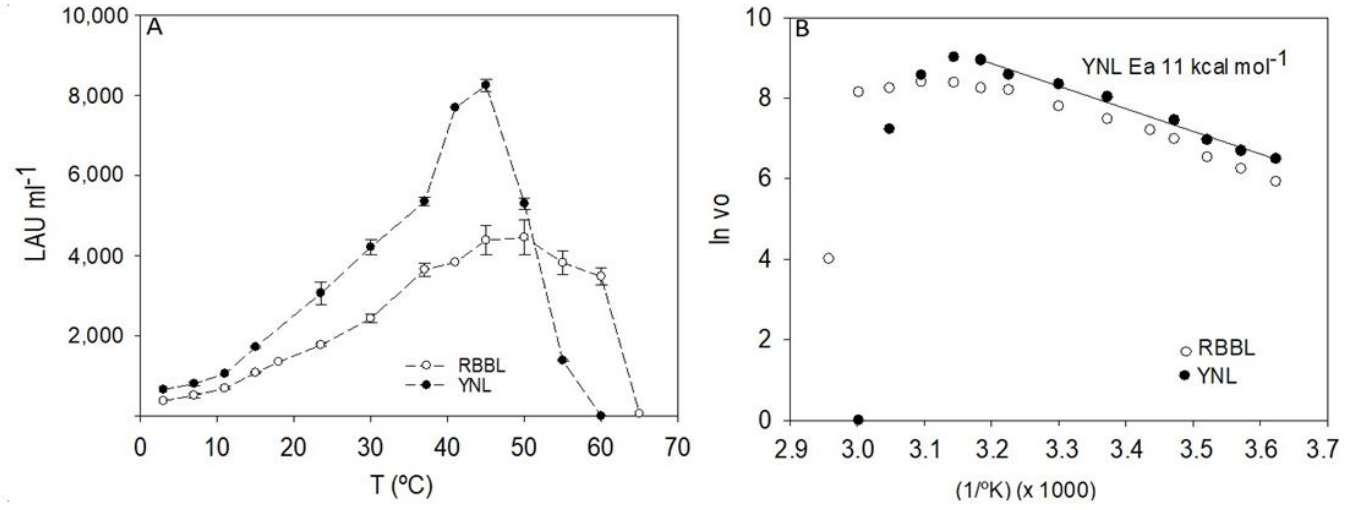

Figure 3. Temperature effect on lactase activities in milk. Panel A: Lactase activity as a function of the temperature. Panel B: Arrhenius plot of the lactase activity Ea: activation energy calculated from the YNL curve.

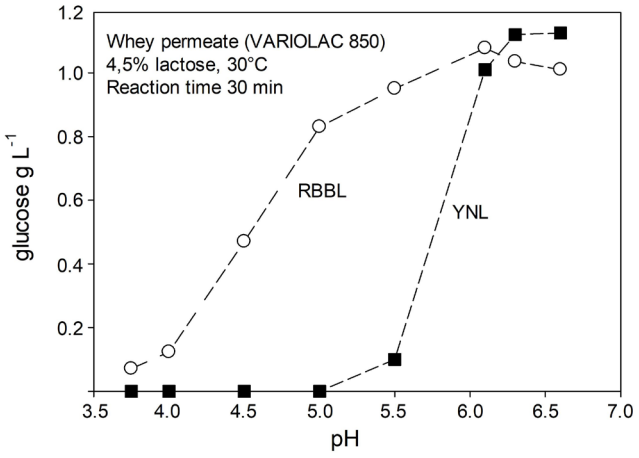

Figure 4. The pH dependence of lactase activity in whey permeate.

$$\text{Glucose}\left(gL^{-1}\right) = C \times Eot \quad (2)$$

where C= B/K.

According to Equation 2, the difference in the Eot value from the one calculated with the nonlinear model is –12.5% at a hydrolysis of 20%.

The model's significance and usefulness lies in its predictive capability. Table 2 lists the model parameters and an estimation of the necessary yields of the commercial product, expressed as standard activity or product volume per liter of milk, in order to obtain hydrolysis values of practical application – such as 20% for the elaboration of Milk Jam (Argentina, 2018) and 95% for reduction in lactose milks (Argentina, 2021). The reaction times considered were those typical of commercial applications. As expected, the RBBL standard activity units necessary to

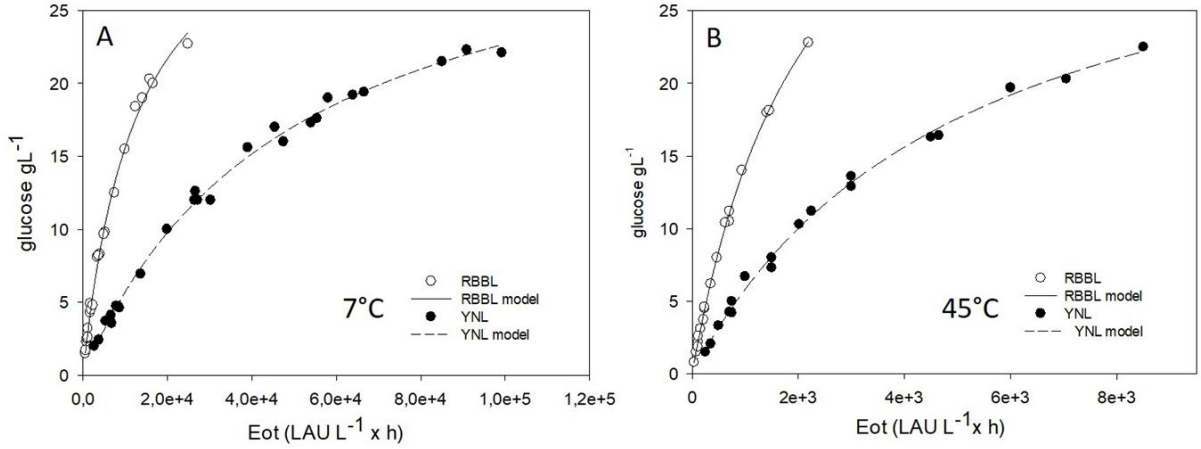

Figure 5. Milk hydrolysis at 7 °C (Panel A) and 45 °C (Panel B) and model fit. The reaction times varied between 3 and 24 h at 7 °C and 10 to 60 min at 45 °C. The data were obtained from three independent experiments. The lines depicts the locus of points generated from the model.

Table 2. Model parameters and dose estimation to obtain different degree of lactose hydrolysis.

$\text{glucose } (gL^{-1}) = \frac{B \times Eot}{K + Eot}$	Model Parameters			
	7 °C		45 °C	
	YNL	RBBL	YNL	RBBL
B	34.0 ± 0.80	35.5 ± 1.13	35.5 ± 1.19	46.1 ± 1.3
K	49450 ± 2431	12790 ± 757	5090 ± 315	2225 ± 94.5
Df	25	21	16	15
SEE	0.440	0.47	0.41	0.25
	Eo [LAUst L^{-1}]*			
	7 °C x 24 h		45 °C x 1 h	
Target glucose gL^{-1} (% hydrolysis)	YNL	RBBL	R	YNL	RBBL	R
4.74 (20)	334	82	0.25	784	255	0.32
22.5 (95)	4031	922	0.23	8810	2121	0.24
	Product dose [mL L^{-1}]**					
	YNL	RBBL	R	YNL	RBBL	R
4.74 (20)	0.022	0.035	1.6	0.052	0.11	2.1
22.5 (95)	0.27	0.39	1.4	0.59	0.90	1.52

SEE: standard error of the estimate; B, K: model parameters ± SEM; df: degrees of freedom; R: (RBBL/YNL). *Standard activity doses required to obtain target glucose concentration according to the model; **Volumetric dose on the basis of the standard activity of the product.

obtain a 20% hydrolysis (4.74 g of glucose.L^{-1}) range between 0.25 (7 °C) and 0.31 (45 °C) with respect to those required by YNL. That relationship was reduced to between 8% (7 °C) and 22% (45 °C) for a 95% hydrolysis. This result indicates that after 20% hydrolysis the reaction presents a slower progress with YNL at both temperatures and, therefore, more standard units are required to achieve – within the same reaction time – a degree of hydrolysis equal to that of RBBL.

From the standard activity of the products, a calcúlation of the volumetric dose necessary to obtain the desired hydrolysis is possible, which estimation is an essential consideration in order to guage the costs of the process. The situation is different if we take the information on hydrolysis at 20% and 95%: in this instance, we need ~1.4-2.0 times more dose per volume for RBBL than for YNL in order to achieve the desired hydrolysis. The higher use of recombinant product is not due to a possible disadvantage inherent in the kinetic properties of lactose hydrolysis, but instead to a lower enzyme concentration (standard units) in the formulated product.

4 Conclusion

We have determined the properties of a commercial recombinant lactase from *B. bifidum* (RBBL; Saphera 2600 L) most relevant to the hydrolysis of milk in comparison to those of a neutral yeast lactase (GODO-YNL2). RBBL exhibits properties that could be advantageous for milk hydrolysis, although the standard activity of the formulated product is lower than that of YNL. Consequently, in order to obtain results similar to those of YNL under certain conditions of milk hydrolysis, a higher volumetric dose is required.

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