Desempeño reológico de la producción de ácido láctico a partir de lactosuero utilizando la levadura *Kluyveromycesmarxianus*. Efecto de concentraciones iniciales de sustrato, inóculo y oxígeno

Rheological performance of lacticacid production from whey using *Kluyveromycesmarxianus* yeast. Effect of initial concentrations of substrate, inoculum and oxygen

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Resumen

El aprovechamiento de la lactosa presente en el lactosuero se puede fermentar para producir un compuesto de interés: ácido láctico, trayendo consigo cambios positivos en las cremerías artesanales. El objetivo de este estudio fue proporcionar información básica sobre la producción de ácido láctico a partir del lactosuero utilizando una cepa silvestre de la levadura Kluyveromycesmarxianus mediante la evaluación del efecto de la concentración inicial de sustrato, inóculo y oxígeno, mientras se investiga las propiedades reológicas de los caldos de fermentación. Los factores se evaluaron mediante la implementación de un diseño factorial 2^3 completamente aleatorizado. Las propiedades reológicas de los caldos se determinaron en estado estacionario usando un reómetro AntonPaarMCR 301. Los resultados obtenidos permitieron concluir que los factores que más influyen en la producción de ácido láctico son la concentración inicial de sustrato, inóculo y la interacción entre los factores inóculo y oxígeno. Los caldos de fermentación presentaron un comportamiento no newtoniano con características dilatantes, ajustando adecuadamente el modelo reológico de Herschel-Bulkley a los datos experimentales (R^2 ˃0.98). La viscosidad aparente de los caldos de fermentación aumentó ligeramente debido al crecimiento de la levadura, asociando los parámetros de esfuerzo de corte inicial e índice de comportamiento reológico al aumento de la biomasa, expresándose mediante un modelo exponencial y lineal respectivamente. Mientras que el parámetro coeficiente de consistencia mostró un comportamiento antagónico, es decir, disminuyó con el aumento de la biomasa.

Palabras claves: *Lactosuero, Kluyveromycesmarxianus, ácido láctico, reología.*

Abstract

The use of the lactose present in the whey can be fermented to produce a compound of interest: lactic acid, bringing positive changes to artisanal creameries. The goal of this study was to provide basic information on the production of lactic acid from whey using a wild strain of the yeast Kluyveromycesmarxianus by evaluating the effect of the initial concentration of substrate, inoculum, and oxygen while investigating the Rheological properties of the fermentation broths. The factors were evaluated through the implementation of a completely randomized 2^3 factorial design. The rheological properties of the broths were determined in steady state using an Anton PaarMCR 301 rheometer. The results obtained allowed concluding that the factors that most influence the production of lactic acid is the initial concentration of substrate, inoculum and the interaction between the factors inoculum and oxygen. The fermentation broths presented a non-Newtonian behavior with dilatant characteristics, fitting the Herschel-Bulkley rheological model adequately to the experimental data (R^2 ˃0.98). The apparent viscosity of the fermentation broths increased slightly due to the growth of the yeast, associating the parameters of yield stress and rheological behavior index to the increase of the biomass, expressed by an exponential and linear model respectively. While the parameter coefficient of consistency showed an antagonistic behavior, i. e. decreased with the increase of biomass.

Keywords: *Whey, Kluyveromycesmarxianus, lactic acid, rheology.*

1 Introduction

Whey is defined as the liquid substance obtained from milk that was not precipitated by the rennet or by acids (organic or mineral) during the process of making the cheese (Prazeres y col., 2012), or any other coagulateddairy products obtained after milk is curdled and strained (Khairey col., 2018). Approximately 90% of the milk used in the cheese industry is converted to whey retaining about 55% of total milk nutrients, of which the most abundant are lactose (0.18-60 g/L), soluble proteins (1.4-33.5g/L), lipids (0.08-10.58 g/L)(Bacenetti y col.,2018). This large nutrient content generates approximately 30 to 50 gBOD and 60 to 80 gCOD for each liter of whey, with lactose being the main component of solids that contributes to high BOD and COD (Risner y col.,2018, Das y col., 2013, 2016, Parra-Huertas 2009). In Mexico, the national production of milk in the year 2017 was estimated at 11,969,879 liters (Sagarpa-Siap,2017) of which the state of Veracruz produced approximately 745,349 liters (Sagarpa-Siap,2017). According to Monteros-Lagunés y col., 2009, 56% of the milk production in the state of Veracruz is destined to the production of cheese, i.e., about 417,395 liters are used in the production of cheese and from this industry around 375,655 liters of whey is disposed of as effluent to the environment, which is equivalent to a polluting power of sewage produced by 169,044 people, taking as a reference that every 1000 liters of whey has a polluting power to waste water produced by 450 people (Padíny y col., 2006).

A viable alternative to this environmental problem is the application of biotechnological processes which seek to take advantage of organic waste, as a source of cheap raw material, rich in carbohydrates for specialized microorganisms to synthesize value-added compounds. A microorganism widely used in the bioprocessing of whey is the yeast *Kluyveromycesmarxianus*. The genus *Kluyveromyces* was named in honor of the Dutch microbiologist Albert Jan Kluyver in 1956. In 1970, the genus comprised 21 species, but a maximum parsimony analysis of genomic sequences in 2003 led to its reorganization so that today it comprises only six species, of which, the most widely used species in the genus *Kluyveromyces* are *Kluyveromyceslactis* and *Kluyveromycesmarxianus* (formerly *Kluyveromycesfragilis*). Unlike the others, these two species have the ability to use xylose, xylitol, cellobiose, lactose, and arabinose, in both solid and liquid media (Spohner y col., 2016). The *Kluyveromycesmarxianus* yeast has been isolated from fruit, cheese, yogurt, milk, human and animal injuries, has characteristics that give it an advantage in biotechnological applications such as the ability to assimilate complex sugars (lactose, galactose, sucrose, raffinose, and inulin), growth rates fast, it is a thermo tolerant yeast grows at temperatures between 20-30 but has the ability to grow at temperatures up to 52°C and at a pH of 4.5-5, obtaining with it ethanol, glycerol, enzymes, unicellular proteins and lactic acid (Saini y col., 2017, Das y col., 2016,Güneşer y col., 2016, PadínyDíaz, 2006).

Lactic acid is one of the 12 most promising value-added basic components that can be obtained from fermentation of sugars and used for the production of several specialized chemical products such as: food additives, chiral synthesizers, lactate ester solvents, acrylic acids, propylene glycol, polyesters, being its most common application, the synthesis of polylactic acid (Kwan y col., 2018, de Oliveira y col., 2018,Bae y col., 2018).In 2012, the amount of lactic acid production worldwide was estimated at 259 thousand metric tons, compared to other years (130-150 thousand metric tons per year, which is estimated to reach367 thousand metric tons by 2017 (Upadhyaya y col., 2014). This increase in its production is due to the growing production of ecological and renewable resources, such as biodegradable plastic produced from polylactic acid, instead of petrochemical products(Germec y col., 2018).

Engineering design and scale-up of fermentation process require an understanding of the rheological properties of culture broths, this because, during the course of fermentation, large changes in the rheological properties of the culture broths may occur, associated with the concentration of substrate, biomass and geometry of the microorganisms and in some cases the concentration of cellular metabolites. These changes imply an increase or decrease in the apparent viscosity and loss of the Newtonian characteristics of the fluid, directly affecting transport processes such as heat and mass transfer, dispersion of products and byproducts, pH adjustment and increase in fermentation time.

The purpose of this study was to provide basic information on the production of lactic acid from whey using a wild strain of the yeast *Kluyveromycesmarxianus* by evaluating the effect of the initial concentration of substrate, inoculum, and oxygen, while investigating the rheological properties of the culture broths to improve the production in a later study.

2 Materials and Methods

2.1 Microorganism

The wild strain of the yeast *Kluyveromycesmarxianus* was obtained from agave musts. It was isolated and identified by the PCR-RFLP method of the ITS-5.8S region and maintained at -80°C with 30% glycerol. Subsequently, it was propagated in Petri dishes containingYPDA solid growth medium (Yeast Extract, Casein Peptone, Dextrose, and Agar). Petri dishes were incubated for 3 days at a temperature of 30°C, then stored until used.

2.2 Whey

Whey used was provided by dairy Plauchú S.A de C.V., located in the city of Camerino Mendoza, Veracruz, Mexico. The whey samples were characterized by measuring pH, density, total solids, volatile solids, nitrogen, proteins, COD_T, COD_S, lactose, fat and color. The whey used in the fermentation series was adjusted to pH 4.5 with 10N HCl to apply a thermo-acid treatment at 115°C in an autoclave (Yamato SM 300) for 15 minutes for deproteinization. Then it was cooled for 24 hours at 4°C, then decanted to remove the precipitated proteins and filtered on Whatman No. 40 filter paper. Finally, the pH was adjusted to 6.5 with 10N NaOH and sterilized in an autoclave at 115°C. for 15 minutes.

2.3 Preparation of the inoculum

It was prepared by passing three inoculating loops of the Petri dishes to a 250 ml Erlenmeyer flask containing 100 ml of the YPD synthetic medium, allowed to incubate for 12 hours with constant agitation at a temperature of 30°C. After 12 hours, a pass was made to the pre-inoculum taking 10 ml of the yeast suspension and sowing it in an Erlenmeyer flask containing 90 ml of whey previously thermodenatured, filtered and with a pH of 6,5. Subsequently, the pre-inoculum was incubated at 30°C for 16 hours, to guarantee the exponential phase of growth. The volume of yeast suspension that was inoculated to have the desired yeast concentration per milliliter was calculated using equation 1. The yeast count per milliliter was obtained by using the Neubauer chamber.

$$C_1V_2 = C_2V_2 \qquad (1)$$

where C_1 (yeast/ml) is the concentration of yeast obtained by the Neubauer chamber count, V_1 (ml) is the volume of the inoculum, C_2 (yeast/ml) is the yeast concentration/ml that is desired, V_2 (ml) volume of the medium to be propagated.

2.4 Fermentations

Volumes of 0.6 and 0.9L of thermo denatured whey were inoculated with initial concentrations of: lactose of 35 and 50 g/L and inoculum of $3x10^6$ and $10x10^6$ yeast/ml at a temperature of 36°C. Subsequently, it was left to ferment for 72 hours. For the development of the fermentation series, a completely randomized design of factorial type 2^3 was used to evaluate the initial concentrations of the substrate, inoculum and oxygen as main factors and their combined effect, in order to determine which factor, it significantly influences the production of lactic acid. The factors were evaluated in 2 levels (low and high) where the variable of response is the maximum production of lactic acid. 8 fermentations were made with its replica giving a total of 16 fermentations. The data obtained experimentally were analyzed statistically in the software StatgraphicsCenturión XVI. The matrix of the design of experiments is presented in Table 1.

2.5 Rheological properties of fermentation broths

The rheological properties of the fermentation broths were determined at steady state using a rotational rheometer Anton PaarMCR 301 with a vane geometry ST22-4V-40. The fitting rheological model was obtained by the RheoPlus /32 V2 software. 81. The type of fluid was established based on the ratio of shear stress and shear rate, determining its behavior with respect to the Herschel-Bulkley three-parameter rheological model (eq.2).

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad (2)$$

where τ (Pa) shear stress, τ_0 (Pa) yield stress (minimum force required for the fluid to move and is related to the internal structure of the material to be broken), K (Pa s^n) coefficient consistency (proportionality constant indicating the degree of non-Newtonian viscosity), $\dot{\gamma}$(s^{-1}) shear rate and, n(-) index of rheological behavior (indicating the proximity of the fluid to a Newtonian fluid; For a Newtonian fluid n =1, for a dilatant fluid n>1 and pseudoplastic fluid n<1).

Table 1. Factorial design matrix 2^3

coded factors					Levels	
Runs	A	B	C		Bajo (-1)	Alto (1)
1	-1	-1	-1	A: Lactose (g/L)	35	50
2	1	-1	-1			
3	-1	1	-1	B: Inoculum ($x10^6$ yeast/ml)	3	10
4	1	1	-1			
5	-1	-1	1	C: Oxygen (ml)	200	500
6	1	-1	1			
7	-1	1	1			
8	1	1	1			

2.6 Analysis methods

The pH: was determined with a Jenway potentiometer model 3310; the density: it was determined with a digital density meter Anton Paar DMA-4500; COD_T: the content of total organic load was determined by the method 5220D Chemical Oxygen Demand/Standard Methods (APHA 2005); COD_S: the content of soluble organic load was determined by centrifuging the whey sample at 3500 rpm for 10 minutes. Subsequently, the supernatant was used to determine the chemical oxygen demand by the method 5220D Chemical Oxygen Demand/Standard Methods (APHA2005); Total Solids: the total solids content was determined by the method 2540 B Total Solids Dried at 103-105°C/Standard Methods (APHA2005); Volatile Solids: the volatile solids content was determined by the method 2540 E Fixed and Volatile Solids Ignited at 550°C/Standard Methods (APHA2005); Total Nitrogen: the nitrogen content was determined under the method 16,036 Kjeldahl (A.O.A.C.1980); Proteins: the protein content was determined as the percentage of nitrogen multiplied by the factor of 6.38, method 16,036 Kjeldahl (A.O.A.C.1980); Lactose: the lactose content was determined with the phenol-sulfuric method of Dubois y col., 1956 at a wavelength of 490 nm.; Fats: the fat content was determined by the method 5520D Soxhlet extraction/Standard Methods (APHA2005); Color: the color was determined through of a HunterLab colorimeter; Biomass: the biomass concentration was determined through of the dry weight method (Rhodes y col.,1997); Lactic acid: the concentration of lactic acid was determined by the chloride-ferric method (Palacios y col., 2009).

3 Results and Discussion

The purpose of this study was to provide basic information on the production of lactic acid from whey using a wild strain of the yeast *Kluyveromycesmarxianus* by evaluating the effect of the initial concentration of substrate, inoculum, and oxygen while investigating the rheological properties of the culture broths to improve the production in a subsequent study. Initially, was carried out the physicochemical characterization of the whey (Table 2), finding similar values for the sweet whey previously published by other authors (Araujo y col., 2013, Callejas-Hernández y col., 2012). The whey samples presenteda yellowish green color, according to Prazares y col., 2012, this color is caused by the presence of riboflavin (vitamin B2).The pH value indicates that is a whey type sweet i.e. is produced by the enzymatic coagulation of casein through the action of renin (Araujo-Guerra y col., 2013,Callejas-Hernández y col., 2012, Parra-Huertas 2009).The density of the whey evaluated at 1.028 g/cm^3 is slightly higher than that reported by Callejas-Hernandez y col., 2012 and corresponds more to the density of fresh milk whose value ranges from 1.028 -1.034 g/cm^3 to 15°C. The value of the organic load (87.66 $gCOD_T$/L) indicates the negative effect of this by-product of the cheese industry on the environment when it is discharged into the ground or in water bodies. Values have been reported ranging from 40 to 50 g/L of lactose present in the sweet whey, in the present study a value of 35.46 g/L of lactose was found which is equivalent to 55% of solids present in the whey, value higher than reported by Nishanthi y col., 2016. The lactose content is followed by fats and the remaining proteins.

Table 2. Physicochemical characterization of whey

Parameters	Valor
pH (-)	$6{,}55^{\pm 0{,}119}$
Density (g/cm^3)	$1{,}028^{\pm 0{,}0265x10^{-3}}$
Total solids (g/L)	$64{,}48^{\pm 0{,}119}$
Volatile solids (g/L)	$62{,}74^{\pm 0{,}0850}$
Total nitrogenKjeldahl(%)	$0{,}16^{\pm 0{,}0164}$
Proteins (%)	$1{,}016^{\pm 0{,}105}$
Lactose (g/L)	$35{,}46^{\pm 0{,}286}$
COD_T(g/L)	$87{,}66^{\pm 1{,}802}$
COD_S(g/L)	$75{,}52^{\pm 1{,}136}$
Fat (g/L)	$3{,}79^{\pm 0{,}0415}$
Color	
L	54,56
a	−0,42
b	9,42

The series of fermentations were carried out using a wild strain of the *Kluyveromycesmarxianus* yeast at constant temperature (36°C), for which volumes of 0.6 and 0.9 L of whey were inoculated at initial concentrations of 35 and 50 g/L of lactose. and with initial concentrations of 3x10^6 and 10x10^6 yeasts/ml. In response, it was found that there was a higher production of lactic acid at high levels of lactose concentration (50g/L), reaching a maximum production of 8.35 ± 0.24 g / L of lactic acid (Fig. 1).

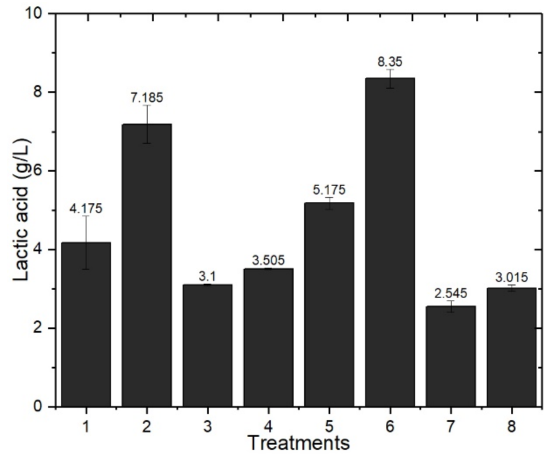

Fig. 1. Lactic acid concentration in each treatment

Recent investigations have reported a production of lactic acid of 130 g/L of L-lactic acid and 122 g/L of D-lactic acid from recombinant strains of *Kluyveromycesmarxianus* yeast obtained by metabolic engineering, using as a source of carbon Jerusalem artichoke tuber powder (Bae y col., 2018).Using whey as a source of carbon and lactic acid bacteria a lactic acid production of: 19.05 g/L with *Lactobacillus bulgaricus*, 18.36 g/L with *Pedioccocusacidilactici* and 12.24 to 17.34 g/L with *Pedioccocuspentosaceus* was reached (Juodeikiene y col., 2016). In comparison Plessasy col., 2008 working on lactic fermentations under similar conditions (37°C and constant agitation) with whey elaborated from a commercial milk with an initial concentration of 50-52 g/L of lactose, obtained a maximum production 8.8 ± 0.2 g/L of lactic acid using the yeast *Kluyveromycesmarxianus*. The data obtained in the development of the fermentations were analyzed statistically by the software Statgraphics Centurion XVI, generating the Pareto chart (Fig. 2) to evaluate the influence of the initial concentration of substrate, inoculum, and oxygen in the production of lactic acid. Figure 2 shows each of the evaluated factorsand their interactions, estimated in descending order of significance with a 95.0% confidence level. The length of each bar is proportional to the standardized effect, which is the estimated effect divided by its standard error, which is equivalent to calculating the t-statistic for each effect. The bars of inoculum, lactose, and the lactose-inoculum, lactose-oxygen interactions extend beyond the vertical line indicating that these factors and interactions have a significant effect on the production of lactic acid.

The main factors were plotted to analyze in more detail the way in which these factors influence the production of lactic acid. It can be seen that the concentration of lactose (Fig. 3) has a positive main effect, that is, an increase in the concentration of lactose displaces the production of lactic acid upwards, this is in agreement with that reported by Plessas y col., 2008, the greater the initial concentration of lactose in the fermentation, the higher will the concentration of lactic acid, the yield and the productivity. While the inoculum size of *Kluyveromycesmarxianus* (Fig. 3) presents a negative main effect, where the low level of this variable causes an increase in the production of lactic acid.Saini y col., 2017 working on alcoholic fermentations from whey and an of *Kluyveromycesmarxianus* strain, found that the maximum production of ethanol (71.8 ± 0.30 g/L) was found with an inoculum level of 6% (v/v) instead of 8% (v/v) (67.2 ± 0.15 g/L). This confirms that the size of the inoculum has a significant effect during the development of fermentations based on whey, finding the best results at low levels of inoculum. On the other hand, it is observed that for the proposed values in the concentration of oxygen it is not very significant since it has very little variability in the production of lactic acid.

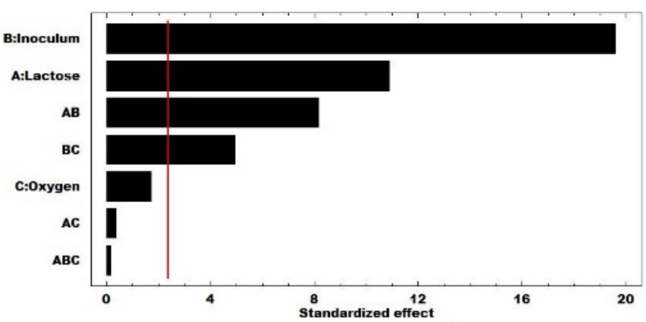

Fig. 2. Pareto chart. Standardized effects

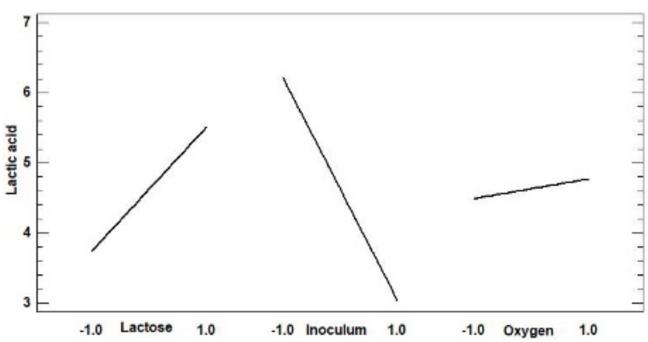

Fig. 3. Main effects: lactose, inoculum and oxygen

The straight, non-parallel lines in the BC interaction (Fig. 4) indicate a strong correlation between these two factors. Obtaining the best result when combining B (initial concentration of inoculum) in the low level and C (initial concentration of oxygen) in the high level. This strong interaction exists because a considerable initial concentration of oxygen is needed for the biomass to start growing. Padín y col., 2006 mention that if the anaerobic conditions begin soon (low level) the density of the yeast population will not be high enough to obtain a higher yield.The rest of the interactions do not have strong effects so they do not significantly influence the production of lactic acid. A regression model was generated, represented by equation 3 with an adjustment of 83% according to the ANOVA (Table 3).

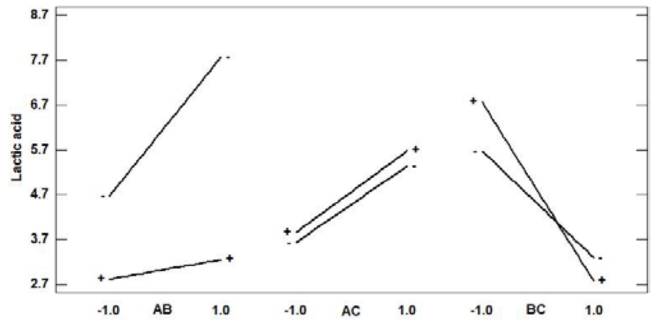

Fig. 4.Significant interaction. Inoculum- oxygen

$$\text{LA} = 4,625 - 1,584\,inoculum + 0,876\,lactose + 0,146\,oxygen \quad (3)$$

Table 3. ANOVA model for the production of lactic acid

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	P-Value	R^2
Regression (Model)	3	53,2241	17,7414	20,39	0,0001	0,8360
Residue	12	10,4393	0,86994			
Total	15	63,6634				

During the course of the fermentations the culture broths of the yeast *Kluyveromycesmarxianus*exhibited a non-Newtonian behavior with dilatant characteristics (Fig. 5). According to Ibarz y Barbosa-Cánovas,2003 the dilatant behavior is due to the presence of particles of different sizes and different shapes that are closely packed making the flow more difficult as the pressure increases, this because when increasing the shear rate, the long and flexible particles stretch. Other authors have reported a non-Newtonian behavior with pseudoplasticcharacteristicsin fungal fermentations, in other words, the apparent viscosity of the culture broths decrease with the increase of the shear rate, adjusting the power law model to the experimental data, where the variation of the apparent viscosity is strongly related to biomass and morphology (Osadolor y col., 2018,Alves da Cunha y col., 2012, Gupta y col., 2007, Lim y col., 2006, Goudar y col., 1999, Landon y col., 1993).For this study, the model that best described the phenomenological behavior of the fermentation broths was the rheological model of Herschel-Bulkley (Table 4) when presenting an adequate adjustment $R^{2}>0.98$, where the yield stress parameter (τ_0) makes evident the presence of solid particles (biomass, lactose, remnant proteins and mineral salts).The effect of the increase of the biomass on the rheological properties of the fermentation broths was obtained from the study of the dependence of the three parameters of the Herschel-Bulkley model (τ_0, K, n) as a function of the biomass concentration (X).

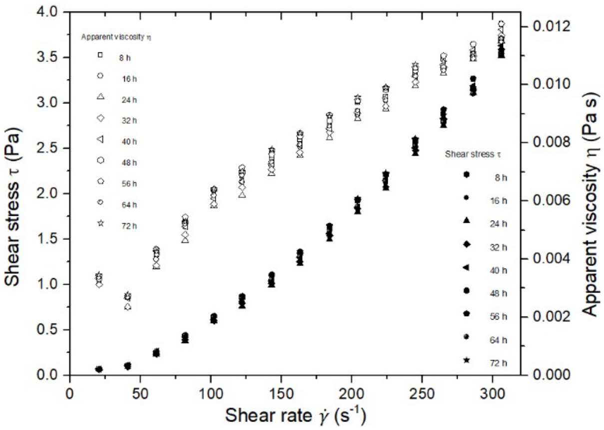

Fig. 5. Shear stress and apparent viscosity of *Kluyveromycesmarxianus* fermentation broth in whey at different time of fermentation

Table 4. Experimental models of *Kluyveromycesmarxianus* fermentation broths in whey at different times

Time (h)	Experimental models	R^2
8	$\tau = 0.07394 + 8.9481x10^{-5}\dot{\gamma}^{1.8486}$	0.99143
16	$\tau = 0.068514 + 8.8221x10^{-5}\dot{\gamma}^{1.8507}$	0.99174
24	$\tau = 0.082881 + 7.7588x10^{-5}\dot{\gamma}^{1.8696}$	0.99216
32	$\tau = 0.079301 + 8.8135x10^{-5}\dot{\gamma}^{1.8503}$	0.99092
40	$\tau = 0.020236 + 1.3493x10^{-4}\dot{\gamma}^{1.7848}$	0.98659
48	$\tau = 0.022892 + 1.4397x10^{-4}\dot{\gamma}^{1.7749}$	0.98469
56	$\tau = 0.034251 + 1.2856x10^{-4}\dot{\gamma}^{1.7918}$	0.98592
64	$\tau = 0.023632 + 1.3406x10^{-4}\dot{\gamma}^{1.7876}$	0.98539
72	$\tau = 0.019989 + 1.5201x10^{-4}\dot{\gamma}^{1.7663}$	0.98396

In Figure 6 it can be seen that the yield stress (τ_0) and the rheological behavior index (n) tend to increase as a function of the biomass concentration, which indicates the non-Newtonian behavior with dilatant characteristics of the fermentation broths, the consistency coefficient (K) showed an antagonistic behavior, i. e. decreased with the concentration of the biomass. It has been reported an opposite behavior in filamentous fermentations where the coefficient of consistency increases and the behavior index remains constant (Lim y col., 2006), in this case, the consistency coefficient is used as the only indicator of the viscosity of the fermentation, since the value of the rheological behavior index is affected by the operational design to a lesser extent than the value of *K*.Other authors such as Goudar y col., 1999,Badino y col., 1999, have modeled the consistency coefficient in function of biomass concentration, fitting power type models to the experimental data, while the yield stress parameter has been correlated as a function of biomass concentration with linear and potential type models (Blanch yBhavaraju, 1976). But authors as Osadolor y col., 2018, PamboukianyFacciotti, 2005 have modeled the consistency coefficient together with the index of rheological behavior as a function of the biomass, adjusting power type models to the experimental data. In the present study the consistency coefficient and the rheological behavior index correlated with the biomass concentration by means of a linear model (eq 4 and 5 respectively), while a model of the exponential type was adjusted to the experimental data of the yield stress (eq. 6).

$$K = 0.0002 - 4x10^{-5}X \qquad R^2 = 0.6175 \qquad (4)$$

$$n = 1.6615 + 0.053X \qquad R^2 = 0.6133 \qquad (5)$$

$$\tau_0 = 0.0033e^{0.8794X} \qquad R^2 = 0.7164 \qquad (6)$$

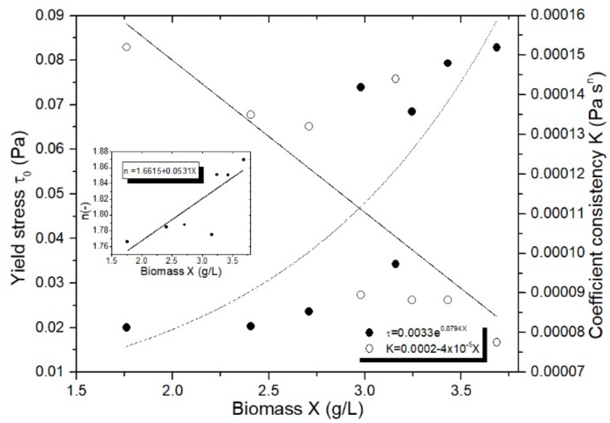

Fig. 6. Relationship between the yield stress, consistency coefficient, rheological behavior index and biomass concentration

4 Conclusions

The physicochemical characterization showed that the whey is a by-product of high organic load, where the compounds that stand out are the lactose followed by fats and proteins. Its biotechnological use from the yeast *Kluyveromycesmarxianus* is a viable alternative since it offers advantages such as low cost of raw material, a product of economic interest such as lactic acid and contributes to the treatment of liquid waste. The results obtained in the series of fermentations allowed us to conclude that whey is an adequate medium for the production of lactic acid from the yeast *Kluyveromycesmarxianus*, where the most important factors are the initial concentration of lactose and inoculum. The higher the concentration of lactose and the lower the concentration of inoculum, the higher the production of lactic acid. The interaction between oxygen and inoculum showed that a considerable initial concentration of oxygen is needed for the biomass to begin to grow, that is, the density of the yeast population is high to obtain a higher yield. The fermentation broths presented a non-Newtonian behavior with dilatant characteristics. The rheological properties of the fermentation broths were influenced by the concentration of biomass, in other words, the increase in the concentration of the biomass causes an increase in the apparent viscosity of the fermentation broths, finding correlations of the linear type and potential in function of the concentration of biomass with the rheological parameters of the Herschel-Bulkley model.

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