Effect of Initial Sugar Concentration on the Production of L (+) Lactic Acid by Simultaneous Enzymatic Hydrolysis and Fermentation of an Agro-Industrial Waste Product of Pineapple (*Ananas comosus*) Using *Lactobacillus casei* Subspecies *rhamnosus*

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Abstract: Production of lactic acid by fermentation process has been studied from glucose solutions and other sources because of many important reasons: biotechnological production is cheaper than chemical synthesis; production of biodegradable materials from L (+) lactic acid and, the use of nutrient-rich agro-industrial wastes as raw material, which helps to reduce the environmental impact. The goal of this study was to evaluate the effect of sugar concentration of a pineapple liquid waste as the carbon source on the capacity of *Lactobacillus casei* subspecies *rhamnosus* to produce lactic acid by simultaneous enzymatic hydrolysis and fermentation. Three different pineapple waste concentrations were evaluated (60, 80 and 100% v/v) from a pineapple juice with 11.3% (m/v) of sugars (sucrose, fructose and glucose). *L. casei* was able to consume all sugars present within the levels tested, and converted all into lactic acid, showing efficient yields of 0.91 g lactic acid/g sugars. Final lactic acid concentration increased significantly ($p<0.05$) with the increase of pineapple waste percentage. Maximum lactic acid concentration (102g/L) was achieved with 100% pineapple waste medium. The highest total productivity (4.0g/h) and volumetric productivity (4.48 g/L*h) were obtained with 60% pineapple waste medium and it decreased significantly ($p<0.05$) when 100% was used. Fermentation time increased with the increment of sugars, and it increased significantly with the medium composed of 100% of pineapple waste in comparison with the other two media. Pineapple waste represents a good alternative as a cheap carbon source for bacterial growth and production of L (+) lactic acid.

Keywords: Lactic acid, polilactic acid, pineapple, agro-industrial waste, repeated-batch fermentation, *L. casei.*

INTRODUCTION

Pineapple has the second highest production volume of all tropical fruits in the world [1]. Costa Rica is one of the main producers and exporters, with approximately 110,000 acres of this fruit [2]. Of all the fresh pineapple harvested, approximately 25% is processed to make added value products like concentrated juice, canned pineapple and jelly [3]. From these processing activities, over 65% of the whole pineapple is unused [4], and big amounts of waste products are produced and need to be treated, turning into an economical and environmental problem for the producers [5].

The use of pineapple waste as an inexpensive underutilized agricultural byproduct for the biotechnological production of lactic acid (LA) is an interesting option. This alternative process can reduce the cost of production of this chemical and, moreover, can add value to an agro-industrial waste as high carbohydrate fermentable source [6].

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LA is a versatile chemical and has many applications in the food, pharmaceutical, leather, textile and chemical industries [7]. Annually 130,000 to 150,000 tones of this additive are produced in the world and considering the global market trends, agro-based chemicals hold a promising future and the demand for LA is expected to shoot up to 200,000 MT by 2011 [7, 8, 9]. More than half of this was used in food (as acidulant and preservative) processing. About one-fifth was processed to produce calcium- and sodium-stearoyl-2lactylate (for baking); the rest was utilized in the pharmaceutical industry (dietary calcium source, blood coagulant) or in technical applications [10].

However, the traditional process by chemical synthesis has the disadvantage that uses toxic solvents and, the LA produced is a racemic mixture of the two optical isomers [11, 12]. Nowadays, 90% of LA produced worldwide is made by fermentation process [11], which main advantage is the high specificity of the product because it produces the desired stereoisomer, L-(+) or D-(-) optically pure LA [7, 13]. This is very important because one of the most recent interests in L (+) LA production is the promising application of its polymer, polylactic acid (PLA), as a biodegradable material and as an alternative to traditional oil based plastics [8, 13, 14, 15].

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Biotechnological production is based on the fermentation of carbohydrate-rich substrates by bacteria or fungi. The most widely used industrially are those of the genus *Lactobacillus* by their high degree of conversion, yield and rate of metabolism [16]. Also, this is the genus most acid-tolerant of all LA bacteria [17]. *Lactobacillus casei* produces the enzyme L-lactate dehydrogenase, so the product of its metabolism will be L (+) LA [17].

The utilization of pure sugars as the carbon source in fermentations is economically unfavorable, because they are expensive and LA is a cheap product [11]. Instead agro-industrial waste products, like whey, starch, sugarcane bagasse, beet molasses, corn cob residues and date juices have been used as the carbon source for the production of L (+) lactic acid [13, 18, 19]. Pineapple residues are rich in sugars, especially in sucrose, glucose and fructose [20] and other components like minerals and vitamins [21], which are important nutrients in fermentations.

According to the metabolism of *L.casei* subsp. *rhamnosus,* this bacterium will use first glucose as a carbon source, followed by fructose [22]. Past studies showed that this bacterium is unable to consume the sucrose in an appropriate period of time [23, 24]. Chan-Blanco *et al*. [23] found that sucrose consumption was insignificant in a banana medium. Besides, Araya-Cloutier *et al*. [6] demonstrated that simultaneous enzymatic hydrolysis in a fermentation process of a pineapple waste, increased significantly the LA yield and the LA production, as a result of the total consumption of the sugars present in the hydrolyzed medium, in comparison with the fermentation without hydrolysis. In the latter case, a residual concentration of 15 g/L of sucrose and a significant higher lag phase were obtained [6]

Even thought, LA concentration is directly proportional to the initial glucose concentration, also it has been demonstrated that the yield is inversely proportional to the initial substrate concentration [25], additionally productivity also starts to decrease gradually with higher substrate concentration needing more hours to get to a maximum production [8]. According to Ghaly *et al*. [26] the use of yeast extract increases the cell number, the growth rate, and cell yield; and reduces the lag period, fermentation time and residual carbon source.

One of the main considerations for the production of LA is the cost of the carbon source and the use of additional nutrients [27], they increase the total cost of the process. This work was focused on using alternative sources of carbon to make the process economically feasible using an inexpensive culture medium based on pineapple waste for the production of LA.

Pineapple waste cannot be used in full. The presence of cell wall components such as lignin, pectin and polysaccharides increase turbidity and viscosity of the juice making the clarification process harder [28]. The growth of the microorganism and production of LA can be affected. In addition, when a lower amount of insoluble solids is used in the fermentation broth, a higher efficiency in the recovering and purification process is expected. Besides, as LA purification involves a concentration step, in order to reduce energy costs in this process, the addition of water to the medium is undesirable [28, 29].

Therefore, the main objective of this research was to evaluate a pineapple liquid waste as the carbon source for the production of LA by simultaneous enzymatic hydrolysis and fermentation, and the concentration of this waste in the culture medium was evaluated to determinate the efficiency of the bacteria using the substrate.

MATERIALS AND METHODS

Microorganism and Culture Conditions

Lactobacillus casei subspecies *rhamnosus* ATCC 11443 used in this study was obtained from American Type Culture Collection (ATCC) (Manassas, USA). This culture is a homofermenter for L-(+)-lactic acid [13, 17, 22, 23], The lyophilized microorganism was transferred to 5mL of *De Man Rogosa Sharpe* (MRS) broth in a test tube, and incubated at 37°C for 18h in static cultivation. The “preculture” was prepared by transferring the 5ml culture to 100mL of fresh MRS in 250ml flask. This preculture was incubated at 37°C for 48h, in static cultivation. Inoculum was prepared by placing 1mL of the preculture in 100mL of fresh MRS, with subsequent incubation at 37°C for 18h. The fermentation medium was inoculated with 0.5% (v/v) of this final culture.

Enzymatic Hydrolysis and Determination of Enzyme Activity

Enzymatic hydrolysis of sucrose was made in all fermentation experiments with a commercial β-fructofuranosidase, *Invertase* (E.C. 3.2.1.26), obtained

from Novo Enzymes (Lund, Sweden). 2L of media was supplied with 2.5 mL of enzyme solution at 1% (w/w).

Enzymatic activity was carried out according to the method described previously [30] by adding 0.5mL of an enzyme solution (0.01% w/v) to 5mL of sucrose 5.4% (w/v) prepared in acetate buffer 0.1M (pH 4.5). Ten test tubes with mixture were then incubated at 35°C, taking one tube as a sample every minute from time 0 min to time 10 min. After the incubation period was completed, the enzyme was inactivated by placing the test tubes in a water bath (85°C/10min). The residual glucose was measured using the Trinder colorimetric method [31]. The enzyme activity obtained was 36800 EU/g of enzyme; where an enzymatic unit (EU) was defined as the milligrams of glucose produced per minute, under the experimental conditions used.

Waste Material

Pineapple waste was obtained from the fruit company *Florida Products*, in Heredia, Costa Rica. This pulp was filtered by applying a pressure of 10 psi on the pineapple waste. The juice obtained, received a pasteurization treatment (75ºC, 5 min) [23] follow by freezing at -20ºC, for microbial stabilization. The liquid waste was composed mostly of water and carbohydrates which were in majority glucose, fructose and sucrose.

Fermentation Medium

Preparation of medium was made following the method described before [5, 32]. The basic fermentation medium consisted of pineapple liquid waste and the following nutrients: 5g/L of yeast extract; 0.6g/L of $MgSO_4 \cdot 7H_2O$ and 0.03g/L of $MnSO_4 \cdot H_2O$. This first mixture was sterilized at 121°C for 15min and 15 psi. In another recipient 10 g of sodium acetate; 20 g of KH_2PO_4 and 20 g of K_2HPO_4 were mixed in 100 mL of distilled water, then this mixture was sterilized too. Finally, 20 mL of this salts mixture was injected on the bioreactor getting a final concentration of 1 g/L of sodium acetate, 2 g/L of KH_2PO_4 and 2 g of K_2HPO_4 in the fermentation medium. The concentration of pineapple waste in the medium varied from 60, 80 and 100% (v/v in distilled water).

Fermentations Mode

Fermentations were carried out in a Bioflo 110 (New Brunswick, USA) bioreactor system (Figure **1**), with a working volume of 2L. Once the equipment was adapted and reached the desired temperature, *L.casei* culture was inoculated simultaneously with 2.5mL of an

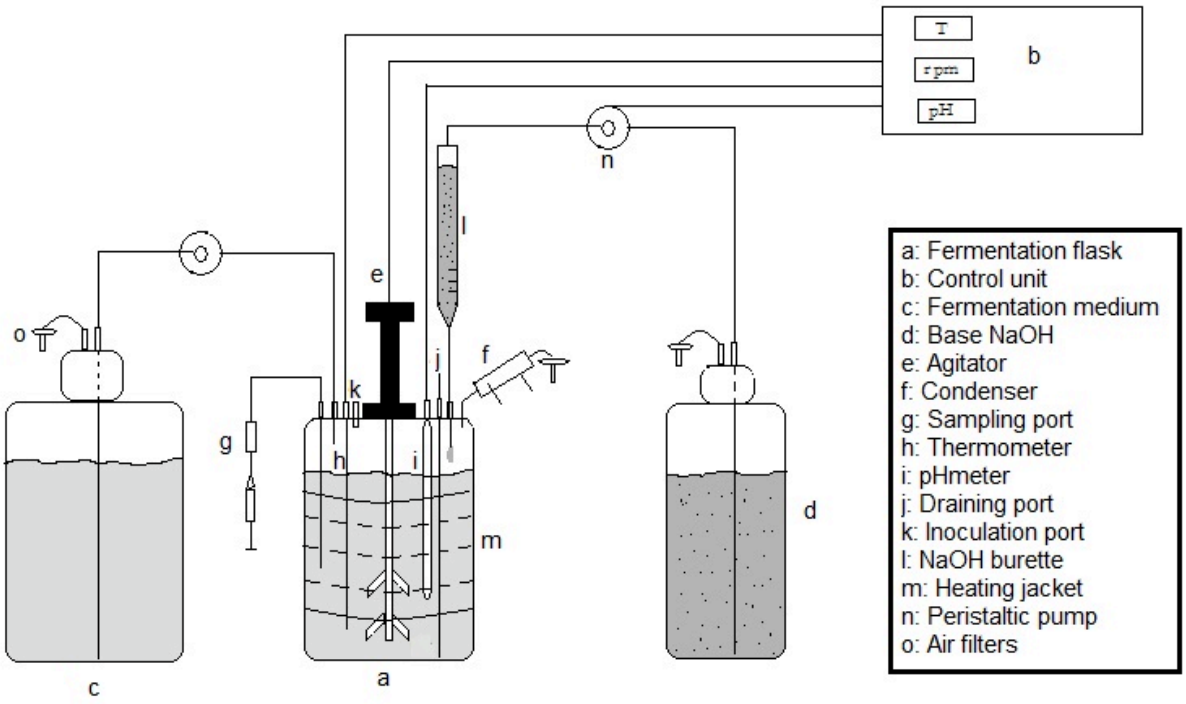

Figure 1: Schematic diagram of a repeated batch fermentation system used for the production of lactic acid with pineapple waste [6].

Invertase solution (1%w/w), in order to hydrolyze the sucrose present in the fermentation medium. The pH was maintained at 5.0 by addition of 5N NaOH, the temperature was kept at 35°C and the stirring speed at 250rpm. The reactor was operated in a batch mode: once the microorganism reached the stationary phase, the fermentation broth was taken out and new fresh medium was introduced to the bioreactor, keeping the system sterile, to start a new fermentation. Cells from the first batch residues were used as the inoculum for the subsequent batch fermentation to improve lactic acid, productivity, cell growth and total glucose utilization when the bacteria is acclimatized first in the medium [33].

Sampling

Samples (5-10 mL each one) were taken from the fermentation medium at different times (every 1-3 h) during the fermentation process. Samples were centrifuged, microfiltered (0.45µm) and frozen at -18°C for further analysis of sugars and LA concentrations.

Chemical Analysis

Moisture, fat, protein, ash, acidity and soluble solids contents were determined by using standard official methods 920.151, 991.20, 920.152, 940.26, 942.15 and 932.12 respectively [34]. Total carbohydrates (TC) content was obtained by difference (TC =100-%Moisture - %Ash - %Protein - %Fat). Starch content was determined following the method described by Southgate [35]. pH was analyzed following the standard official method 981.12 [34].

Glucose, fructose and sucrose were determined by an enzymatic analysis using the kit K-SFRUGL from Megazyme (Napa, USA) [36]. Lactic acid was measured by high performance liquid chromatography (HPLC). The analysis was conducted on a Shimadzu Scientific HPLC (Kyoto, Japan), equipped with a quaternary pump (model LC20AD) with degasser built and coupled to a diode array detector Shimadzu (SPD-M20A). LA was analyzed with a BioRad Aminex HPX-87H column (300x7.8mm; BioRad, Richmond, USA) using H_2SO_4 0.005mol/L as the mobile phase in an isocratic gradient. Separation of LA was carried out with an injection volume of 20 µL, a flow rate of 0.9mL/min, and a pressure 90kgf/cm^2 and column temperature set at 35 º C. Chromatograms were processed at a wave length of 210 nm. LA was quantified using the external calibration curve of L-(+)-lactic acid (Sigma-Aldrich, St. Louis, MO, USA) in a range of 0.2 g/100 g – 1.0 g/100 g and at a minimum of five levels of concentration. Each concentration was injected into the HPLC and the linear regression equation was acquired by plotting the quantity of standard injected against the peak area. Good correlation was obtained (r^2 = 0.995).

Optical Density

Cell growth was determined by optical density. An aliquot of 5 mL was taken from the fermentation medium. The analysis was conducted with a UV-visible spectrophotometer (Shimadzu UV-1700 PharmaSpec, Kyoto, Japan), using a wavelength of 660 nm.

Parameters and Statistical Analysis

The parameters calculated were the final LA concentration, LA yield, total productivity, and volumetric productivity using the formulas A1-A4 respectively, described by Chan-Blanco *et al*. [23], and, fermentation time and lag phase.

Final lactic acid concentration (g/L) = Lactic acid produced (g) / Volume (L). A.1

Lactic acid yield (g/g) = Lactic acid produced (g) / Initial sugars (g)* A.2

$$\text{Total productivity}\left(\frac{g}{h}\right) = \frac{\text{Lactic acid (g)}}{\text{Fermentation time - Lag phase (h)}} \quad \text{A.3}$$

$$\text{Volumetric productivity}\left(\frac{g}{L*h}\right) = \frac{\text{Lactic acid production}\left(\frac{g}{h}\right)}{\text{Volume (L)}} \quad \text{A.4}$$

*Note: The amount of initial sugars (g) refers to the sum of glucose, fructose and sucrose of the medium.

A variance analysis (ANOVA) was carried out to determined significant differences ($p<0.05$) between the three different concentrations of pineapple waste evaluated, using JMP 4.1 software (SAS, Cary, NC, USA). Values were means of triplicate experiments. Variability was expressed by the confidence interval (CI).

RESULTS

Composition of juice from pineapple waste is presented in Table **1**. The main component of pineapple juice is water followed by TC. The sum of the sugars sucrose, glucose and fructose represented up to 90% of the TC , which is very convenient because

these sugars are carbon sources for lactic acid bacteria [22]. Glucose and fructose were present in similar content (4.1% and 4.0%, respectively) and higher than sucrose (2.8%). On the other hand, ash content of juice from pineapple waste (0.23%) can be a source of minerals which are micronutrients for the bacteria.

Table 1: Composition of Juice Obtained from Pineapple (*Ananas comosus*) Waste as a Substrate for Fermentation[1]

Component	Content % w/v
Moisture	87.2 ± 0.5
Soluble solids	10.36 ± 0.08
Fat	Not detected
Protein	0.33 ± 0.05
Ash	0.23± 0.03
Total Carbohydrates	11.8 ± 0.6
Starch	Not detected
Total sugar:	10.9 ± 0.
Sucrose	2.8 ± 0.5
Glucose	4.1 ± 0.1
Fructose	4.0 ± 0.1
Acidity (expressed as g of citric acid)	0.42 ± 0.4
pH	3.8 ± 0.2

[1]Results are expressed as the mean of three replicates with their respective standard desviation.

Concentration of the pineapple waste varied from 60 to 100% (v/v in distilled water) in the fermentation medium. Also simultaneous hydrolysis of sucrose was carried out; therefore levels of this sugar were very low and glucose and fructose were high from the beginning of the fermentation process. Figure **2** shows the cell growth in the three different levels of sugars evaluated.

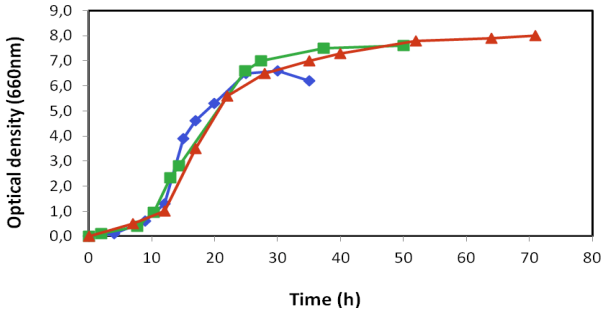

Figure 2: Cell growth during the fermentation of a medium composed of 60 (♦), 80 (■) and 100% (v/v) (▲) of pineapple waste juice (mean of triplicates).

Higher levels of sugars resulted in a longer lag and log phases. Figure **3** shows the kinetics of fermentations for the same levels of sugars evaluated. Exponential production of LA was observed in all cases, meanwhile sugars (glucose and fructose) concentration declined simultaneously.

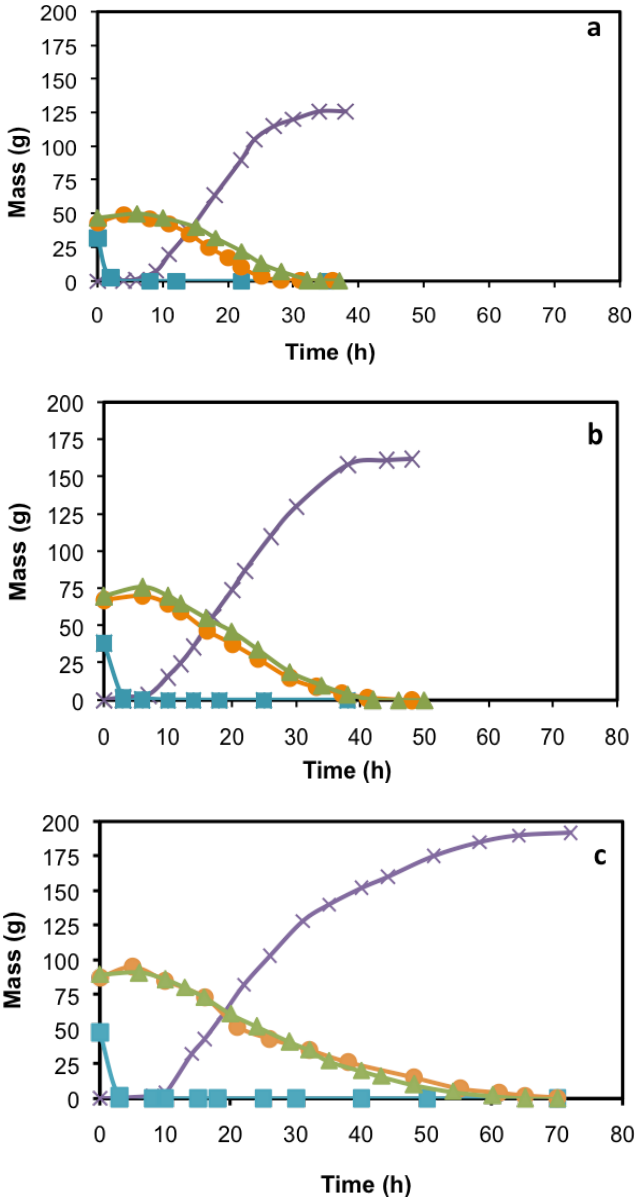

Figure 3: a-c. Lactic acid production and sugars consumption during the fermentation of a medium composed of 60 (**a**), 80 (**b**) and 100% (v/v) (**c**) of pineapple waste juice (mean of triplicates); × lactic acid, ▲ fructose, ● glucose, ■ sucrose.

In all cases, sucrose was hydrolyzed in two hours reaching a final concentration of 0 g/L. An increase in the LA production was observed when the content of pineapple waste increased up to 100% (v/v), and in the

Table 2: Comparison of the Fermentation of Different Media Composed of Pineapple Waste with *L.casei* Subsp. *Rhamnosus*

Parameters	Pineapple juice content % v/v		
	60	80	100
Lactic acid (g/L)	60 ± 3[a]	79 ± 7[b]	102 ± 2[c]
Yield (g/g)	0.91 ± 0.3[a]	0.90 ± 0.4[a]	0.92 ± 0.2[a]
Total productivity (g/h)	4.0 ± 0.1[a]	3.94 ± 0.06[a]	3.25 ± 0.06[b]
Volumetric productivity (g/L*h)	4.48 ± 0.06[a]	3.31 ± 0.05[b]	3.58 ± 0.05[c]
Fermentation time (h)	35 ± 2[a]	47 ± 2[b]	70.3 ± 0.5[c]
Lag phase (h)	6.2 ± 0.3[a]	7.3 ± 0.6[a,b]	8 ± 1[b]
Initial total sugar (g/L)	68 ± 3	88 ± 4	113 ± 1
Sucrose (g/L)	17 ± 3	19 ± 4	24 ± 4
Glucose (g/L)	25 ± 6	34 ± 3	44 ± 1
Fructose (g/L)	26 ± 5	35 ± 3	45 ± 1

[a]Results are expressed as the mean of three replicates with their respective confidence interval (α=0.05).
[b]Means in the same line with different letter are significantly different ($p < 0.05$).
[c]Yield and Total productivity formulas are described in section *Parameters and statistical analysis*.

three cases residual sugars were minimal at the end of the fermentation process.

The final LA concentration increased proportionally with the increment in the level of pineapple waste in the medium. The highest concentration obtained was 102 g/L of LA produced from 113 g/L of initial sugars (Table **2**). In relation with the productivity of the process, the medium composed of 60% of pineapple juice showed the highest total productivity (4.0 g/h of LA), but this value wasn't different ($p<0.05$) from the total productivity of the 80% medium. Whereas, the medium composed of 100% (v/v) of pineapple juice presented a significant lower total productivity of 3.25 g/h of LA. Productivity was calculated at the same period of time for all media, 38 hours, the time it took for the 60% (v/v) pineapple juice medium to end the LA production (end of phase log).

The medium of 80% of pineapple juice presented the highest productivity (4.16 g/h of LA). The medium composed of 100% (v/v) of pineapple juice presented a significant lower productivity of 3.8 g/h of LA. Nevertheless, at 38 hours 100% (v/v) pineapple juice medium was on log phase (Figure **3c**) and LA production was still increasing. The fermentation time was longer in regards to the increments of pineapple waste concentration in the medium within the range of work. The increment of time observed from 80 to 100% (23h) is almost twice than from 60 to 80% (12h). The lag phase was proportional to the amount of initial sugars present. However, the lag phase did not increase significantly ($p>0.05$) from 60 to 80% of pineapple juice in the medium and neither from 80 to 100%.

Figure **4** shows the yield and volumetric productivity during the fermentation process. *L.casei* was able to convert all the sugars to LA, showing in all three cases

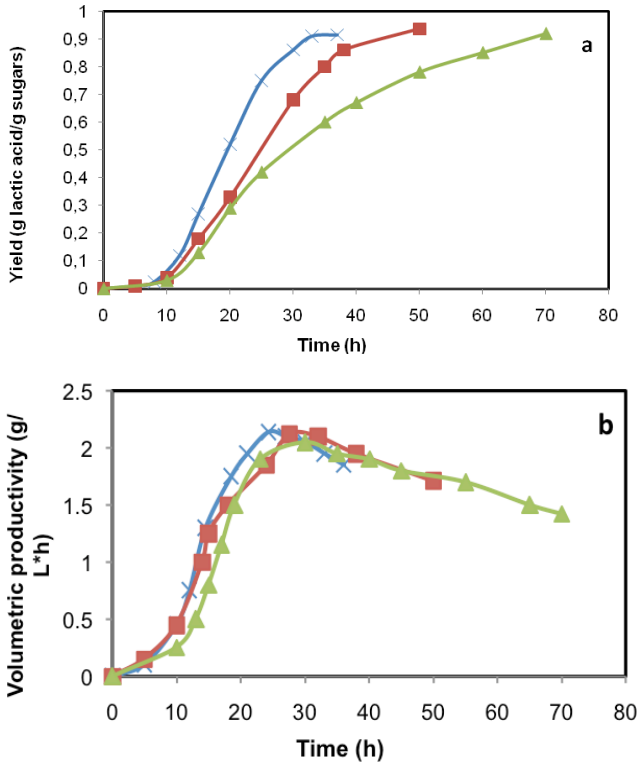

Figure 4: Lactic acid yield (**a**) and volumetric productivity (**b**) as a function of time with different pineapple waste media: 60 (**×**), 80 (■) and 100% (▲) (v/v in distilled water).

yields higher than 0.90 g LA per g sugar. The highest yield (0.92) was achieved with the medium composed of 100% pineapple juice, but the differences between the three media by the end of the fermentation were not significant ($p<0.05$) (Figure **4a**).

Regarding the volumetric productivity (Figure **4b**), all three media reached their maximum between 25 and 30h of fermentation. Thereafter, the productivity started to decrease gradually. The highest volumetric productivity (4.48 g/L*h), was reached at 25h with the 60% pineapple waste medium. With 80% and 100% of pineapple juice maximum productivity (3.31 and 3.58 g/L*h) was obtained at 28h and 31h, respectively, and the difference between the three media were significant ($p<0.05$).

DISCUSSION

Compared with other carbon sources, pineapple waste was a good alternative. Chan-Blanco *et al*. [23] reported a higher fermentable sugar content (20%) for banana substrate than pineapple, but its high insoluble content and high viscosity makes it necessary to apply a dilution of 20:80 in water. In this condition, sugar content is similar, but the high viscosity in the banana substrate makes the recuperation and concentration processes of the metabolite more expensive [28].

Due to low polysaccharides content on juice from pineapple waste, enzymatic treatment for increasing fermentable sugars is not necessary. Other substrates such as apple waste require enzymatic and heat treatments to reduce turbidity and viscosity due to the presence of polysaccharides [37].

Sucrose is not used by *L. casei* in its first metabolism [6?]. Araya-Cloutier *et al*. [6] demonstrated that, if no hydrolysis is applied, this sugar remains in the medium even after 40 hours of fermentation, in a medium composed of 80% (v/v) of pineapple waste. In these conditions, less consumed sugars and lower yields were obtained. On the other hand, according to Araya *et al*. [6], LA yield, LA production and sugar consumption increased (16%, 17% and 20%, respectively) when enzymatic treatment was used to hydrolyzed sucrose. Same authors reported that lag phase decreased 4 hours when sucrose was hydrolyzed while fermentation occurred. For these reasons, in this study all three mediums (60, 80 and 100% v/v) were treated with an invertase enzyme at a concentration (1% m/m) that allowed hydrolysis of all sucrose in the medium in less than two hours (Figure **2a-c**).

The advantages of the invertase treatment on the pineapple waste were possible due to the catabolic pathway of the bacteria. *L. casei* was able to efficiently consume glucose and fructose (two main carbon sources in the medium) almost at the same time. Nancib *et al.* [24] also observed a parallel consumption of glucose and fructose with the fermentation of date juice with *L.casei*, and Chan-Blanco *et al*. [23] with a banana enriched medium. On the basis of these results, it is clear that *L.casei* subsp. *rhamnosus* has the ability to produce simultaneously the enzymatic machinery necessary for the utilization of these sugars, even though the metabolic pathway used (*Embden-Meyerhof-Parnas*) starts from the glucose molecule [17]. *L. casei* is able to consume glucose and fructose simultaneously by applying PEP-phosphotransferase systems getting as final product the salt form: lactate [22].

It could be stated that the utilization of all the sugars was exclusively for the production of LA, even thought perfect yields of 2 mol of LA per mol of glucose are unusual. Hujanen *et al*. [25] obtained just 80g/L of LA with an initial concentration of glucose of 100g/L using *L.casei*; and Kotzamanidis *et al*. [38] obtained a maximum LA concentration of 90g/L with a medium composed of 100g/L of initial sugars, in the fermentation of beet molasses with *L.delbrueckii*. Whereas, the pineapple medium of 113 g/L initial sugars permitted the production of a higher amount of LA. The simplicity of the carbon sources may have been beneficial for bacterial growth and help the conversion into solely LA, providing higher LA concentrations with the same initial sugar concentration that previews works. It is important to note that the yield was estimated as the relationship between LA and the sum of glucose, fructose and sucrose which were the total sugars present in the substrate. However, there are other nutrients present in the pineapple juice in lower concentrations, like galactose, citric acid and malic acid [20], which the bacteria may use as the carbon source for biomass or energy [17] and therefore produced the observed yields.

Although, the decrease of the total productivity with the highest sugar concentration can be due to substrate inhibition: above a critical substrate concentration, the osmotic pressure and low water activity can cause the decrease of the rate of production of LA [8, 38]. Also the accumulation of toxic byproducts (e.g. high concentration of LA) can cause autolysis of the cells resulting in a decrease of bacterial growth and thus, a decrease in the production of LA [38], especially after around 30h of fermentation. This

can be also extrapolated from the results of the fermentation time, where the increment in initial sugars caused an increment in the total fermentation time. Despite the increment of time, the bacteria was able to consume all the sugars present in the medium composed of 100% pineapple waste and produced more LA than the others media. During the lag phase the bacteria is adapting to the new environment [39]. The bacteria is recognizing the metabolism needed for the consumption of the sugars present [6] and no LA production takes place; so the reduction of this unproductive time is good for the overall process in order to minimize energetic costs. The lag phase for the highest sugar concentration evaluated was longer than the other two medium probably because the higher osmotic pressure and so the difficulty to adapt. In this way, the option of 80% medium was the best since it showed a significant shorter ($p<0.05$) lag phase than the 100% medium and, total productivity was not different ($p>0.05$) from the highest total productivity obtained in 60% medium.

The pineapple medium was efficiently used by *L.casei* subsp. *rhamnosus*. At each pineapple juice concentration studied, practically all the substrate was consumed by the end of fermentation. But not only the utilization of the substrate was maximum; also the yield of LA was high, which is essential to minimize purification costs.

According to the results present in Table **2**, the best option for production of L (+) LA could be from a medium composed of 80% (v/v in distilled water) of pineapple waste since the lag phase and total productivity did not differ significantly from those obtained in the medium of 60 % of pineapple waste. Even when the medium composed of 80% of pineapple waste reached a lower LA concentration than that of 100% waste; it required less fermentation time to achieve the highest yield, and to achieve a similar volumetric productivity obtained for the medium composed of 100% of pineapple waste.

In summary, the pineapple waste utilized in this study was suitable as a cheap carbon source for *L.casei* subsp. *rhamnosus* and for the production of L (+) LA. This liquid waste obtained from the processing of the fresh fruit, is very easy to handle, has no seeds and little insoluble fiber, and presents very low viscosity and a high concentration of simple sugars.

The hydrolysis of sucrose permitted a total consumption of the sugars and gave high yields (0.91g LA/g sugar). Even though, the productivity and time of fermentation are affected when 100% of pineapple juice is used, high concentrations of LA are obtained, which is desired for a cheap purification process. This pineapple waste, which is produced in high volumes in the world and requires little pretreatment and has good potential in the production of LA. The utilization of pineapple waste products to produce L (+) LA by fermentation can be a good alternative for the disposal of such industrial residues and also can reduce the cost of production of this additive.

CONCLUSION

Potential application of pineapple waste as a substrate to produce LA by fermentation with *Lactobacillus casei* subspecies *rhamnosus* was demonstrated. The bacterium was able to consume all the sugars present without any apparent inhibition, even in 100% pineapple juice medium. Pineapple juice waste is a good alternative for the industrial production of LA through fermentation due to its high final LA concentration which is very important for an economic process. Also, the use of a 100% pineapple juice in the substrate increases the amount of impurities in the broth and therefore the 80% juice is preferred regarding recuperation costs involved. The 80% pineapple juice was the best choice since there was no significant difference in total productivity and in time of lag phase between 60% and 80% pineapple juice while the 100% pineapple juice showed the lowest total productivity.

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