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OBTENTION OF A LACTOSE HYDROLYSATE FROM NANOFILTRATION OF SWEET WHEY: CHARACTERIZATION AND PROCESS OPTIMIZATION

OBTENCIÓN DE UN HIDROLIZADO DE LACTOSA A PARTIR DE LACTOSUERO DULCE USANDO NANOFILTRACIÓN: CARACTERIZACIÓN Y OPTIMIZACIÓN DEL PROCESO

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Abstract

Lactose has been a sugar of limited applicability in the food industry due to its low level of sweetness, low solubility and poor digestion in most of the global population. However, from the world production of whey, a high production volume of this disaccharide could be obtained and be enzymatically hydrolysate to produce sweeter sugars, very soluble in water and easily digestible like glucose and galactose. This study was proposed to optimize the process of hydrolysis of a commercial enzyme (β-galactosidase of *Kluyveromyces lactis*) in a concentrate of lactose (207 g L^{-1}), obtained by nanofiltration of sweet whey. A Box-Behnken response surface design allowed the evaluation of the influence of several factors on the percentage of hydrolysis, indicating that it is possible to obtain up to 84.5% by combination of the following process conditions: pH (6.11); temperature (37.20 ºC); enzyme concentration (1.36 g L^{-1}) and time (176 min). The hydrolysate obtained was valued, finding minerals such as potassium: 0.3 g $100g^{-1}$, magnesium: 0.020 g $100g^{-1}$, calcium: 0.060 g $100g^{-1}$, phosphorus: 0.09 g $100g^{-1}$, glucose: 89 g L^{-1}, galactose: 66 g L^{-1} and CIELAB coordinates: L^* = 32.3, a^* = -0.5 and b^* = 15.

Keywords: Whey, nanofiltration, lactose, hydrolysis, lactase

Resumen

La lactosa ha sido un azúcar de limitada aplicabilidad en la industria de alimentos debido a su bajo nivel de dulzura, baja solubilidad y deficiente digestión en la mayoría de la población global. Sin embargo, un alto volumen de producción de este disacárido podría obtenerse de la producción mundial de lactosuero y ser hidrolizado enzimáticamente para generar azúcares más dulces, muy solubles en agua y fácilmente digeribles como la glucosa y galactosa. Este estudio se propuso optimizar el proceso de hidrólisis de una enzima comercial (β-galactosidasa de *Kluyveromyces lactis*) en un concentrado de lactosa (207 g L^{-1}), obtenido por nanofiltración de lactosuero dulce. Un diseño de superficie de respuesta Box-Behnken permitió evaluar la influencia de diferentes factores sobre el porcentaje de hidrólisis, indicando que es posible obtener hasta el 84.5% mediante las siguientes condiciones de proceso: pH (6.11); temperatura (37.20 ºC); concentración de enzima (1.36 g L^{-1}) y tiempo (176 min). El hidrolizado obtenido fue analizado; encontrándose minerales como potasio: 0.3 g $100g^{-1}$, magnesio: 0.020 g $100g^{-1}$, calcio: 0.060 g 100 g^{-1}, fósforo: 0.09 g $100g^{-1}$), glucosa: 89 g L^{-1}, galactosa: 66 g L^{-1} y coordenadas CIELAB: L^* = 32.3, a^* = -0.5 y b^* = 15.

Palabras clave: Lactosuero, nanofiltración, lactosa, hidrólisis, lactasa.

1 Introduction

Lactose is a reducing disaccharide formed by one molecule of glucose and one of galactose linked by a β 1-4 glycosidic bond, which is found mainly in the milk of mammal females at a concentration of 40-50 g L^{-1}, it has a low sweetness level (30% of sucrose), a solubility of 17% compared to sucrose at 15 ºC (Durham, 2009; Wong and Hartel, 2014), and a poor digestion in the majority of the adult population worldwide (70-75%); which has limited its use as a sweetener in the food industry and promoted its reduction in milk and dairy products (Dainese-Plichon *et al.*, 2014).

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Hydrolysis products (glucose and galactose) obtained from this disaccharide are sweeter, very soluble in water and easily digestible (Ghosh *et al*., 2013). In general, β-galactosidases recognized as GRAS (*Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Aspergillus niger* and *Aspergillus oryzae*) catalyze the hydrolysis reaction of lactose with wide acceptance in the food industry due to its safety, thermal stability and low cost (Beltran and Acosta, 2012; García-Reyes *et al*., 2017). Its use in the removal of lactose from milk and production of whey hydrolysates has motivated studies on the influence of process variables, efficiency of enzyme immobilization methods and hydrolytic potential of commercial β-galactosidase preparations (Ansari and Husain, 2010; Guerrero *et al*., 2015).

At present, whey has been the subject of numerous investigations as it is a natural source for obtaining lactose with approximately 6.3 million tons available from the production of whey that are generated each year worldwide (Paterson, 2011), to which Colombia contributes with at least 18 thousand tons from the 445 million liters of whey produced annually (calculated as 90% of the total milk collected in a formally during the year 2017 and used in cheese making - Statistics of Colombian Dairy Products Association, ASOLECHE). However, there have been few investigations reported on lactose hydrolysis by sweet whey nanofiltration retentate. That is why this study aimed to evaluate the influence of pH, temperature, time and concentration of a commercial enzyme (β-galactosidase from *Kluyveromyces lactis*) on the hydrolysis of a lactose concentrate derived from whey nanofiltration and determine its optimal value. Physicochemical and techno-functional properties of the hydrolysate obtained were evaluated.

2 Materials and methods

2.1 Reagents and raw material

Lactose concentrate (LC) was supplied by the dairy laboratory of the Universidad Nacional de Colombia-Sede Medellin. A spiral type polyethersulfone membrane (3838 K131-HYV with a cut-off range of 10000 Daltons) and a spiral polyamide membrane (3839 SR3D-VYV with a cut-off range of 200 Daltons) coupled to a membrane filtration pilot plant (Perinox, Spain) were used. The operation of the equipment in ultrafiltration mode allowed to separate proteins from whey and obtain a permeate as effluent, which served to feed the nanofiltration process and concentrate the lactose to 207 g L^{-1}; using a transmembrane pressure (TMP) of 2.84 MPa, feed flow temperature of 25 °C and a volumetric concentration factor (VCF) of five. The analytical grade reagents: α-lactose monohydrate (≥ 99%), D (+) anhydrous glucose (≥ 96%) and D (+) anhydrous galactose (≥ 99%) were obtained from Sigma Aldrich. The commercial β-galactosidase LACTAPROQ from the Proquiga group, which corresponds to a preparation of β-galactosidase purified from *Kluyveromyces lactis* in a solution of glycerol and water, was used as an enzyme source.

2.2 Characterization of raw material

The lactose concentrate was analyzed in its pH (electrometric method using an OHAUS STARTER 3100 pH meter), *titratable acidity* (% lactic acid, according to AOAC 947.05: 2012), *density* (pycnometer method), *color* (tristimulus spectrophotometry method evaluated in CIELAB coordinates (L*,a*,b*) with Konica Minolta CR-400 series where the parameter L* indicates luminosity whereas a* axis shows the variation of red (+a*) to green (-a*) and axis b* is the variation from yellow (+b*) to blue (-b*)), Protein (Kjeldahl method, according to AOAC 991.20: 2012) with Protein = Total Kjeldahl Nitrogen x 6.38, Minerals (using atomic absorption spectrometry, internal procedure based on AOAC 968.08: 2012), *Phosphorus* (colorimetric method, internal procedure based on AOAC 964.06: 2012) and *ashes* (internal procedure gravimetric method based on AOAC 945.46: 2012), *lactose, glucose, and galactose* were quantified by high-performance liquid chromatography (HPLC) using a chromatograph AGILENT TECHNOLOGIES 1200 series with an AMINEX HPX-87H ion exchange column (300 x 7.8 mm), and as mobile phase solution of H_2SO_4 0,008N at a constant flow of 0.6 mL min^{-1} (Beltran and Acosta, 2012).

Physicochemical analyzes in whey, permeate and LC were developed on 15 independent samples which obtaining methodology had been standardized.

2.3 Hydrolysis of lactose concentrate

The hydrolytic reaction was conducted in batch mode to study the effect of the following process parameters: temperature (25 - 55 ºC), pH (6.0 - 7.5), enzyme concentration (0.5 - 1.25 mL L^{-1}) and time (30 - 180 min), in 50 mL of solution (lactose concentrate plus

Kluyveromyces lactis enzyme extract) making use of NaOH or citric acid to adjust pH according to the case. Each reaction bottle placed in an orbital shaker with temperature control was subjected to experimental process conditions to complete the reaction time (fixed by increasing the temperature of the solution at 85 °C for 10 min). Sugars generated during the reaction: glucose, galactose and the remaining lactose, were quantified by HPLC according to the methodology described above. Finally, equation 1 was used to determine the percentage hydrolysis (% H).

$$\% = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where, C_i is the concentration of lactose in the sample without hydrolyzing and C_f the concentration of lactose in the sample after hydrolysis process.

2.4 *Enzymatic activity*

It was defined as the amount of enzyme that catalyzes the conversion of 1 μmol of lactose per minute. The activity of soluble β-galactosidase from *Kluyeromyces lactis* was determined under experimental conditions, from whey nanofiltration retentate.

2.5 *Characterization of the lactose hydrolysate*

Physicochemical parameters such as: *pH, titratable acidity* (% lactic acid), *density, color, mineral content* (calcium, magnesium, potassium, and phosphorus), *Ash, protein, lactose, glucose and galactose* were determined in the lactose hydrolysate according to the methodology described for the lactose concentrate (LC).

2.6 *Statistical analysis*

A Box-Behnken design with a split-plot structure and 48 treatments allowed identifying the factors (pH, temperature, enzyme concentration and time) affecting response variables: % Lactose hydrolysis, glucose concentration (g L^{-1}) and enzymatic activity (μmol/min), and estimate their functional relationship using a second-order polynomial regression model (Das *et al*., 2015). Design Expert statistical software (Version 8.0.6, Statistical Ease Inc., Minneapolis, MN, USA) was used to perform data analysis. A significance level of $\alpha = 0.05$ was used for all analyses.

3 Results and discussion

Table 1 shows the results of whey physicochemical characterization, ultrafiltration permeate (PUF) and lactose concentrate (LC). According to the results, whey is described as a slightly acid liquid, rich in lactose and greenish yellow (according to CIELAB parameters) attributable to the water-soluble vitamin B2, known as riboflavin (Pizzichini, 2006; Chatterjee *et al*., 2015). The pH of sweet whey was 6.53, this value is usually used as a parameter of whey type differentiation. Callejas *et al*., (2012) mention that pH > 6 characterizes sweet whey resulting from the coagulation of casein present in fresh milk without the addition of organic or mineral acids, nor fermentative processes conducive to coagulation. A lactose content = 46.0 g L^{-1} in whey, shows the affinity of the disaccharide with the aqueous part of the cheese making process, and a value of 0.85 g $100g^{-1}$ protein the propensity of amino acids towards the insoluble "casein" part (Sánchez *et al*., 2009; Banaszewska *et al*., 2014). Also, there are minerals such as: magnesium = 0.007 g 100 g^{-1}, potassium = 0.14 g 100 g^{-1}, ash = 0.53 g 100 g^{-1} and calcium = 0.037 g 100 g^{-1} with approximately 30% of its milk content due to the fact that the majority remains insoluble interacting with casein, which forms the basic structure of cheese (Inda, 2000; Kreczmann *et al*., 2015).

During ultrafiltration process, the permeate was obtained; a liquid of translucent appearance with CIELAB coordinates: L* = 44.9, a* = -1.2, and b* = 4, in which major components were lactose = 50 g L^{-1} and mineral salts such as: potassium = 0.14 g 100 g^{-1} and calcium = 0.030 g 100 g^{-1} with values similar to those reported by Cuartas-Uribe *et al*., (2009). The increase in lactose concentration, after passing through the ultrafiltration membrane, was due to the decrease in the total mass by discount of soluble protein that also influenced ºBrix decreasing them to 5.40 (Kleinhenz and Bumgarner, 2015). Finally, no significant changes were observed in titratable acidity and pH values of whey and PUF, which is indicative of microbial stability in the product, possibly as a result of whey pasteurization and the capacity of retention of microorganisms in the membrane (Chacón, 2006).

A polyamide nanofiltration membrane with a cut-off molecular weight of 200 Daltons allowed to concentrate PUF lactose to 207 g L^{-1} and minerals such as calcium, magnesium and potassium up to 0.097; 0.026 and 0.29 g 100 g^{-1}, respectively.

Table 1. Composition of sweet whey, ultrafiltration permeate (PUF) and lactose concentrate (LC) (average values ± standard deviation).

Parameter	**Sweet whey**	**PUF**	**LC**
pH	6.53 (± 0.07)	6.33 (±0.09)	6.1 (±0.1)
Acidity (g lactic acid $100g^{-1}$)	0.09 (± 0.01)	0.08 (±0.01)	0.22 (±0.02)
Soluble solids (°Brix)	7.0 (± 0.2)	5.40 (±0.09)	20.2 (±0.3)
Density (g mL^{-1})		1.111 (±0.002)	
Lactose (g L^{-1})	46.0(± 0.3)	50 (±1.47)	207 (± 8.00)
Glucose (g L^{-1})	0	0	0
Galactose (g L^{-1})	0	0.121(±0.092)	0
Protein (g $100g^{-1}$)	0.85 (±0.07)	< 2.5	<2.5
Ash (g $100g^{-1}$)	0.53 (± 0.04)	0.48 (±0.03)	1.04 (± 0.04)
Calcium (g $100g^{-1}$)	0.037(±0.005)	0.030 (±0.003)	0.097(±0.005)
Magnesium (g $100g^{-1}$)	0.007(±0.001)	0.0060 (±0.0005)	0.026(±0.001)
Potassium (g $100g^{-1}$)	0.14 (± 0.02)	0.14 (±0.01)	0.29 (±0.02)
Phosphorus (g $100g^{-1}$)		0.100(±0.009)	
Color:			
L*	49.62 (±0.23)	-2.2 (±0.2)	6.9 (±0.3)
a*	44.9 (±0.8)	-1.2 (±0.4)	4.0 (±1.0)
b*	49.0 (±1.0)	-5.1(±0.4)	18.5 (±0.7)

The high permeability of the membrane to monovalent salts and the low permeability to organic compounds and divalent salts has been corroborated by Oatley-Radcliffe *et al*., (2017) and by Nath *et al*., (2018). However, some authors highlight the existence of electrostatic and steric factors that take place during the saline solution permeation process and their determining role in ions retention; which explains the preference of high valence ions against monovalent ions and the possibility that the latter are retained or released to guarantee electroneutrality in the system (Rice *et al*., 2011). The reduction of pH and increase in titratable acidity of the lactose concentrate (LC) was due to the presence of lactic acid and other acids from the microbial degradation of this disaccharide as suggested by Schmidt *et al*., (1996). Finally, CIELAB coordinates: L* = 49, a* = - 5.1 and b* = 18.5 described for LC show an intense yellow color that warns about the concentration of vitamin B2 during nanofiltration process (Pizzichini, 2006).

3.1 % hydrolysis of lactose

This parameter varied in the range of 26.05% - 88.66%; some authors have reported hydrolysis of up to 100% in concentrated whey samples (128 g L^{-1} of lactose) when using similar enzyme sources (Beltran and Acosta, 2012), while others registered up to 88% with β-galactosidases from *Bacillus circulans* (Das *et al*., 2015). Table 2 presents coefficients of the model equation that best fit each response variable and have been simplified by eliminating non-significant variables (p value > 0.05).

Figure 1 shows an initial increase of % hydrolysis of lactose at pH 6.0-6.3 and temperature between 25 - 40 °C, but once the optimum temperature and pH were reached, a decrease in the percentage of hydrolysis was observed (figures (1a) - (1c)).

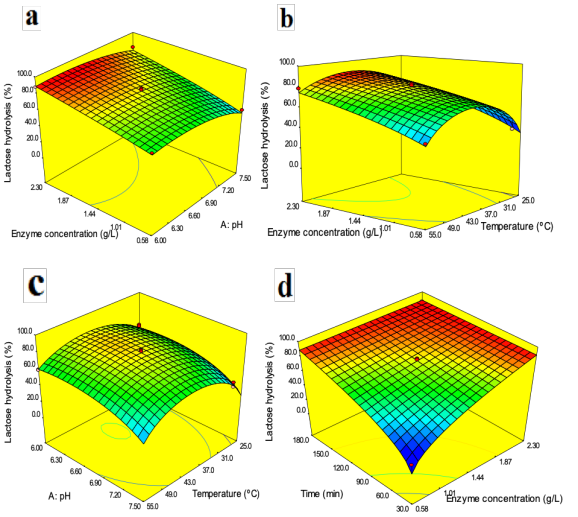

Fig. 1. Response surface: (a) effect of enzyme concentration and pH, (b) effect of enzyme concentration and temperature, (c) effect of pH and temperature, (d) effect of time and enzyme concentration.

Table 2. Regression coefficients (polynomial model of second order) for the responses variables of the lactose hydrolysis process.

Coefficient	Lactose hydrolysis (%)	Glucose (g L^{-1})	Enzymatic activity (μmol/min)
B (intercept)	-422971	-20397.35	-110.08
β_{A-pH}	97967.14	4040.68	34.07
$\beta_{B-Temperature}$	4382.47		
$\beta_{C-enzymeconcentration}$	214322.15	1547.09	9.36
β_{D-Time}	348.52	34.86	-0.1
β_{AD}			0.013
β_{BD}	-1.95	-0.27	-0.00232
β_{CD}	-120.13	-9.2	-0.07
β_{A^2}	7672.64	-334.72	-2.78
β_{B^2}	-51.25	-3.86	-0.0075
β_{D^2}			0.00071
R^2	0.9731	0.9227	0.9604
Adj R^2	0.9673	0.9059	0.9491
P-value (lack of fit)	< 0.0001	0.0001	< 0.0001
C.V. %	10.43	14.62	5.42
P-value (model)	< 0.0001	< 0.0001	< 0.0001

According to Pizzichini (2006) this behavior is attributed to the thermal denaturation experienced by β-galactosidases at temperatures above the optimal activity and that the enzyme has two carboxylic groups in its active site; a protonated one (OH^-) and an ionized one (COO^-) activated at the same time (as a proton donor and as a nucleophile) at pH values close to neutral. Das *et al*., (2015) revealed in their study on optimization of a lactose hydrolysis process that this type of enzymes are more stable in the range of pH 6.5-7.0 and temperatures around 30 °C, because additional increases in temperature can alter the conformation of the enzyme. On the other hand, Beltran and Acosta (2012) in their research on whey hydrolysis with a commercial β-galactosidase from *Kluyveromyces lactis* concluded that the amount of active enzyme was higher at pH 6.5 and temperatures around 45 ºC. In addition, a progressive increase in the proportion of lactose hydrolysis was observed increasing the initial concentration of enzyme, and the percentage of hydrolysate substrate with longer incubation times (Figure 1d) which may be due to the increase of active sites to unfold to the disaccharide and to greater opportunities to interact with lactose molecules present in the nanofiltration retentate; as suggested by Kaur *et al*., (2009) in their investigations on whey hydrolysis and Chen *et al*., (2009) when analyzing the behavior of thermostable β- galactosidases in the lactose hydrolysis from milk.

The coefficient of determination (R^2 = 97.31%) indicates that only 2.69% of the variability in the % hydrolysis of lactose cannot be explained by the model. Additionally, the adjusted determination coefficient (R^2 adjusted = 0.97) and a low coefficient of variation (C.V. = 10.43%) seem to confirm that it provides an adequate adjustment to the data. However, a significant lack of fit (< 0.0001) means that the model may not adequately describe the functional relationship existing between factors and response variable and therefore cannot be used as a predictor, only to explain its tendency.

3.2 Glucose concentration (g L^{-1})

One of the products resulting from the hydrolytic reaction was glucose, which production depended significantly on pH, initial concentration of the enzyme and incubation time (Table 2), reaching proportions between 21.50 g L^{-1} - 90.57 g L^{-1}; concentrations of 0.2 mol/L (~ 36 g L^{-1}) and 0.5 mol/L (~ 90 g L^{-1}) of glucose have been obtained by Palai and Bhattacharya (2013) when evaluating the products of trans-galactosylation reaction of a β-galactosidase from *Bacillus circulans* from a lactose concentrate of 200 g L^{-1}, and by Jenab *et al*., (2018) when evaluating the enzymatic conversion of lactose to galacto-oligosaccharides (GOS).

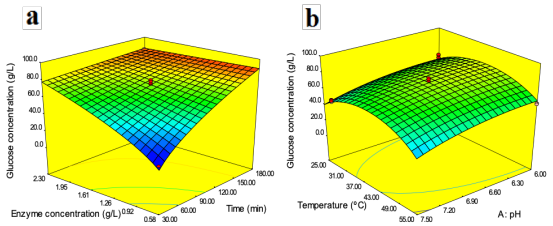

Fig. 2. Response surface: (a) effect of enzyme concentration and time, (b) effect of temperature and pH on glucose production.

The maximum glucose concentration in the nanofiltration retentate was obtained by combining the following factors: pH = 6.00; enzyme concentration = 1.44 g L^{-1}; temperature = 40 ºC and time = 180 minutes. Figure 2 illustrates the characteristic behavior of glucose production; the quantity of product generated per unit of time is initially greater, followed by a state of apparent leveling or stationary as the substrate is depleted; its increase being the consequence of a greater lactose hydrolysis and favorable conditions for the optimal functioning of the enzyme; as corroborated by Palai and Bhattacharya (2013) when observing the conversion kinetics of lactase in a lactose concentrate of 200 g L^{-1} for 30 hours and Hatzinikolaou *et al*., (2005) when evaluating glucose concentration during 3 hours of whey hydrolysis.

3.3 Enzymatic activity (U)

It depended significantly on pH, the initial concentration of enzyme and the incubation time (Table 2), with values comprised between 75.129 to 571.034 (μmoles/min); higher than the values reported for lactose hydrolysis in milk (Obón *et al*., 2000).

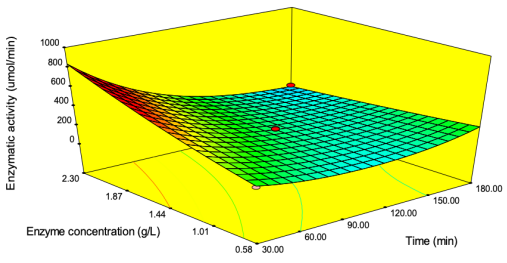

Fig. 3. Response surface for the effect of enzyme concentration and time on the enzymatic activity of *Kluyveromyces lactis*.

Figure 3 illustrates two behaviors, at the beginning (30 - 120 minutes) and the end of response surface. The first is an increase in enzymatic activity with higher initial concentrations of enzyme; possibly caused by the increase of active sites in incubation periods in which there is abundant substrate in the reaction medium; and a second behavior in which enzymatic activity is reduced as the concentration of enzyme increases, probably due to an incubation period with greater depletion of lactose in the medium, which limits the function of the enzyme. The decay of enzymatic activity with longer incubation times is also observed in Figure 3; behaviors with a similar activity profile were reported by Obón *et al*., 2000 when they used enzyme concentrations between 0.0 - 0.7 g L^{-1} and incubation times of 0 - 100 minutes when hydrolyzing milk lactose with β- galactosidase from *Kluyveromyces lactis* and by Mariotti *et al*., (2008) to evaluate the enzymatic activity of a β - galactosidase immobilized in microfiltered whey for 30 days.

3.4 Optimization

The process was optimized by maximizing the % hydrolysis of lactose and the concentration of glucose (g L^{-1}) using the multiple responses method by desirability approach. The optimum point of the process with global desirability of 0.94 was obtained by combining the following conditions: pH = 6.11; temperature = 37.20 °C; enzyme concentration = 1.36 g L^{-1} and time = 176 min. The experimental validation showed a degree of lactose hydrolysis of 84.5% and a glucose concentration of 88.63 g L^{-1} against the values predicted by the model: 88.67% and 90.64 g L^{-1}, respectively. The test was performed in triplicate.

3.5 Lactose hydrolysate

It has been prepared considering the hydrolysis process optimal conditions to produce glucose (89 g L^{-1}) and galactose (66 g L^{-1}); sweeter sugars, very soluble in water and easily digestible, as is characteristic of the hydrolysis process (Beltran and Acosta, 2012). The product presented an increase in acidity (% lactic acid) due to the acids resulting from the microbial degradation of lactose (Schmidt *et al*., 1996), and a decrease in calcium content, caused by the precipitation of phosphates of this mineral (Durham, 2009). The physicochemical parameters of the lactose hydrolysate are given in table 3.

Table 3. Composition of the lactose hydrolysate (average values ± standard deviation).

Parameters	**Lactose hydrolysate**
pH	5.63 (±0.02)
Acidity (g lactic acid $100g^{-1}$)	0.33 (±0.01)
Soluble solids (°Brix)	20.2 (±0.3)
Density (g mL^{-1})	1.100 (±0.002)
Lactose (g L^{-1})	32.0 (± 3.0)
Glucose (g L^{-1})	89 (± 8.0)
Galactose (g L^{-1})	66 (±5.2)
Protein (g $100g^{-1}$)	< 2.5
Ash (g $100g^{-1}$)	1.03 (±0.05)
Calcium (g $100g^{-1}$)	0.060 (±0.005)
Magnesium (g $100g^{-1}$)	0.020 (±0.001)
Potassium (g $100g^{-1}$)	0.3 (±0.10)
Phosphorus (g $100g^{-1}$)	0.09 (±0.01)
Color:	
L*	32.3 (±0.33)
a*	-0.5 (±0.4)
b*	15 (±1.430)

Conclusions

The hydrolytic reaction has industrial importance because sugars formed contain greater sweetness than lactose.

The techno-functional properties, the content of minerals (potassium, magnesium, and calcium) and easily absorbed energy sources such as glucose make the lactose hydrolysate an ideal product for the development of flavored drinks, which can also be used as a base in the development of hydrating drinks. Response surface methodology was effective to analyze the influence of different factors (pH, temperature, time and initial concentration of enzyme) on a nanofiltration retentate of sweet whey hydrolysis. Temperature and pH were the critical factors that affected the hydrolysis process.

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Nomenclature

C_i	Concentration of lactose in the sample without hydrolyzing (g L^{-1})
C_f	Concentration of lactose in the sample after hydrolysis process (g L^{-1})
C.V	Coefficient of variation (%)
Pa	Pascal (N/m^2)
TMP	Transmembrane Pressure (MPa)
U	Enzymatic activity (μmoles/min)
VCF	Volumetric Concentration Factor

References

Ansari, S. A., and Husain, Q. (2010). Lactose hydrolysis by β galactosidase immobilized on concanavalin A-cellulose in batch and continuous mode. *Journal of Molecular Catalysis B: Enzymatic 63*, 68-74. doi: 10.1016/j.molcatb.2009.12.010.

Asoleche. (2018). Acopio mensual de leche por la industria formal al sector primario (2017 - 2018). Disponible en:

https://asoleche.org/leche-en-cifras/. Accesado 21 octubre 2018.

Banaszewska, A., Cruijssen, F., Claassen, G. D. H., and van der Vorst, J. G. A. J. (2014). Effect and key factors of byproducts valorization: The case of dairy industry. *Journal of Dairy Science 97*, 1893-1908. doi: 10.3168/jds. 2013-7283.

Beltran, L. J., and Acosta-Cárdenas, A. (2012). Empleo de una β-galactosidasa comercial de *Kluyveromyces lactis* en la hidrólisis de lactosuero Usage of a commercial β-galactosidase from *Kluyveromyces lactis* in the hydrolysis of whey. *Hechos Microbiológicos 3*, 25-35.

Callejas, J., Prieto, F., Reyes, V. E., Marmolejo, Y., and Marzo, M. A. M. (2012). Caracterización fisicoquímica de un lactosuero: potencialidad de recuperación de fósforo. *Acta Universitaria (México) 22*, 11-18.

Chacón-Villalobos, A. (2014). Tecnologías de membranas en la agroindustria láctea. *Agronomía Mesoamericana 17*, 243. doi: 10.15517/am.v17i2.5164.

Chatterjee, G., De Neve, J., Dutta, A., Das, S. (2015). Formulation and statistical evaluation of a ready-to-drink whey based orange beverage and its storage stability. *Revista Mexicana de Ingeniería Química 14*, 253-264.

Chen, W., Chen, H., Xia, Y., Yang, J., Zhao, J., Tian, F., Zhang, H. (2009). Immobilization of recombinant thermostable β-galactosidase from *Bacillus stearothermophilus* for lactose hydrolysis in milk. *Journal of Dairy Science 92*, 491-498. doi: 10.3168/jds.2008-1618.

Cuartas-Uribe, B., Alcaina-Miranda, M. I., Soriano-Costa, E., Mendoza-Roca, J. A., Iborra-Clar, M. I., and Lora-García, J. A. (2009). Study of the separation of lactose from whey ultrafiltration permeate using nanofiltration. *Desalination 241*, 244-255. doi: 10.1016/j.desal.2007.11.086.

Dainese-Plichon, R., Schneider, S., Piche, T., and Hébuterne, X. (2014). Malabsorption et intolérance au lactose chez l'adulte. *Journal de Nutrition Clinique et Métabolisme 28*, 46-51. doi: 10.1016/j.nupar.2013.12.002.

Das, B., Roy, A. P., Bhattacharjee, S., Chakraborty, S., and Bhattacharjee, C. (2015). Lactose hydrolysis by β-galactosidase enzyme: Optimization using response surface methodology. *Ecotoxicology and Environmental Safety 121*, 244-252. doi: 10.1016/j.ecoenv.2015.03.024.

Durham, R. J. (2009). Modern approaches to lactose production. En: *Dairy-Derived Ingredients: Food and Nutraceutical Uses*. (M. Corredig, ed), Pp 103-144. Woodhead Publishing Limited, United States of America. doi: 10.1533/9781845697198.1.103.

García-Reyes, M., Beltrán-Hernández, R. I., Vázquez-Rodríguez, G. A., Coronel-Olivares, C., Medina-Moreno, S.A., Juárez-Santillán, L. F., Lucho-Constantino, C. A. (2017). Formation, morphology and biotechnological applications of filamentous fungal pellets: a review. *Revista Mexicana de Ingeniería Química 16*, (2017) 703-720.

Ghosh, M., Pulicherla, K. K., Rekha, V. P. B., Vijayanand, A., and Sambasiva, K. R. S. (2013). Optimisation of process conditions for lactose hydrolysis in paneer whey with cold-active β-galactosidase from psychrophilic *Thalassospira frigidphilosprofundus*. *International Journal of Dairy Technology 66*, 256-263.

Guerrero, C., Vera, C., Conejeros, R., and Illanes, A. (2015). Transgalactosylation and hydrolytic activities of commercial preparations of β-galactosidase for the synthesis of prebiotic carbohydrates. *Enzyme and Microbial Technology 70*, 9-17. doi: 10.1016/j.enzmictec.2014.12.006.

Hatzinikolaou, D. G., Katsifas, E., Mamma, D., Karagouni, A. D., Christakopoulos, P., and Kekos, D. (2005). Modeling of the simultaneous hydrolysis-ultrafiltration of whey permeate by a thermostable β-galactosidase from *Aspergillus niger. Biochemical Engineering Journal, 24*, 161-172. doi: 10.1016/j.bej.2005.02.011.

Jenab, E., Omidghane, M., Mussone, P., Armada, D. H., Cartmell, J., and Montemagno, C. (2018). Enzymatic conversion of lactose into galacto-oligosaccharides: The effect of process parameters, kinetics, foam architecture, and product characterization.

Journal of Food Engineering 222, 63-72. doi: 10.1016/j.jfoodeng.2017.11.015.

Kaur, G., Panesar, P. S., Bera, M. B., and Kumar, H. (2009). Hydrolysis of whey lactose using CTAB-permeabilized yeast cells. *Bioprocess and Biosystems Engineering 32*, 63-67. doi: 10.1007/s00449-008-0221-9.

Kleinhenz, M. D., and Bumgarner, N. R. (2015). Uso de los grados brix como indicador de calidad. Disponible en: http://www.agroindustria360.com/2015/04/uso-de-los-grados-brix-como-indicador.html. Accesado: 07 Febrero 2019.

Kreczmann, B., Alonso, A., Liloia, M., Zamboni, E., Cerutti, R., Baroni, D., and Poluján, D. (2015). Procesamiento del lactosuero: elaboración de lactosa y aprovechamiento de proteínas. *Tecnología Láctea Latinoamericana 87*, 44-49.

Mariotti, M. P., Yamanaka, H., Araujo, A. R., and Trevisan, H. C. (2008). Hydrolysis of Whey Lactose by Immobilized β-Galactosidase. *Brazilian Archives of Biology and Technology 516*, 1233-1240.

Nath, K., Dave, H. K., and Patel, T. M. (2018). Revisiting the recent applications of nanofiltration in food processing industries: Progress and prognosis. *Trends in Food Science and Technology 73*, 12-24. doi: 10.1016/j.tifs.2018.01.001.

Oatley-Radcliffe, D. L., Walters, M., Ainscough, T. J., Williams, P. M., Mohammad, A. W., and Hilal, N. (2017). Nanofiltration membranes and processes: A review of research trends over the past decade. *Journal of Water Process Engineering 19*, 164-171. doi: 10.1016/j.jwpe.2017.07.026.

Obón, J. M., Castellar, M. R., Iborra, J. L., and Manjón, A. (2000). β-Galactosidase immobilization for milk lactose hydrolysis: A simple experimental and modelling study of batch and continuous reactors. *Biochemical Education 28*, 164-168. doi: 10.1016/S0307-4412(99)00097-7.

Palai, T., and Bhattacharya, P. K. (2013). Kinetics of lactose conversion to galacto-oligosaccharides by β-galactosidase immobilized on PVDF membrane. *Journal of Bioscience and Bioengineering 115*, 668-673. doi: 10.1016/j.jbiosc.2012.12.014.

Paterson, A. H. J. (2011). Lactose and oligosaccharides; Lactose production, aplications. En: *Encyclopedia of Dairy Sciences*. (Fuquay, J. W. eds), Pp.196-201, Elsevier Ltda.

Pizzichini, M. (2006). *Tecnologie di Processo per il Recupero e la Valorizzazione delle Componenti del Siero di Latte*. Editorial ENEA, Italia.

Rice, G., Barber, A. R., O'connor, A. J., Stevens, G. W., and Kentish, S. E. (2011) Rejection of dairy salts by a nanofiltration membrane. *Separation and Purification Technology 79*, 92-102. doi: 10.1016/j.seppur.2011.03.022.

Sánchez, G. L., Gil, M. J., Gil, M. A., Giraldo, F. J., Millán, L. de J., and Villada, M. E. (2009). Milk serum use in a company from the Antioquian Northern region by the use of efficient microorganisms. *Producción + Limpia 4*, 65-74.

Schmidt, K. A., Stupar, J., Shirley, J. E.; and Adapa, S. (1996). "Factors affecting titratable acidity in raw milk" Kansas Agricultural Experiment Station. *Research Report*. doi: 10.4148/2378-5977.3265.

Wong, S. Y., and Hartel, R. W. (2014). Crystallization in lactose refining-a review. *Journal of Food Science 79*, 257-272. doi: 10.1111/1750-3841.12349.