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Valorization of acid whey through processing as a substrate for lactic fermentation

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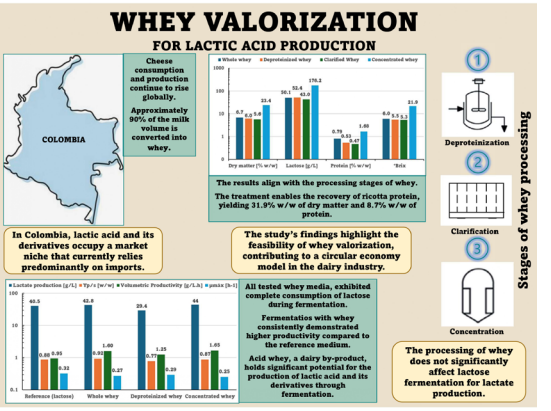
Graphical abstract



Keypoints:

1. Whey processing reduces equipment fouling and transport/storage volumes, resulting in significant cost savings and environmental benefits.
2. Processed whey improves fermentation productivity, enhancing overall efficiency in dairy production facilities without compromising key components.
3. Whey valorization supports sustainable lactic acid production and by-product recovery, contributing to reduced waste and increased economic value.

Abstract
In Colombia, whey substrate has great potential to produce lactic acid and its derivatives by fermentation. Moreover, it could contribute to the development of an emerging industry that takes advantage of by-products from the dairy industry and covers a market niche that today depends mostly on imports. In this research work, a scheme of valorization of acid whey (result of the production of string cheeses), based on stages of deproteinization, clarification, and concentration, for use in lactic fermentation was studied. The results obtained in this study show a reduction of 40 % of the protein (recovered for other uses), while concentrated whey was obtained up to a Volumetric Concentration Factor (VCF) of 4, increasing the concentration of lactose and other nutrients. Fermentations were carried out with whey: whole (untreated), deproteinized and concentrated/diluted, all at a VCF of 1, to evaluate the effect of the degree of processing on the performance of the bioprocess. Fermentations with lactose reagent grade were made as references. All the evaluated media presented complete consumption of lactose, with product/substrate yields ($Y_{p/s}$) between 0.77 ± 0.03 g/g and 0.92 ± 0.03 g/g for whey media, and 0.88 ± 0.04 g/g for the reference medium. In general, it was observed that fermentations with whey reached higher productivity (between 1.25 ± 0.21 and 1.65 ± 0.35 g/L/h) compared with 0.95 ± 0.35 g/L/h for the reference medium. The statistical analysis, conducted using Tukey's test, shows that the processing stages do not significantly affect the conversion of whey to lactate.

Keywords: *By-product; Dairy industry; Lactic acid; Processing scheme; Substrate.*

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Valorización de lactosuero ácido mediante procesamiento para su uso como sustrato en la fermentación láctica

Resumen
En Colombia, el lactosuero tiene un gran potencial para producir ácido láctico y sus derivados mediante fermentación. Además, podría contribuir al desarrollo de una industria emergente que aproveche los subproductos de la industria láctea y cubra un nicho de mercado que hoy en día depende principalmente de importaciones. En este trabajo de investigación, se estudió un esquema de valorización del lactosuero ácido (resultado de la producción de quesos hilados), basado en etapas de desproteinización, clarificación y concentración, para su uso en la fermentación láctica. Los resultados obtenidos en este estudio muestran una reducción del 40 % de la proteína (recuperada para otros usos), mientras que se obtuvo lactosuero concentrado hasta un Factor de Concentración Volumétrica (VCF) de 4, aumentando la concentración de lactosa y otros nutrientes. Se realizaron fermentaciones con lactosuero: entero (sin tratar), desproteinizado y concentrado (diluido a un VCF de 1 para la fermentación), para poder evaluar el efecto del grado de procesamiento en el rendimiento del bioproceso. Las fermentaciones con lactosa de grado reactivo se realizaron como referencia. Todos los medios evaluados presentaron un consumo completo de lactosa, con rendimientos de producto/sustrato ($Y_{p/s}$) entre 0,77 ± 0,03 g/g y 0,92 ± 0,03 g/g para los medios con lactosuero, y 0,88 ± 0,04 g/g para el medio de referencia. En general, se observó que las fermentaciones con lactosuero alcanzaron una mayor productividad (entre 1,25 ± 0,21 y 1,65 ± 0,35 g/L/h) en comparación con 0,95 ± 0,35 g/L/h para el medio de referencia. El análisis estadístico, realizado mediante la prueba de Tukey, muestra que las etapas de procesamiento no afectan significativamente la conversión de lactosuero a lactato.

Palabras clave: *Subproducto; Industria láctea; Ácido láctico; Esquema de procesamiento; Sustrato.*

Valorização de soro de leite ácido através do processamento para seu uso como substrato na fermentação lática

Resumo
Na Colômbia, o soro de leite tem um grande potencial para produzir ácido lático e seus derivados por meio de fermentação. Além disso, poderia contribuir para o desenvolvimento de uma indústria emergente que aproveita os subprodutos da indústria de laticínios e atende a um nicho de mercado que hoje depende principalmente de importações. Neste trabalho de pesquisa, foi estudado um esquema de valorização do soro de leite ácido (resultado da produção de queijos hilados), baseado em etapas de desproteinização, clarificação e concentração, para seu uso na fermentação lática. Os resultados obtidos neste estudo mostram uma redução de 40 % da proteína (recuperada para outros usos), enquanto o soro concentrado foi obtido com um Fator de Concentração Volumétrica (VCF) de 4, aumentando a concentração de lactose e outros nutrientes. Foram realizadas fermentações com soro: integral (não tratado), desproteinizado e concentrado/diluído, todos com VCF de 1, para avaliar o efeito do grau de processamento no desempenho do bioprocesso. As fermentações com lactose de grau reagente foram feitas como referência. Todos os meios avaliados apresentaram consumo completo de lactose, com rendimentos de produto/substrato ($Y_{p/s}$) entre 0,77 ± 0,03 g/g e 0,92 ± 0,03 g/g para os meios com soro de leite, e 0,88 ± 0,04 g/g para o meio de referência. Em geral, foi observado que as fermentações com soro de leite atingiram maior produtividade (entre 1,25 ± 0,21 e 1,65 ± 0,35 g/L/h) em comparação com 0,95 ± 0,35 g/L/h para o meio de referência. Além disso, verificou-se que as etapas de processamento não afetaram significativamente a conversão do soro de leite em lactato. A análise estatística, realizada por meio do teste de Tukey, mostra que as etapas de processamento não afetam significativamente a conversão de soro de leite em lactato.

Palavras-chave: *Subproduto; Indústria láctea; Ácido lático; Esquema de processamento; Substrato.*

Introduction

Cheese is the second dairy product in terms of dairy solids content, and its consumption is expected to continue rising globally [1]. Its production generates a significant amount of whey, corresponding to approximately 90 % of the milk volume used, in which about 55 % of milk nutrients are retained by whey, primarily lactose (4.5-5 %w/v) [2]. Such a generation implies economic losses and a serious environmental impact when it is not given an adequate disposition. In Colombia, whey is used in animal nutrition and the human food industry for meat, bakery, and moisturizing beverage applications [3].

The high availability of lactose and the presence of other nutrients that stimulate microbial growth makes whey a substrate with interesting potential for producing various compounds through fermentation, thereby increasing its value as a by-product [4]. One of these alternatives is the production of lactic acid, a product with an attractive market nationally and internationally, used as a raw material in the food, leather and textile, pharmaceutical, and biodegradable plastics industries [5], with annual global production of lactic acid in the order of 490,000 tons per year [6]. About 75 % of its production is concentrated in the companies Galactic© (Belgium), Carbion© (Netherlands), Cargill Inc. © (USA), and Archer Daniels Midland Co. © (USA) [7]. Similarly, lactic acid salts (lactates), produced during fermentation by controlling pH, are of industrial interest [6]. The Departamento Administrativo Nacional de Estadística (DANE) notes an important consumption of lactic acid and sodium and calcium lactates, with almost zero production in Colombia [8]; so, it is important to promote its production, considering the high demand and availability of raw material.

Lactic acid is one of the smallest optically active molecules in nature, with the isomers (D-) and (L+) [9]. In the food industry, lactic acid (L+) is the most widely used; its (D-) form can be toxic in the human organism at certain concentrations [10]. This compound can be obtained chemically, but in a mixture of its two isomers (approximately in equal proportion), which is its main disadvantage, while by biotechnological route, it is possible to obtain optically pure lactic acid; depending on the microorganism, the substrate and the conditions arranged within the fermenter [11].

Certain pretreatment stages are necessary for the biotechnological conversion of whey to lactic acid. In this sense, the literature does not report the study of the effect of these key stages of the process on the performance of lactic fermentation. Reports only focuses on laboratory-scale studies about the denaturation (thermal or acidic) of whey proteins to induce their precipitation [12], and small-scale filtrations (with filter paper) to separate them [13]. These pretreatments are made to minimize the foaming capacity of the medium, in addition, to recovering this fraction that can be used in the formulation of nutritional supplements or processed to be used as a low-cost nitrogen source in fermentation processes [14]. Moreover, later stages of concentration and drying can be used to improve the stability and storage of whey by reducing its volume.

For the denaturation stage, Vázquez-Puente *et al*. [13] defined the best-operating conditions for the separation: pH of 4.4, a temperature of 93 °C, and a time of 40 min. The concentration of whey was performed by falling film vacuum evaporation, a technique that favors the elimination of water by evaporation at moderate temperatures. Muvdi-Nova *et al*. [15] concentrated microfiltered whey using this technique, with an evaporation temperature between 65 and 70 °C, and pressure of 20 kPa, reaching a Volumetric Concentration Factor (VCF) of approximately 3.6 (concentrates up to 18 °Brix). Other studies such as that of Tanguy *et al*. [16] reached VCF up to 8.6 in a falling film evaporator with a larger evaporation surface.

To obtain lactic acid L (+) by fermentation, a wide variety of microorganisms has been studied, highlighting the acidic bacteria (BAL) belonging to the genera *Bacillus*, *Lactobacillus*, *Lactococcus*, *Enterococcus* and *Streptococcus* [17]. Within the bacteria of the genus *Lactobacillus*, it is possible to select a homofermentative strain producing lactic acid L (+). Its main disadvantage is the strong inhibition in its growth and production, at a pH less than 5 [18]. This implies the addition of neutralizing agents, an aspect that makes more difficult the subsequent recovery of lactic acid; this has been the main limiting factor of its production at an industrial level using these microorganisms [6]. However, as mentioned before, the salts generated in the neutralization are a product of industrial interest, which could be attractive from the economic perspective, considering lower costs associated with their separation.

The pH control during the process is very important to avoid microorganism inhibition. In the study of Ríos-Castro [19], fermentations were carried

out without pH control, and it was observed that fermentation production stopped at 18 h, reaching a low yield of 0.25 g lactic acid/g lactose. Similarly, in the study of García *et al*. [20], a yield of 0.53 g lactic acid / g lactose was achieved in 21 h, using *Lacticaseibacillus casei* ATCC 393.

The objective of this study was to analyze the effect of the proposed processing scheme for whey (deproteinized and concentrated), on its conversion to lactate via fermentation. This analysis compared its performance with commercial fermentation media, focusing on microbial growth, lactose consumption, and product formation. The comparison was based on the estimated experimental kinetic parameters.

Materials and methods

Stages of the whey processing: Protein precipitation was carried out in an industrial boiling pan of 150 L of total capacity, processing a volume of 70 L of acid whey from the production of stretched-curd cheeses in the SENA C.A.S.A. Piedecuesta, Santander. In it, the whey was acidified up to a pH of 4.4 by adding commercial lactic acid, keeping temperature at 93 °C, for 40 min [13]. Subsequently, the two phases obtained, "ricotta" on the surface and whey on the bottom, were separated by draining the latter and collecting the "ricotta" on canvas. The deproteinization yield (γd) was calculated using Equation 1.

$$\gamma d = \frac{kg\ of\ wet"ricotta"}{L\ of\ processed\ whey} \qquad (1)$$

Clarification was performed by filtration in filter press, using as a filter medium drill type of 70 threads/warp and 42 threads/weft, to separate the protein insolubilized in the precipitation stage, working with batches of 20 L, at room temperature and pressure of 172.4 kPa. It should be noted that these tests were performed by reducing the load of the insolubilized protein; whey filtration with full insolubilized-protein load, induced a rapid blocking of the filter media, stopping the process in a short time. Both the cake and the filtrate were characterized in %dry matter, %protein, %fat, %ash, %carbohydrates, °Brix, and turbidity; the last two for filtering only. The determination of filtration resistances was performed by correlating the experimental data of cumulative filtration volume (V) and filtration time (t) and adjusting them to Equation 2 [21].

$$\frac{dt}{dV} = \frac{\alpha\mu c}{A^2\Delta P}V + \frac{R_m\mu}{A\Delta P} \qquad (2)$$

Where: t is the filtration time [s]; V is the accumulated filtered volume [m^3]; α the resistance of the cake [m/kg]; μ the viscosity of the whey [$Pa.s$]; c the concentration of protein in the solution [kg/m^3]; A the filtration area [m^2]; ΔP the pressure drop in the filter [Pa]; and Rm the resistance of the filter medium [m^{-1}].

In the falling film vacuum evaporation, the deproteinized and clarified whey was concentrated up to an approximate FCV of 4, in a falling film vacuum evaporation pilot, operated at evaporator tube temperature of 70 °C, and pressure of 20 kPa and processing lots of 30 L.

Characterization of lactic fermentation. Fermentations were characterized with 3 distinct media based on: whole (untreated) whey, deproteinized whey, and concentrated/diluted whey, together with a reference medium from reagent grade lactose, all at an initial lactose concentration (S_0) of approximately 50 g/L.

Activation of microorganisms. The freeze-dried strain *L. casei* ATCC 393 in *kwik-stick* presentation, was activated in Petri dishes with MRS agar (Merck) under anaerobic conditions and incubated at 37 °C for 24 h. The working strains (passage 4) were refrigerated at 4 °C (for up to 20 days), while the reserve strain was preserved at -70 °C in cryovials with pearls.

Characteristics of the preculture medium. For each set of fermentations, 200 mL of preculture were prepared with the following composition: 10 g/L of yeast extract (Merck), 0.5 g/L of K_2HPO_4 (Merck), 0.5 g/L of KH_2PO_4 (Merck), 0.2 g/L of $MgSO_4 \cdot 7H_2O$ (Merck), 0,05 g/L of $MnSO_4 \cdot H_2O$ (Panreac), 50 g/L of lactose (Panreac), or 200 mL of deproteinized whey for whey-based media. All of them were sterilized in an autoclave at 121 °C, for 15 min, then their initial pH was adjusted to 6.5 with NaOH 1N and an aliquot of the working strain was transferred. They were incubated without agitation, at 37 °C for 12 h. Preculture was standardized by counting Colony Forming Units per milliliter (CFU/ mL) and optical density (O.D.) at 660 nm.

Characteristics of fermentation media. To start the fermentation, 30 mL of the preculture in the exponential phase of growth were transferred to 270 mL of each test, each one with the same concentration of nutrients in the medium (yeast extract, KH_2PO_4, $MnSO_4$. H_2O, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $MnSO_4$. H_2O, lactose, or pretreated whey), and including 30 g/L of $CaCO_3$ (Panreac) to maintain the pH at approximately 6.5. Fermentations were carried out in Erlenmeyer flasks of 500 mL with a working volume of 300 mL, sealed with an adaptation to avoid contamination during sampling, at a temperature of 37 °C and orbital agitation of 130 rpm. Medium samples were taken at 0, 6, 12, 24, 30, 36, and 48 h, in all tests, to quantify lactose consumption and lactate production by High-Performance Liquid Chromatography of (HPLC), and microbial growth by viable cell count. The product/substrate yield ($Y_{p/s}$) was calculated using Equation 3, volumetric productivity (Ꝑ) with Equation 4, and the specific maximum growth rate (μ_{max}) with the slope of the exponential phase obtained by plotting Equation 5.

$$Y_{p/s} = \frac{Net\ lactate\ production\ [g/L]}{lactose\ consumption\ [g/L]} \quad (3)$$

$$P = \frac{Net\ lactate\ production\ [g/L]}{Production\ time\ [h]} \quad (4)$$

$$\mu_{máx} = \frac{In(N/N_0)}{t - t_0} \quad (5)$$

Quantification methods. %dry matter (A.O.A.C. 990,19) [22]; %protein (A.O.A.C. 991,20) [23], %fat (A.O.A.C. 2000) [24], %ash (A.O.A.C. 923.03) [25]; °Brix with Fisher portable refractometer No. 13964, turbidity with Hanna HI93703 portable turbidimeter. Quantification of lactose and lactate: Quantification by HPLC using a Coregel 107 column, coupled to a UFCL LC 20D (Shimadzu) equipment followed by a RID-10th refractive index detector. It was operated in isocratic mode, at a temperature of 30 °C, a flow of 0.6 mL/min, for 20 min, using as a mobile phase H_2SO_4 8 mM. The samples were filtered using a 0.45 µm, 25 mm PTFE syringe filter for HPLC.
Bacterial count: It was performed by counting viable cells sown in-depth in MRS agar at different decimal dilutions, after 32 h of growth at 37 °C; optimal growth temperature of the microorganism and time established as a result of preliminary tests.

Tukey test. This test was conducted to identify significant differences between the various treated whey samples and their possible influence on lactic fermentation, in terms of productivity, yields, and concentrations.

Results and discussion

Stages of pretreatment of whey

Thermal and acid denaturation. In this stage of pretreatment, a reduction of 33 % in the protein content of the whey was achieved. The physicochemical characterization of the initial and deproteinized whey and the ricotta obtained are presented in Table 1. The initial composition of acid whey is within what was reported by other authors [15,26]. In general terms, a separation of 47.1 g of wet protein/L of whey was achieved, equivalent to a yield (γ_D) of 4.7 %, which is close to that reported by Vázquez-Puente *et al*. [13], with a (γ_D) of 4,9 % in tests carried out at flask level (volume of 1 L) under the same conditions of pH, temperature, and time of the present study. Riera-Rodríguez *et al*. [27] achieved a yield of 5.6 % in tests performed with sweet whey, acidified to a pH between 4.6-4.8 and a temperature between 85-90 °C, for 1 h. This 0.9 % increase in (γ_D), implied a 50 % increase in processing time over the current study, which seen from an economic perspective could significantly increase the energy expenditure associated with this stage.

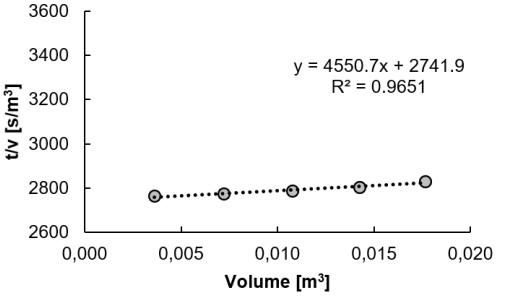
Clarification. Deproteinized whey was clarified, and the stage was characterized in terms of estimating the resistances to filtration and determining physicochemical permeate parameters. The filtration curve obtained fare shown in Figure 2, which, together with Equation 2, allowed the calculation of the resistances of both the filter medium and the cake formed and the characterization of the permeate. From the correlation shown in Figure 1, it could be estimated that the resistance of the filter medium was $1.18*10^{11}$ m^{-1}, while the resistance of the cake was $1.40*10^{5}$ m/kg. From what was observed in the figure, it is concluded that the filtration was dominated by the resistance of the filter medium.

At this stage, the protein content was reduced by 15 % and turbidity by 14 %, with respect to the deproteinized whey from the previous stage. Muvdi-Nova *et al*. [15] reported a reduction of 17.8 % in protein, and obtaining a clarified whey with final turbidity of 5.31 ± 2,32 NTU. However, they used the technique of microfiltration with ceramic membranes with a cut-off of 0.22 µm, in which the retention of the protein is much higher compared to the filter medium used in the filter press, which has to do with the smaller cut-off and porous structure found in membranes.

Table 1. Physicochemical characterization denaturation stage (mean deviation).

Parameter	Whole whey	Deprot. whey	Ricotta cheese	Parameter	Whole whey	Deprot. whey	Ricotta cheese
Humidity [%p]	93.95 ± 0.03	93.98 ± 0.01	68.13 ± 0.00	Ash [%w]	0.48 ± 0.01	0.58 ± 0.01	1.27 ± 0.01
Dry matter [%w]	6.74 ± 0.03	6.02 ± 0.01	31.87 ± 0.00	Lactic acid [g/L]	4.05 ± 0.35	5.50 ± 1.50	<LOD
Carbohyd. [%w]	5.20 ± 0.02	4.91 ± 0.01	5.93 ± 0.00	pH	5.68 ± 0.01	4.40 ± 0.01	4.43 ± 0.01
Lactose [g/L]	50.13 ± 1.8	52.43 ± 1.9	3.36 ± 0.00	Brix	6.0 ± 0.0	5.5 ± 0.5	-
Protein [%w]	0.79 ± 0.01	0.53 ± 0.01	8.67 ± 0.01	Turbidity [NTU]	2348 ± 11	95.1 ± 1.0	-
Fat [%w]	0.27 ± 0.05	<LOD	16.00 ± 0.00	Density [g/mL]	1.024 ± 0.004	1.023 ± 0.003	-

Deprot. (deproteinized), carbohyd. (carbohydrates), LOD (limit of detection).



Property	Value
Dry matter [% w/w]	5.60 ± 0.00
Carbohydrates [% w/w]	4.57 ± 0.01
Protein [% w/p]	0.47 ± 0.00
Ash [% w/w]	0.56 ± 0.01
Fat [% w/w]	<LOD
Turbidity [NTU]	83.3 ± 1.5

Figure 1. Physicochemical characterization and filtration curve of deproteinized clarified whey. Filtration area = 0.0039 m^2; V= 20 L; room temperature; P= 137.9 kPa. LOD (limit of detection).

Concentration. The concentration of deproteinized and clarified whey was performed in batches of 30 L taking it to a VCF of 4. The evolution of °Brix and VCF as a function of time, as well as the relationship between °Brix and VCF and the appearance of concentrates at different VCF are shown in Figure 2.

It is observed that °Brix and VCF exhibit the same growth trend, where there is a faster increase of values after reaching an FCV of 2, at an approximately constant evaporation rate of 2.3 L/h; this is associated with a lower volume content of water to be removed. The relationship between °Brix and FCV was similar to that obtained by Muvdi-Nova *et al*. [15] who reported °Brix of 5, 10, 14, and 19, for FCVs of 1, 2, 3, and 4, respectively. On the other hand, the bromatological characterization performed for the whey to VCF of 1, 1.9, 3.1, and 4.1 are presented in Figure 3. There is a linear increase in the content of dry matter, carbohydrates, protein, and ashes with VCF. The final concentrates reached a total solids content between 4.1 and 3.5 times higher than the initial one, for the two processed batches, with compositions like those reported by Tanguy *et al*. [16] for concentrates of micro and ultrafiltrates of

milk, with a VCF of 4.1, 22.4 % of dry matter, 2.5 % of protein and 2.0 % of ashes.

In general, an important separation of whey protein was achieved, which has proven to be an interesting alternative as a low-cost nitrogen source, to be evaluated in subsequent studies. In addition, this removal reduced considerably the fouling of equipment, mainly for the vacuum descending film evaporator, an aspect that had a significant positive effect on the productivity of this unitary operation and allowed to obtain concentrates up to a VCF of 4.1.

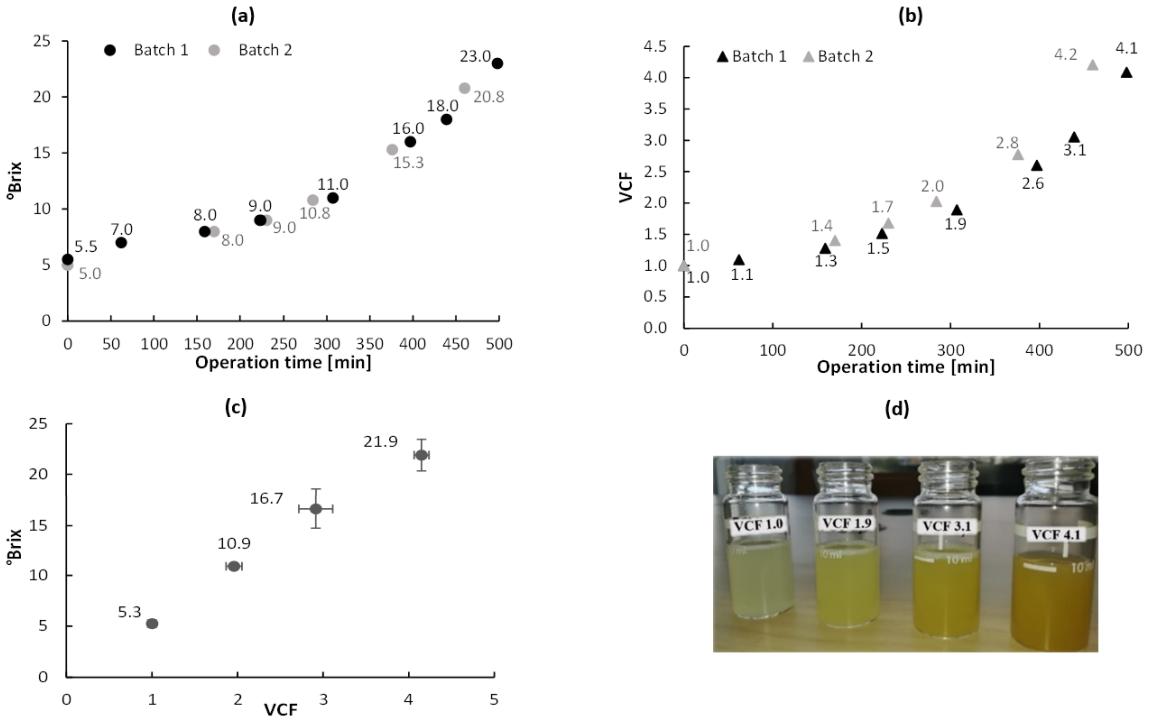


Figure 2. Evolution of a) °Brix and b) VCF, depending on the operating time of the evaporator; (c) relation between VCF and °Brix, and (d) appearance of deproteinized/clarified whey concentrates to VCF of 1.0, 1.9, 3.1 and 4.1. (T_{FEED}= 70 °C, T_{WALL}=70 °C and P = 200 kPa). Mean deviation.

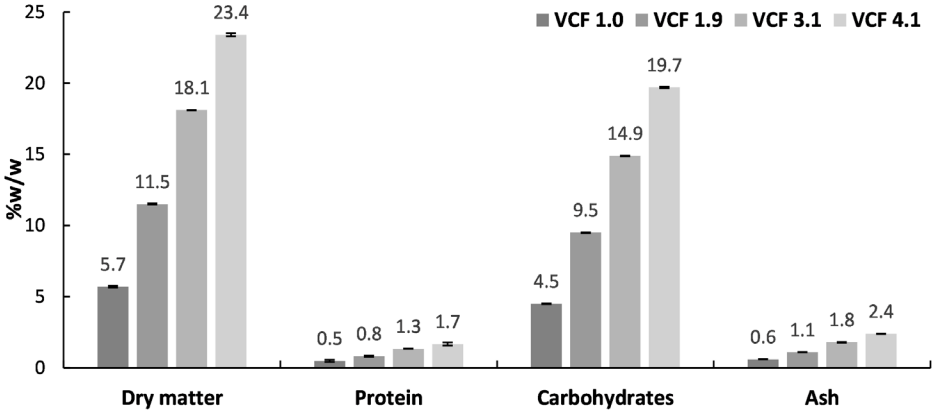


Figure 3. Bromatological characterization of whey concentrates obtained by evaporation of descending film under vacuum. (T_{POWER}= 70 °C, T_{WALL}= 70 °C and P = 200kPa). (Mean deviation).

Characterization of lactic fermentation

The growth of *L. casei* in the preculture medium was standardized by a correlation between CFU/mL and the Optical Density ($O.D._{660\ nm}$) thus ensuring that the inoculum added to the media was in the phase of exponential growth, with a microbial population in the same order of magnitude in all trials.

Reference fermentations: The monitoring overtime of the concentration of lactose, lactic acid, biomass (dry weight), pH, and $O.D._{660\ nm}$, for fermentation with reference medium (lactose of reagent degree) without pH control are shown in Figure 4. It is appreciated that only 24.1 % of the available lactose was consumed after 48 h, and low production of lactic acid (8.4 g/L). *L. casei* grew exponentially until 28 h, with a cellular mass concentration of 1.7 g/L. In the first 24 h of fermentation, there was a decrease in pH, from 6.7 to 3.7, the latter related to the finish of lactose consumption. The concentration of lactic acid showed a significant increase after 24 h, from 6 to 8.4 g/L. That could be related to a formation of mixed product associated with growth, a typical behavior of lactic fermentation [28]. Moreover, the kinetics of lactose consumption, lactate production, and microbial growth for fermentation with pH control from the reference medium using lactose reagent grade are shown in Figure 5. Unlike tests without pH control, the total of available lactose in the medium was consumed, with a production of 43.1 g/L of lactate and a maximum microbial population of $3x10^{11}$ CFU/mL. This evidence the importance of pH control in fermentation, considering the strong inhibitory effect exerted in general on the genus *L. casei,* when the pH of the medium approaches the pk of lactic acid (3.86), as evidenced in Figure 4.

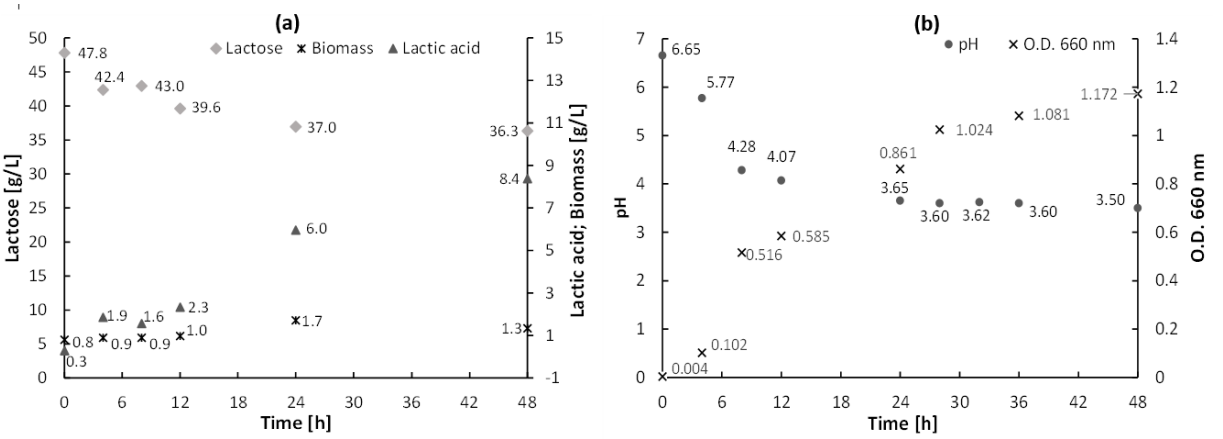


Figure 4. Kinetics of a) lactose consumption, lactic acid production, and microbial growth; b) pH and $O.D._{660\ nm}$, in fermentation with the reference medium without pH control with *L. casei* ATCC 393.

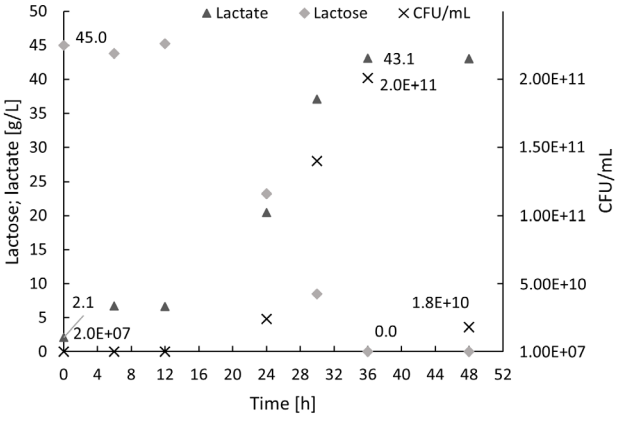


Figure 5. Kinetics of lactose consumption, lactate production, and microbial growth, in reference fermentation with pH control at 6.5 ± 0.3.

Fermentations with pretreated whey. Fermentations were carried out with pH control, using as a substrate whey with different degrees of pretreatment: whole (untreated), deproteinized, and concentrated (all to VCF = 1), at S_0 close to 50 g/L. The kinetics of lactose consumption, lactate production, and microbial growth are presented in Figure 6, and the comparison between production and growth parameters obtained in all cases are presented in Table 2.

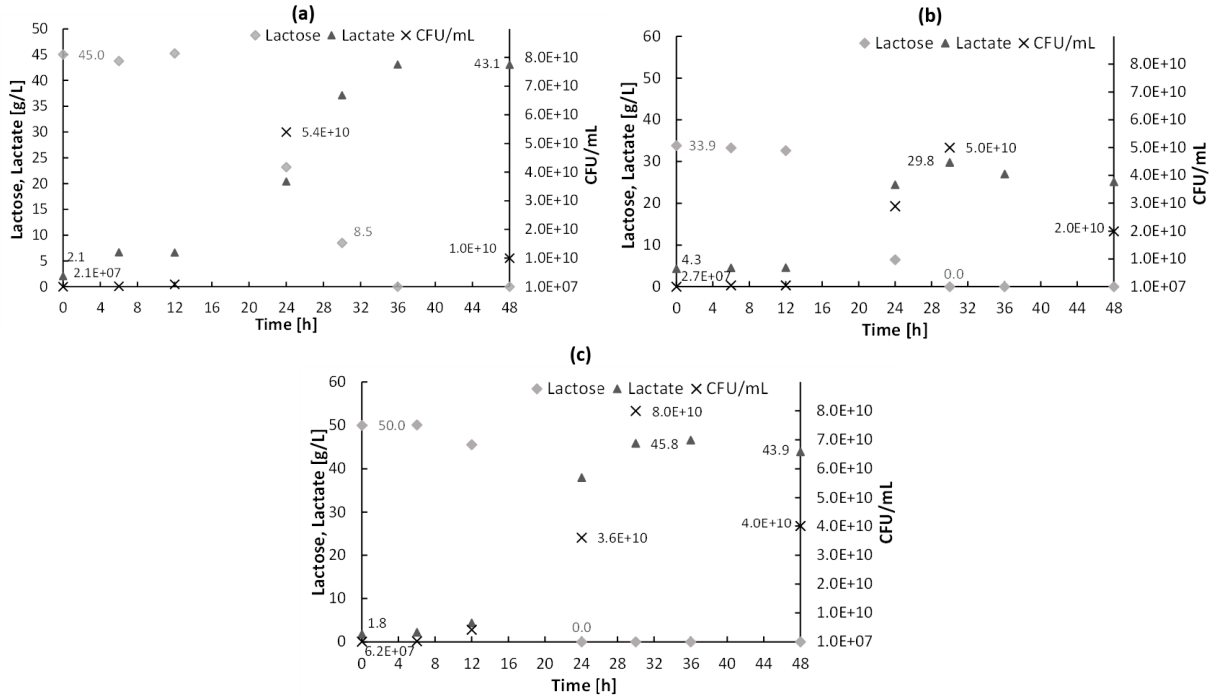


Figure 6. Kinetics of lactose consumption, lactate production, and microbial growth for fermentations with a) whole whey, b) deproteinized whey, and c) concentrated whey.

Table 2. Productive parameters in fermentations with the reference media and based on whey with different degrees of pretreatment (mean deviation).

Fermentation medium	Lactate production [g/L]	$Y_{p/s}$ [g/g]	Volumetric Productivity [g/L.h]	$\mu_{máx}$ [h^{-1}]
Reference (lactose)	40.5 ± 0.7$^{(a,b)}$	0.88 ± 0.04$^{(c)}$	0.95 ± 0.21$^{(d)}$	0.32 ± 0.01
Whole whey	42.8 ± 2.8$^{(a,b)}$	0.92 ± 0.03$^{(c)}$	1.60 ± 0.14$^{(d)}$	0.27 ± 0.04
Deproteinized whey	29.4 ± 5.7$^{(b)}$	0.77 ± 0.03$^{(c)}$	1.25 ± 0.21$^{(d)}$	0.29 ± 0.04
Concentrated whey	44.0 ± 2.5$^{(a)}$	0.87 ± 0.08$^{(c)}$	1.65 ± 0.35$^{(d)}$	0.25 ± 0.01

$Y_{p/s}$ (product/substrate performance); $\mu_{máx}$ (specific maximum growth rate). Different exponent letters indicate significant differences (Tukey test, $p < 0.05$).

As with the reference medium, the entirety of the lactose was used by the microorganism, in times less than or equal to 36 h (time of depletion of lactose in the reference medium). The final cellular concentration was in the same order of magnitude 10^{11} CFU/mL. Statistical analysis, conducted using Tukey's test, shows that the processing stages do not significantly affect the conversion of whey to lactate. In general, lactate concentrations (greater than 40 g/L in most cases), together with the product/substrate yields ($Y_{p/s}$), showed high selectivity towards product formation and are in accordance with Büyükkileci and Harsa [29] in the fermentation of whey powder. In their study, they achieved a $Y_{p/s}$ of 0.93 g/g and volumetric productivity of 1.87 g/L.h with *L. casei* NRRL B-441, with operating conditions and medium composition like those used in the present study.

Conclusions

This work on whey processing stages revealed important advantages from a productive perspective, including reductions in equipment fouling and transport/storage volumes, translating into cost savings and environmental benefits. This approach enhances resource efficiency and enables the recovery of value by-products, such as whey proteins, while increasing lactose content and other nutrients up to fourfold. These improvements contribute to waste reduction and add marked economic value.

Importantly, kinetic parameters from fermentation trials using processed whey showed no negative impact on key components, serving as an initial approach to validate this strategy's robustness for further applications. The state-of-the-art conducted in this work reveals that, to date, no studies have specifically assessed the impact of whey processing on lactose fermentation into lactic acid, highlighting the novelty and relevance of this work. These results support designing of a sustainable and efficient process for producing of lactic acid and its derivatives. The recovery, recycling, and processing of whey -still often considered waste- open new research pathways, including high-lactose fermentations from the whey, potential applications of whey protein in lactic fermentation, and novel uses in food formulations. These pathways set the groundwork for future scaling and product purification, ultimately supporting Sustainable Development Goals through innovative waste valorization and resource optimization.

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Disclaimers

All authors made significant contributions to the document, agreed with its publication, and declare no conflicts of interest in this study.

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