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HIGHLY EFFICIENT PRODUCTION OF L (+)-LACTIC ACID USING MEDIUM WITH POTATO, CORN STEEP LIQUOR AND CALCIUM CARBONATE BY *Lactobacillus rhamnosus* ATCC 9595

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Abstract - The objective of this study was to optimize the composition of the fermentation medium through a Central Composite Rotational Design (CCRD) for the production of L (+)- latic acid (LA) using *Lactobacillus rhamnosus* ATCC 9595 and potato, corn steep liquor (CSL) and calcium carbonate as raw materials. A CCRD with three independent variables (potato flour (PF), CSL and $CaCO_3$ concentrations) was performed for a total of 17 treatments. Kinetic studies were conducted, and samples were taken at time intervals of 0 to 72 h. Cell count, pH, reducing sugars and LA concentrations were determined. LA productivity and yield, sugar consumption and cell growth factor were calculated. The highest concentration of LA was 183.8 g/L, with 2.553 g/L.h productivity, 94.2% yield and 96.1 % sugar consumption.

Keywords: Lactic acid; Fermentation; Potato flour; Corn steep liquor; Response surface.

INTRODUCTION

Lactic acid (LA) has attracted great attention due to its widespread application, especially in the food, chemical, cosmetic and pharmaceutical industries. In addition, this organic acid has great potential for the production of polylactic acid (PLA), a biodegradable and biocompatible polymer driving the expansion of the LA market. However, compared to petrochemical plastic, PLA production is considered to be a relatively recent technology on an industrial scale; this is attributed mainly to its high cost of production (Abdel-Rahman *et al*., 2013).

Most of the LA in the world is produced using the fermentative pathway by the action of lactic bacteria (Abdel-Rahman *et al.*, 2011). However, these bacteria have significant nutritional requirements of minerals, vitamins and peptides specific to ensuring their growth (Hofvendahl and Hahn-Hägerdal, 2000). Research is now focused on the search for alternative and renewable substrates such as agro-industrial byproducts, including starch, cellulose, whey and molasses (Wee *et al.*, 2006).

Pure sugars have been the traditional carbon source for the production of LA (Hofvendahl and Hahn- Hägerdal, 1997), and yeast extract (YE) is the most commonly used nitrogen source (Altaf *et al.*,

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2007). One of the advantages of the use of refined products is to obtain pure LA, reducing pre-treatment and recovery costs (Hofvendahl and Hahn-Hägerdal, 2000).

However, in industrial processes, the cost of raw materials is an important factor for the economic production of LA (Tinoi *et al.*, 2005), and the search for low-cost raw materials to be used in the production of LA by fermentation aims to promote the development of more competitive processes (Martinez *et al.*, 2013).

Additionally, LA production by fermentation is an environmentally friendly process, according to the principles of green chemistry (Poliakoff *et al.*, 2002) and produces the optically pure isomer (D or L-lactic acid) (Chauchan *et al.,* 2007), since the optical purity of LA is important for the synthesis of PLA (Hofvendahl and Hahn-Hägerdal, 2000; Karp et al., 2011; Södergärd and Stolt, 2002).

The high level of losses and waste, or agro-industrial byproducts generated by the food industry, has led researchers to seek viable alternatives. With the expansion of potato processing industries, complete utilization of the raw material becomes more and more important due to the urgent demand for reducing feedstock waste and releasing the environmental pressure from potato residue. Industrial processing generates between 70 and 140 thousand tons of peels worldwide annually (Wu, 2016).

The potato (*Solanum tuberosum* L.) contains approximately 60-80% starch on a dry basis (Fernandes *et al*., 2010). Due to its high starch content, it is often used in the fermentation process. However, starch is not directly fermentable because it requires a prior hydrolysis of its chains. This hydrolysis may occur in two ways, either by acid treatment or enzymatic treatment. Enzymatic treatment has advantages over acid treatment in that it is more selective, expends less energy and produces glucose that can be directly fermented (Delgado *et al.*, 2009).

The existing literature has reported nitrogen sources of low-cost agricultural byproducts as an alternative to achieve a partial or complete replacement for YE (Yu *et al.*, 2008). Corn steep liquor (CSL), a byproduct of the corn manufacturing process (Gao and Yuan, 2011), is a potentially useful resource for YE substitution (Li *et al.*, 2010). CSL is a low-cost byproduct and has nutrients and minerals that are effective for fermentation (Martinez *et al.*, 2013).

This study aimed to optimize the production of L (+)-LA by *Lactobacillus rhamnosus* ATCC 9595 in a fermentation medium containing potatoes, CSL and calcium carbonate using response surface analysis methodology.

MATERIALS AND METHODS

Raw materials

Potatoes of the species *Solanum tuberosum* ssp. *tuberosum* (Agata cultivars) were bought in the city of Lavras, MG, Brazil. The CSL was donated by the company Ingredion Brazil - Industrial Ingredients Ltda., located in Mogi Mirim, SP, Brazil.

Microorganism

LA bacteria

The microorganism *L. rhamnosus* ATCC 9595, the non-amylolytic and homofermentative species used in this study, was provided by the Oswaldo Cruz Foundation - FioCruz (Rio de Janeiro, Brazil). This microorganism was previously selected by Alves (2014) for its good productivity of L (+)-LA.

Maintenance, standardization and stock culture

The microorganism was purchased in lyophilized form; therefore, an activation step was necessary. Activation of the *L. rhamnosus* ATCC 9595 culture was begun by transferring the lyophilized microorganism to a test tube containing 10 mL of MRS Broth (Man Rogosa and Sharpe Himédia®, comprising 10 g/L peptone, 5 g/L yeast extract, 10 g/L meat extract, 20 g/L glucose, 2 g/L dipotassium phosphate, 5 g/L sodium acetate, 2 g/L triammonium citrate, 0.20 g/L magnesium sulfate, 0.05 g/L manganese sulfate and 1 g/L Tween 80), that had been previously sterilized in an autoclave (121 ºC/15 min). Then, the cultures were kept in a BOD oven at 37 ºC/ 48 h. The temperature used is considered optimal for LA bacteria (LAB) (Axelsson, 2004).

The subsequent step was the activation of bacteria propagation. After verifying the increase in inoculum turbidity, the inoculums were then transferred to a 125mL Erlenmeyer flask containing 50 mL sterile MRS broth in an oven and were maintained in a BOD oven at 37 ºC/ 24 h.

Then, to prepare the stock cultures, a volume of 1 mL of new liquor, which was growing in MRS broth, was transferred to Eppendorf tubes, previously sterilized (121 ºC/15 min), and centrifuged (Spinlab model SL- 5AM) at 71,250 g/min in order to separate

the microorganism. The supernatant was discarded, and the microorganism was centrifuged and kept in Eppendorf tubes. Subsequently, 1 mL sterile freezing medium was added to each Eppendorf tube and, after identification, the cultures were stored under freezing conditions for future use as stock cultures.

The freezing medium was prepared by adding 15 mL glycerol, 0.5 g bacteriological peptone, 0.3 g yeast extract and 0.5 g NaCl to 100 mL of distilled water. After mixing, the pH was adjusted (7.2 to 7.4) using 0.1 mol/L NaOH, and the solution was sterilized (121 ºC/15 min) (Souza *et al.*, 2015).

Inoculum preparation

The inoculum was made from the stock culture of *L. rhamnosus* ATCC 9595. In a test tube containing 10 mL sterile MRS broth, 1 mL of stock culture was added and kept in an incubator at 37 ºC/ 24h. Subsequently, in a 125mL Erlenmeyer flask containing 50 mL sterile MRS broth, 10% (v/v) of the above seed culture broth (5 mL) was added and maintained at 37 ºC. After 12 h, the samples were collected for reading in a spectrophotometer (600 nm) and, according to the absorbance value, 1% (v/v) inoculum was transferred to an Erlenmeyer flask containing 300 mL of fermentation medium corresponding to a count of 10^7 CFU/mL at the beginning of the assays (Alves, 2014).

Experimental design

A central composite rotational design (CCRD) for three variables was employed to determine the optimum conditions for LA production, based on preliminary studies by the authors. Three independent variables were studied in a total of 17 experiments: carbon source concentration (PF), nitrogen source concentration (CSL) and $CaCO_3$ concentration. The relationship between the coded and real values of the independent variables for the CCRD is shown in Table 1.

All treatments were performed in duplicate, and the flasks were sealed with cotton and incubated in a BOD oven at 37 ºC, which is the optimal temperature for LAB (Axelsson, 2004). For each treatment, a kinetic study was performed in which 15-mL samples were taken aseptically at time intervals of 0, 12, 24, 48 and 72 h. Volumes of 1 mL were removed from each treatment aseptically for cell counts by plating at time intervals of 0, 24, 48 and 72 h using the pour plate method; they were then incubated in a BOD oven at 37 ºC/72 h. The plates considered for counting contained between 25 and 250 colonies (Silva *et al.*, 2010). The withdrawn samples were centrifuged at 2260 g/30 min; the supernatant was stored in freezing conditions (-5 ºC) and was then subjected to reducing sugar, pH and LA analysis. LA productivity and yield, sugar consumption and cell growth factor were calculated. Statistical analyses were performed using Statistica 8.0 (Statistica, 2008) software at 5% significance. Cell growth factor was determined as the ratio between final and initial lactic acid bacteria cell concentration.

Upon completion of the CCRD, the models were adjusted (Equation 1) and response surfaces, contour curves and desirability profiles were determined, according to the methodology recommended by Rodrigues and Iemma (2014).

$$y = \beta'_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad (1)$$

where β'_0 is the intercept term, β_1, β_2 e β_3 are the linear coefficients; β_{12}, β_{13} e β_{23} are the interaction coefficients; β_{11}, β_{22} e β_{33} are the quadratic coefficients and x_1, x_2 e x_3 are the coded variables.

The model validation was performed through the repetition of a test in triplicate under conditions for the best production of L (+)-LA. The predicted concentration of LA was then compared with experimental data.

Fermentation medium preparation

Forty-five kilograms of potato were acquired, washed in potable water, peeled, chopped and left in an oven with air circulation (FANEM, Brazil) at 65 ºC/72 h. The material was turned over every 24 h. After being dried, the potatoes were ground in a Willye Star Model FT 80/1 knife mill, and the flour was sieved on a 14-mesh screen casting (1.41 mm). After being ground, the batch of material was homogenized by shaking and was stored in PET plastic containers. To

Table 1. Relationship between the coded and real values of the independent variables for LA production by *L. rhamnosus* ATCC 9595.

Variables	-1.68	-1	0	1	1.68
X_1= PF (g/L)	113	140	180	220	247
X_2 = CSL (g/L)	15	25	40	55	65
X_3=$CaCO_3$(g/L)	5	15	30	45	55

obtain the hydrolysate, the PF was weighed according to the experimental design. The enzymes used in this study were provided by LNF Latin American Company - Brazil. The process of enzymatic hydrolysis of starch was based on that found in Menezes *et al.* (2016).

After completion of the hydrolysis step, the mash was filtered using a cloth filter and supplemented according to the design; after homogenization, the mash was sterilized by autoclaving (121 ºC/15 min). The must was cold, and the inoculum was added. Fermentation was conducted in a BOD oven without stirring at 37 ºC/72 h in flasks sealed with cotton. The CCRD treatments were performed in duplicate.

Batch fermentation in a bioreactor

After verifying the best condition with CCRD, a scale up was performed using a 5L bioreactor (Sartorius Stedim Biotech bioreactor Biostat A plus, Germany). The same procedures used previously in the study to prepare the inoculum and the potato fermentation medium were used.

A volume of 3L of fermentation medium was prepared and autoclaved at 121 ºC/20 min in the bioreactor. The inoculum was added at a concentration of 1% (v/v), and the batch fermentation was carried out at 37 ºC without agitation or aeration, according to Alves (2014).

A kinetic study was performed in which 30 mL samples were taken aseptically at time intervals of 0, 12, 24, 48 and 72 h. For cell counting, 1 mL was removed by plating at time intervals of 0, 24, 48 and 72 h. The withdrawn samples were centrifuged at 2260 g/30 min, and the supernatant was stored in freezing conditions (-5 ºC) and then subjected to analysis in triplicate for reducing sugars, pH and LA. LA productivity and yield, sugar consumption and cell growth factor were calculated.

Analytical methodology

All analyses were performed in triplicate, and the results were expressed as the mean and standard deviation of these values.

Characterization of raw materials

The potatoes, PF and CSL were characterized by analysis of the moisture content, lipids, proteins, ash, crude fiber and density using the AOAC method (AOAC, 2005), and the results were expressed on a wet basis of g/100 g. The starch content was measured by the modified Somogyi method used by Nelson (1944).

Determination of reducing sugar

The concentration of reducing sugar was determined in the enzymatic hydrolysis process and in samples from fermentation following the methodology described by Miller (1959) using the 3,5-dinitrosalicylic acid method (DNS). The absorbance values were measured in a spectrophotometer (FEMTO 700 S Soft., Brazil) at 540 nm and were expressed in g glucose/L.

Determination of pH and titratable acidity

The pH values were determined directly in the sample supernatant using a digital pHmeter (Tecnopon, MPA-model 210). The acidity was determined by the AOAC method (AOAC, 2005).

Determination of LA

The LA content was analyzed by high-performance liquid chromatography (HPLC). The samples were thawed and centrifuged (2K15 Sigma®) at 4 ºC with 15052 g/10 min. The supernatant was separated and filtered through a PVDF membrane (0.22 µM pore size and 25 mm diameter); the samples were then diluted with deionized water before being injected into the chromatograph and were kept under freezing conditions for later analysis.

The chromatograph used was a Shimadzu model LC-10Ai (Shimadzu Corp., Japan) equipped with refractive index detectors (RID-10A model, ultraviolet, model SPD-10Ai). The column used was a Shimpack SCR-101H ion exchange model with 7.9 mm diameter X 30 cm length (Shimadzu).

The LA was analyzed using modified methods from Schwan *et al.,* (2001) in duplicate. A UV detector (210 nm) was used, and the column was operated at 50 ºC. The mobile phase used 100 mM perchloric acid at a flow rate of 0.6 mL/min. The quantification was performed in comparison with the calibration curve for LA, as determined with Sigma® brand certified standards. The presence of L (+)-LA was confirmed using an enzymatic kit (Megazyme®, Wicklow, Ireland) with 98% confidence.

RESULTS AND DISCUSSION

Characterization of raw materials

The chemical analysis results of the potato used for the preparation of flour, the chemical composition of PF and of the CSL used in the formulation of the fermentation media are presented in Table 2.

Table 2. Results of chemical composition analysis of potato cv. Agata, PF and CSL.

Parameters (%)	Potato paded	PF 1st batch	PF 2nd batch	CSL
Humidity	85.7 ± 0.2	7.0 ± 0.4a	4.12 ± 0.01b	57.8 ± 0.4
Lipid	0.02 ± 0.00	0.37 ± 0.05a	0.33 ± 0.01a	0.07 ± 0.02
Protein	1.87 ± 0.14	8.35 ± 0.44a	9.0 ± 0.8a	19.68 ± 0.25
Ashes	0.78 ± 0.27	4.01 ± 0.08a	4.4 ± 0.4a	6.05 ± 0.09
Fiber	0.43 ± 0.02	2.42 ± 0.72a	2.2 ± 0.8a	0.00 ± 0.02
Starch	7.8 ± 0.8	77 ± 6a	79 ± 4a	n.d.
Reducing sugars (g/ L glucose)				1.112 ± 0.005
Density (g/mL)		n.d.		1.202 ± 0.005
pH				3.98 ± 0.05
Acidity in LA (g/L)				14.0 ±0.7

The 1st batch of PF was used in the CCRD, and the 2nd batch of PF was used in the model validation on a larger scale.
The means followed by the same letter between lines do not differ significantly at 5% significance using Tukey's test.
n.d. not determined

Evangelista *et al.* (2011) found 14.46 g/100 g dry matter, 10% starch, 0.86% ash, 1.42% protein and 0.34% fiber in potatoes from the Agata cultivar. The values found for potato chemical compositions are close to those in the literature; however, several factors can interfere with the chemical composition of potato tubers, including climate, soil type, temperature, maturity of the tubers, environmental conditions of each crop, and other factors (Evangelista *et al.,* 2011; Fernandes *et al.,* 2010).

The values of moisture for each PF batch are within the values stipulated by Brazilian legislation (the maximum humidity is 15% for flour). Freitas *et al.,* (2015) found levels of 7.90% humidity for PF from the Monalisa cultivar. There was no significant difference between the batches of PF except for moisture.

The results of the chemical composition analysis of the CSL are shown in Table 3 and are close to those found by Chiani *et al.* (2010), who obtained 47.5% moisture, 1% fat, 20.5% protein, 8.8% ash, 1% fiber and a pH between 4 and 5 for CSL. CSL possesses great variability in relation to its chemical composition (Ligget and Koffler, 1948). This variability is due to different geographical origins, harvest seasons and corn manufacturing processes (Gao and Yuan, 2011).

CCRD

For all treatments in the CCRD, after the process of PF starch hydrolysis, glucose yields above 95% were obtained, and for treatments with a concentration of 113 g/L, 140 g/L, 180 g/L, 220 g/L and 247 g L potato flour, the glucose concentration obtained was 104 g/L, 129 g/ L, 166 g/L, 203 g/L and 228 g/L respectively.

Table 3 shows the real (in parentheses) and coded values of the independent variables of PF concentration, CSL and $CaCO_3$, as well as the dependent variables of LA production, yield, glucose consumption and cell growth factor.

The highest LA concentration (183.8 g/L) after 72 h of fermentation was obtained in treatment 8 with 220 g/ L PF, 55 g/L CSL and 45 g/L $CaCO_3$; treatment 8 also presented the highest value of productivity during the fermentation process (2,553 g/L.h), 96.1% sugar consumption and 94.2% yield. It was observed that the LAB increased by 2 log units (between 4.4 and $6x10^7$ CFU/mL to $2.3x10^9$ CFU/mL), as can be seen in Figure 1.

Satisfactory results were obtained in treatment 14 (157 g/ L LA, 97.49% yield, 2.181 g/L.h productivity and 96.9% sugar consumption), in which the fermentation medium consisted of 180g/L PF, 40 g/L CSL and 55 g/L $CaCO_3$. It was also found that the microorganism *L. rhamnosus* increased its cellular concentration by 3 logarithmic units (from $1.6x10^7$ CFU/ mL to $1.3x10^{10}$ CFU/ mL) throughout the fermentation.

Wang *et al.*, (2010) found efficient production of L (+)-LA from cassava flour by *L. rhamnosus*. The highest concentration of LA (175.4 g/L) was obtained using 275 g/ L of manioc flour (a total of 222.5 g sugar/L) during a simultaneous saccharification and fermentation process (SSF) in a batch with 15 g/L of YE and 165 g/L $CaCO_3$ added; the productivity was 1.8 g/L.h, and the yield was 0.71 g/g. This study thus had a greater production of LA in relation to productivity and yield.

From the experimental data presented in Table 3, a multiple regression analysis was conducted for the variables production of LA (g/L) yield (%), consumption of sugar (%), and cell growth

Table 3. Matrix CCRD (real and coded values) of the independent variables and the results of the dependent variables LA (gL^{-1}) yield (%), glucose consumption (%) and cell growth factor.

Treat.	PF(g/L)	CSL (g/L)	$CaCO_3$(g/L)	LA (g/L)	Yield (%)	Consumption glucose (%)	Cell growth factor
1	-1 (140)	-1 (25)	-1 (15)	72.0 ± 0.0	90.0 ± 3.5	62.0 ± 2.3	233 ± 64
2	-1 (140)	-1 (25)	1 (45)	134.7 ± 0.0	100 ± 0.0	92.2 ± 1.2	178 ± 15
3	-1 (140)	1 (55)	-1 (15)	78.5 ± 0.0	90.3 ± 2.6	67.2 ± 1.9	93 ± 17
4	-1 (140)	1 (55)	1 (45)	136.5 ± 0.0	100 ± 0.0	98.6 ± 0.0	86 ± 4
5	1 (220)	-1 (25)	-1 (15)	83.9 ± 0.0	79.6 ± 2.9	52.0 ± 2.0	122 ± 3
6	1 (220)	-1 (25)	1 (45)	93.7± 1.1	50.4 ± 0.6	91.5 ± 1.0	230 ± 27
7	1 (220)	1 (55)	-1 (15)	82.0 ± 1.9	62.2 ± 2.7	65.0 ± 4.0	74 ± 8
8	1 (220)	1 (55)	1 (45)	183.8 ± 0.2	94.2 ± 1.3	96.1 ± 0.8	45 ± 1
9	-1.68(113)	0 (40)	0 (30)	104.9 ± 0.0	100 ± 0.0	90.7 ± 0.9	130 ± 28
10	1.68 (247)	0 (40)	0 (30)	98.3 ± 0.0	75.6 ± 0.1	57.0 ± 1.0	132 ± 3
11	0 (180)	-1.68 (15)	0 (30)	97.0 ± 6.4	100 ± 0.1	51.7 ± 6.5	97 ± 25
12	0 (180)	1.68 (65)	0 (30)	137.4 ± 1.0	100 ± 0.4	74.2 ± 0.3	139 ± 53
13	0 (180)	0 (40)	-1.68 (5)	57.0 ± 5.5	86.9 ± 1.8	39.4 ± 0.8	302 ± 117
14	0 (180)	0 (40)	1.68 (55)	157.0 ± 1.9	97.4 ± 0.2	96.9 ± 0.2	496 ± 161
15	0 (180)	0 (40)	0 (30)	118.2 ± 1.7	97.0 ± 3.2	67.0 ± 5.0	100 ± 14
16	0 (180)	0 (40)	0 (30)	113.74± 1.2	97.0 ± 3.2	64 ± 5.0	74 ± 14
17	0 (180)	0 (40)	0 (30)	109.86 ± 2.3	94.4 ± 3.2	70 ± 5.0	78 ± 14

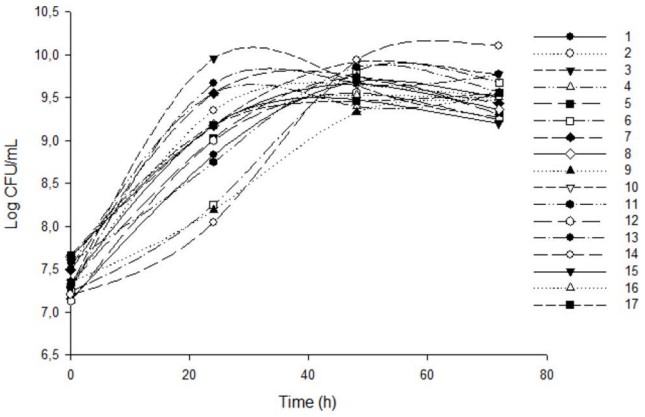


Figure 1. Growth kinetics of *L. rhamnosus* ATCC 9595 for each treatment after 72 h of fermentation

factor. Parameters were considered significant at $p < 0.05$. Table 4 shows the regression coefficients for the production of LA and sugar consumption, and significant parameters are shown in bold. Table 5 shows the coefficients of the model with only the significant terms for the production of LA and sugar consumption.

According to the results shown in Tables 4 and 5, the linear terms of the concentrations of CSL and $CaCO_3$ had a significant positive effect on the production of LA ($p < 0.05$). For the consumption of sugar, the linear term of the concentration of $CaCO_3$ had a significant positive effect at 95% confidence, meaning that an increase in $CaCO_3$ provides increased

Table 4. Regression coefficients for the production of L (+)-LA and sugar consumption by *L. rhamnosus* ATCC 9595.

	LA (g/L)		Sugar consumption (%)	
Factor	Regress coeff.	p	Regress coeff.	p
Mean/ Interc.	113.960	0.000	66.356	0.000
X_1(L)	0.774	0.838	-5.292	0.067
X_1(Q)	-4.390	0.312	4.581	0.133
X_2(L)	**12.047**	**0.013**	4.906	0.085
X_2(Q)	1.131	0.787	0.719	0.797
X_3(L)	**29.333**	**0.000**	**16.775**	**0.002**
X_3(Q)	-2.491	0.555	2.573	0.372
X_1 X_2	9.989	0.074	0.740	0.823
X_1 X_3	-1.141	0.818	1.124	0.735
X_2 X_3	10.942	0.055	-0.902	0.786
R^2	0.92		0.89	

X_1: PF, X_2: CSL, X_3:$CaCO_3$.

Table 5. ANOVA of reparameterized models for the production of L (+)-LA and sugar consumption by *L. rhamnosus* ATCC 9595.

LA (g/L)						
Factor	**SS**	**df**	**MS**	F_{value}	$F_{(5\%)}$	**p**
Regression	13720.63	2	6860.31	28.40	3.74	0.00
Error	3381.31	14				
Total	17101.93	16				
R^2= 0.80						
Sugar consumption (%)						
Factor	**SS**	**df**	**MS**	F_{value}	$F_{(5\%)}$	**P**
Regression	3840.08	1	3840.07	36.65	4.54	0.00
Error	1571.44	15	104.76			
Total	5411.52	16				
R^2= 0.71						

[SS]Sum of squares, [df]Degrees of freedom, [MS]Mean square.

LA production and consumption of sugar response and that an increase in CSL concentration indicates that there will be an increase in LA production.

The encoded regression model obtained for LA production is expressed in equation 2, and equation 3 shows the regression model obtained for the consumption of sugar (CS (%)).

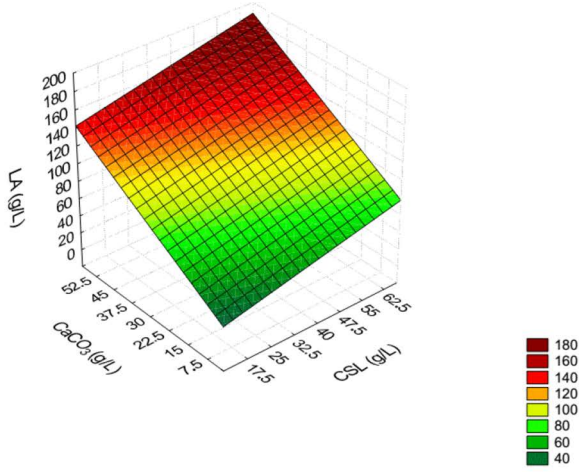
$$LA(g/L) = 109.34 + 12.047x_2 + 29.333x_3 \quad (2)$$

$$CS(\%) = 72.67 + 16.775x_3 \quad (3)$$

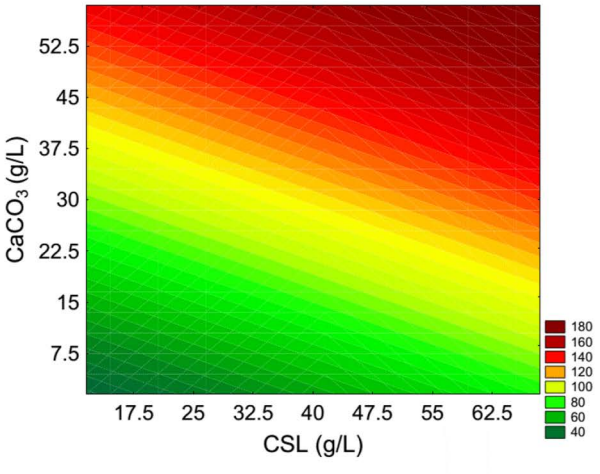
Figure 2 shows the response surface and contour curve to produce LA by the microorganism *Lactobacillus rhamnosus* ATCC 9595.

By analyzing Figure 2 and Table 3, a region with very high values for the production of L (+)-LA can be defined that corresponds to fermentation media at concentrations greater than 45 g/L CaCO3, greater than 47.5 g/L CSL and greater than 180 g/L PF. The PF level can be explained because Figure 2 was built for 180 g/L PF in the fermentation medium (central point of the CCRD) and the best results for LA production were obtained using 180 and 220 g/L of potato flour (Table 3). The addition of $CaCO_3$ for neutralizing LA formation allowed fermentation to proceed for a longer time under optimal pH conditions and increased the production of L (+)-LA by *L. rhamnosus* ATCC 9595.

LABs are fastidious microorganisms that require multiple amino acids and vitamins for their growth (Yu *et al.*, 2008). As the synthesis of lactic acid by fermentation is associated with cell growth, there is no product formation if the medium does not have an adequate concentration of nitrogen for promoting growth (Pritchard and Coolbear, 1993). In this study, an efficient production of L (+)-LA was obtained from



(A)



(B)

Figure 2. Response surface (A) and contour curve (B) for LA production by *Lactobacillus rhamnosus* ATCC 9595 as a function of the variables CSL and $CaCO_3$, for fermentation media with 180 g/L PF.

the addition of CSL as a source supplementing the fermentation medium made of hydrolyzed PF.

Yu *et al.* (2008) used CSL in place of YE, glucose, molasses, Tween 80 and $MnSO_4$ for LA production by *L. rhamnosus* CGMCC 1466. The maximum concentration of LA was 113.05 g/L using 118.20 g/L glucose, 37.27 mL/L of molasses, 42.54 g/L CSL, 1.52 mL/L Tween 80 and 0.30g/L $MnSO_4$. The authors also compared the medium optimized with CSL to another medium comprising YE as a nitrogen source, yielding an increase of 30.4% in LA production.

Liu *et al.* (2010) studied the effects of five alternative sources of nitrogen: malt sprouts, corn steep liquor (CSL), NH_4Cl, NH_4NO_3 and diamine citrate on the production of L (+) lactic acid by *Lactobacillus plantarum* As1.3. Malt buds and CSL showed significant effects on lactic acid production and their optimum values were 16.0 g/L and 12.0 g/L, respectively, with 3.20 g/ L.h LA productivity and 0.98 g/g LA yield. The results obtained by the authors indicate that the production of LA can be improved with alternative sources of low cost nitrogen.

Thus, CSL may be used to replace YE because the high cost of YE has a negative impact on industrial processes (Oh *et al.*, 2005). It is known that CSL has in its composition proteins, amino acids, B vitamins and other nutrients (Marták *et al.*, 2003) and has been widely studied for supplementation in fermentation processes (Coelho *et al.*, 2010).

Wee *et al.* (2004) evaluated the productivity, yield, consumption of sugar and production of L (+)-LA by *Enterococcus faecalis* RKY1 using 200 g/L molasses (equivalent to 102 g/L glucose) under different pH (5 to 9). The authors noted that the highest production (96.1 g/L) and highest yield obtained from LA (96.3%) was at a pH of 6. In the present study fermentation began at pH values between 6 and 7, and the best production of LA was obtained using the highest concentrations of $CaCO_3$ between 45 and 55 g/L with a final pH ranging from 4.20 to 4.85. Figure 3 shows the decay in pH among the treatments for a 72h fermentation process.

Nakano *et al.* (2012) studied the effects of $Ca(OH)_2$, NH_4OH and NaOH as neutralizing agents for LA production by *L. delbrueckii* from broken rice during SSF. According to the authors, the molarity of lactate in the fermentation medium is lower when adding $Ca(OH)_2$, because to form 1 mol calcium lactate it is necessary to combine two mols of lactate ions and 1 mol calcium cation. This results in high production efficiency in the neutralization of LA. The same is true when using $CaCO_3$ in the fermentation medium for pH control. The authors observed that when 25 % (w/v)

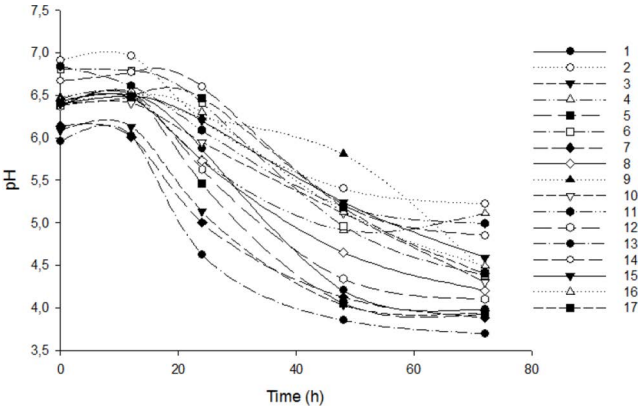


Figure 3. pH evolution among the treatments for LA production by *Lactobacillus rhamnosus* ATCC 9595 for 72 h.

of $Ca(OH)_2$ was used, the maximum concentration of lactic acid produced after 22 h fermentation was 79.0 g/L. In comparison, the maximum concentrations of lactic acid produced using NH_4OH and NaOH were 63.3 g/L and 64.1 g/L, respectively. When $Ca(OH)_2$ was used as the neutralizing agent, the lactic acid yield was considerably higher (80.6%) and high viable cell count was maintained for at longer period compared to those of NH_4OH and NaOH (Nakano *et al.*, 2012).

Mussatto *et al,* (2008) evaluated the pH control, with addition of 5M NaOH, and the supplementation of MRS medium with barley hydrolyzate. The pH-controlled medium reached production of 35.54 g/L of lactic acid with 0.99 g/g of glucose consumed, while without pH control the LA production was 13.02 g/L. The authors observed that, without pH control, metabolism of the microorganism *Lactobacillus delbrueckii* UFV H2B20 was affected, resulting in lower cell growth during fermentation and glucose consumption for the production of LA ceased after 12 hours of fermentative process.

With regard to LA yield (Table 3), high yields (above 90%) were obtained in the majority of the treatments. Fermentation assays containing calcium carbonate concentrations above 30 g/L showed higher lactic acid yields, while higher potato flour contents in the media tended to decrease the yield. For *L. rhamnosus* growth factor, the values ranged from 45 (Treatment 8) to 496 and, on average, cell count increased by 2 logarithmic units.

Table 6 shows the regression coefficients for the yield of LA and growth factor *L. rhamnosus* ATCC 9595, and the significant parameters are shown in bold.

According to the statistical results presented in Table 6, only the linear term of PF concentration was statistically significant for LA yield ($p < 0.05$). The sign of the coefficient is negative, and the decrease

Table 6. Regression coefficients for yield of LA and growth factor *L. rhamnosu* s ATCC 9595.

	Yield		Cell growth factor	
Factor	Regress coeff.	p	Regress coeff.	p
Mean/ Interc.	98.779	0.000	90.112	0.132
X_1 (L)	**-9.893**	**0.014**	-8.475	0.743
X_1 (Q)	-5.822	0.128	-3.803	0.893
X_2 (L)	1.949	0.544	-28.907	0.282
X_2 (Q)	-1.500	0.670	-8.406	0.767
X_3 (L)	2.949	0.367	25.131	0.345
X_3 (Q)	-4.281	0.245	**91.151**	**0.012**
X_1 X_2	3.250	0.443	-0.125	0.997
X_1 X_3	-2.100	0.616	17.625	0.603
X_2 X_3	7.625	0.098	-11.125	0.741
R^2	0.74		0.70	

X_1: PF, X_2: CSL, X_3: $CaCO_3$.

in PF concentration provides an increased LA yield. It is known in the literature that there is a reduction of the concentration of LA due to inhibition of the microorganism by high substrate concentration (Abdel-Rahman *et al.*, 2013). The use of glucose concentrations up to 200 g/L in the fermentation medium did not inhibit LA production by *Lactobacillus* (Min-tian *et al.*, 2005).

The only significant coefficient ($p > 0.05$) for the variable growth factor *L. rhamnosus* ATCC 9595 was the quadratic term for the concentration of $CaCO_3$ (g/L). The coefficient is positive, indicating that the growth of *Lactobacillus* was greater at higher concentrations of $CaCO_3$. The ANOVA for LA yield and cell growth factor is shown in Table 7. It is observed that, although the regression models were significant for both responses, LA yield and growth factor *L. rhamnosus* ATCC 9595, using the Fisher test ($F > F_{5\%}$), the models did not give a good fit, which can be verified from the coefficient of determination (R^2), and it was not possible to generate a graphical response surface for these dependent variables.

Model validation

The estimate of the optimal conditions for the validation of LA production and consumption patterns of sugar was based on the proposed statistical models with the aid of a simultaneous optimization technique called "function desirability". Optimized concentrations of the variables studied were greater than120 g/L of PF, up to 55 g/L CSL and greater than 45 g/L $CaCO_3$.

To validate the model, Table 8 shows the actual values in g/L of PF concentration, CSL and $CaCO_3$ as well as the responses obtained for experimental and predicted LA concentration and relative error, and for the experimental and predicted sugar consumption and relative error.

It can be seen from Table 8 that the relative error for both LA and the consumption of sugar were low, indicating that the results obtained in the validation test were satisfactory compared to the model predictions.

Identification of isomers produced

The determination of the isomer L (+) using an enzymatic kit confirmed the presence of this isomer in the sample. In previous studies conducted by Lu *et al.* (2010) and Wang *et al.* (2010), it was found that *L. rhamnosus* can produce the isomer L (+)-LA at a high level of purity.

The ability to produce optically pure L (+)-LA from agricultural byproducts is important for industrial application (Wee *et al.*, 2008). The allocation of LA in the industrial sector varies according to the degree of optical purity. LA with purity from 20-50% is labeled technical grade, above 80% is labeled food grade and above 90% is labeled pharmaceutical grade plastic (Vijayakumar *et al.*, 2008).

Bioreactor fermentation kinetics

LA production by *L. rhamnosus* ATCC 9595 was performed in a bioreactor using the same concentration employed in the model validation step. The kinetics of substrate consumption could be observed, as well as the formation of LA (Figure 4A), changes in pH and the cell viability of *L. rhamnosus* ATCC 9595 (Figure 4B).

It can be seen in the graphs that there was no consumption of glucose from the hydrolyzed PF up to 24 h, and there was not a significant lowering of the pH or LA formation during this period. The viability

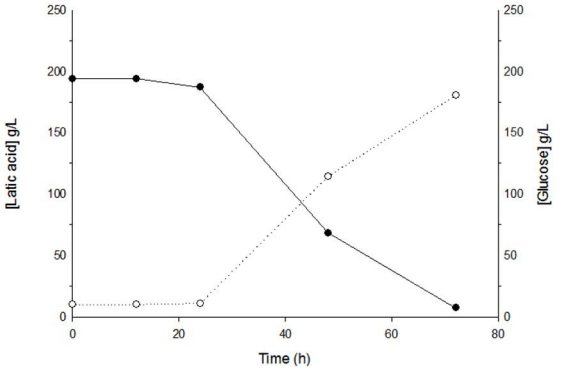
Table 7. ANOVA of reparameterized models for yield of LA and growth factor *L. rhamnosus* ATCC 9595.

Yield of LA						
Factor	**SS**	**df**	**MS**	**F**	**$F_{(5\%)}$**	**p**
Regress	1335.51	1	1335.51	9.43	4.54	0.00
Error	2123.16	15	141.54			
Total	3458.67	16				
R^2= 0.39						
Growth factor *L. rhamnosus* ATCC 9595						
FV	**SS**	**df**	**MS**	**F**	**$F_{(5\%)}$**	**p**
Regress	114517.58	1	114517.57	20.39	4.54	0.00
Error	84214.7	15	5614.3			
Total	198732.2	16				
R^2= 0.55						

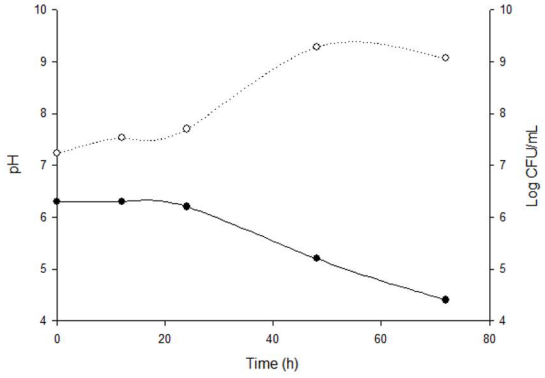
[SS]Sum of squares, [df]Degrees of freedom, [MS]Mean square.

Table 8. Validation of the model for the production of L (+)-LA and sugar consumption by *L. rhamnosus* ATCC 9595.

Medium composition			LA (g/L)			Sugar consumption (%)		
Components	Real	Cod.	Exp.	Pred.	Error	Exp.	Pred.	Error
PF (g/L)	210	0.75						
CSL (g/L)	65	1.68	179.2 ± 2.8	178.8	0.2	97.2 ± 0.7	100	-3.7
$CaCO_3$ (g /L)	55	1.68						



(A)



(B)

Figure 4. (A) Kinetics of substrate consumption and production of L (+)-LA. (•) glucose and (o) LA (B) Evolution of pH and growth kinetics of *L. rhamnosus* ATCC 9595 during fermentation in a bioreactor for the production of L (+)-LA. (o) log CFU/mL and (•) pH

of cells, however, increased after the addition of the inoculum ($1.7x10^7$ CFU/mL to $5.0x10^7$ CFU/mL) in the first 24 h.

Beginning at 24 h of fermentation, a continual decrease in glucose uptake can be seen up to 72 h, when the substrate was consumed almost completely. The opposite occurred for LA formation, which increased continuously after 24 h of fermentation and had produced 180.4 g/L of LA at the end of 72 h.

The evolution of pH, according to Figure 4B, shows a reduction after 24 h of cultivation with the concomitant production of LA. During the period from 24 to 48 h of fermentation, LA production increased from 11 g/L to 114.36 g/L, and the pH ranged from 6.2 to 5.2. According to Yuwono and Kokugan (2008), a pH between 5 and 6 is ideal for the growth and production of LA for most *Lactobacillus*.

The pH at the end of fermentation was 4.4. This value agrees with Panesar *et al.* (2007), who claim that low pH values lead to the inhibition of LA production and the cessation of fermentation at pH values below 4.5. The addition of $CaCO_3$ for neutralizing LA formation allowed fermentation to proceed for a longer time under optimal pH conditions for the production of L (+)-LA by *L. rhamnosus* ATCC 9595.

In the present study, 180.45 g/L LA, 96.7% yield, 2.506 g/L.h LA productivity and 68 growth factor were obtained in the bioreactor after 72 h, similar to the results found with the same composition of

fermentation medium held in flasks (179.2 g/L LA, 97.2% yield, 2.490 g/L.h LA productivity and 110 growth factor).

CONCLUSION

The carbon and nitrogen sources obtained from agro-industrial byproducts have proven to be suitable for LA production, lowering the cost of production and contributing to more sustainable processes that follow the principles of green chemistry. PF may be added in amounts up to 220 g/L without altering LA production. CSL was shown to be an excellent substitute for YE and calcium carbonate and contributing to higher performance and productivity of the process. The application of response surface methodology proved to be a useful tool for obtaining high yield and production of *L. rhamnosus* ATCC 9595 using agro-industrial byproducts.

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NOMENCLATURE

BOD - Biochemical Oxygen Demand
CCRD - Central Composite Rotational Design
CFU - Colony Forming Units
CSL - Corn Steep Liquor
FioCruz - Oswaldo Cruz Foundation
HPLC - High Performace Liquid Chromatography
L(+) - Levorotatory
L. - *Lactobacillus*
LA - Lactic Acid
LAB - Lactic Acid Bacteria
PET - Polyethylene terephthalate
PF - Potato Flour
PLA - Polylactic acid
PVDF - Polyvinylidene difluoride
R^2 - Determination coefficient

REFERENCES

Abdel-Rahman, M. A., Tashiro, Y., Sonomoto, K., Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: Overview and limits. J. Biotechnol., 156, 286-301(2011).

Abdel-Rahman, M. A., Tashiro, Y., Sonomoto, K., Recent advances in lactic acid production by microbial fermentation processes. Biotechnol. Adv., 31, 877-902 (2013).

Altaf, M. D., Naveena, B. J., Reddy, G., Use of inexpensive nitrogen sources and starch for L (+)-lactic acid production in anaerobic submerged fermentation. Bioresource Technol., 98, 498-503(2007).

Alves, M. L. F., Produção de L (+) ácido lático por bactérias láticas utilizando meios com batata. Federal University of Lavras. Dissertation (2014).

Association of Official Analytical Chemists. Official methods of analysis of Association Analytical Chemists, 18th edn, Gaithersburg, Maryland (2005).

Axelsson, L., Lactic acid bacteria: classification and physiology. In: Salminen S, Von Wright A, Ouwehand A (ed) Lactic acid bacteria: microbiological and functional aspects. 3rd edn. New York (2004).

Barampouti, E.M.P., Vlyssides, A.G., Dynamic modeling of biogas production in an UASB reactor for potato processing wastewater treatment. Chemical Eng. J., 106, 53-58 (2005).

Chauhan, K., Trivedi, U., Patel, K. C. Statistical screening of medium components by Plackett-Burman design for lactic acid production by *Lactobacillus* sp. KCP01 using date juice. Bioresource Technol., 98, 1, 98-103, (2007).

Chiani, M., Akbarzadeh, A., Farhangi, A., Mehrabi, M.R. Production of desferrioxamine B (desderal) using corn steep liquor in *Streptomyces pilosus*. Pak. J. Biol. Sci., 13, 1151-1155 (2010).

Coelho, L. F., Lima, C. J. B., Bernardo, M.P., Alvarez, G. M., Contiero, J., Improvement of L (+)-latic acid production from cassava wasterwater by *Lactobacillus rhamnosus* B 103. J. Sci. Food Agri., 90, 1944-1950 (2010).

Delgado, R., Castro, A. J., Vazquez, V. A kinetic assessment of the enzymatic hydrolysis of potato (*Solanum tuberosum*). Food Sci. Technol., 42, 797-804 (2009).

Evangelista, R. M., Nardin I.,Fernandes, A. M., Soratto, R. P., Nutritional quality and postharvest greening of tubers from potato cultivars. Pesq. Agropec. Bras., 46, 953-960 (2011).

Fernandes, A. M., Soratto, R. P., Evangelista, R. M., Nardin, I., Qualidade físico-química e de fritura de tubérculos de cultivares de batata na safra de inverno. Horti. Bras., 28, 299-304 (2010).

Freitas, A. A., Kwiatkowski, A., Tanamati, A. A. C,Fuchs, R. H. B. Use of white potato flour (*Solanum tuberosum* L.) cv. Monalisa in mixture to chicken breasts. Exact and Earth Sciences, Agrarian Sciences and Engineering 11, 17-26 (2005)

Gao, Y., Yuan, Y. J., Comprehensive quality evaluation of corn steep liquor un 2-keto-L-gulonic acid fermentation. J. Agri. Food Chem., 59, 9845-9853 (2011).

Hofvendahl, K., Hahn-Hägerdal, B., L-lactic acid production from whole wheat flour hydrolysate using strains of *Lactobacilli* and *Lactococci*. Enzyme Microb. Tech., 20, 301-307(1997).

Hofvendahl, K., Hahn-Hägerdal, B., Factors affecting the fermentative lactic acid production from renewable resources. Enzyme Microb.Tech., 26, 87-107(2000).

Karp, S. G., Igashiyama, A. H., Siqueira, P. F., Carvalho, J.C., Vandenberghe, L. P. S., Tomaz-Soccol, V., Coral, J., Tholozan, J. C., Pandey, A., Soccol, C. R. Application of the biorefinery concept to produce L-lactic acid from the soybean vinasse at laboratory and pilot scale. Bioresource Technol., 102, 1765-1772 (2011).

Li, Z., Han, L., Ji, Y., Xiaonan, W., Tan, T., Fermentative production of L-lactic acid from hydrolysate of wheat bran by *Lactobacillus rhamnosus*. Biochem. Eng. J., 49, 138-142 (2010).

Liggett, W. R., Koffler, H., Corn steep liquor in microbiology. Bacteriol. Rev., 12, 297-311(1948).

Liu, B., Yang, M., Qi, B., Chen, X., Su, Z., Wan, Y. Optimizing L-(+)-lactic acid production by thermophile *Lactobacillus plantarum As.1.3* using alternative nitrogen sources with response surface method. Biochem. Eng. J., 52, 212-219 (2010).

Lu, Z., He, F., Shi, Y., Lu, M. L., Yu, L., Fermentative production of L (+)-lactic acid using hydrolyzed a corn starch, persimmon juice and wheat bran hydrolysate as nutrient Bioresource Technol., 101, 3642-3648 (2010).

Marták, J., Schlosser, S., Sabolová, E., Kristofíková, L., Rosenberg, M., Fermentation for lactic acid with *Rhizopus arrhizus* in a stirred tank reactor with a periodical bleed and feed operation. Process Biochem.,38, 1573-1583(2003).

Martinez, F. A. C., Balciunas, E. M., Salgado, J. M., González, A. C., Oliveira, R. P. Z., Lactic acid properties, applications and production: A review. Trends in Food Sci. Tech., 30, 70-83 (2013).

Menezes, A. G. T., Menezes, E. G. T., Alves, J. G. L. F., Rodrigues, L. F., Cardoso, M. G., Vodka production from potato (*Solanum tuberosum* L.) using three *Saccharomyces cerevisiae* isolates. J. Inst. Brew., 122,76-83 (2016).

Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31:426-428 (1959).

Min-tian, G.,Koide, M., Gotou, R., Takanashi, H., Hirata, M., Hano, T., Development of continuous electrodialysis fermentation system for based production of lactic acid by *Lactobacillus rhamnosus*. Process Biochem., 40, 1033-1036 (2005).

Nakano, S., Ugwu, C. U., Tokiwa, Y., Efficient production of D(-)-lactic acid from broken rice by *Lactobacillus delbrueckii* using $Ca(OH)_2$ as a neutralizing agent. Bioresource Technol., 104,791-794 (2012).

Nelson, N. A., A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153, 375-380 (1944).

Oh, H., Wee, Y. Y., Yun, J. S., Han, S. H., Jung, S., Ryu, H. W., Lactic acid production from agricultural resources as cheap raw materials. Bioresource Technol., 96, 1492-1498 (2005).

Panesar, P. S., Kennedy, J. F., Gandhi, D. N. K., Bunko, K., Bioutilisation of whey for lactic acid production. Food Chem., 105, 1-14 (2007).

Pereira, C. A., Carli, L., Beux, S., Santos, M. S., Busato, S. B., Kobelnik, M., Barana, A. C., Utilization of flour obtained from potato wastes in the preparation of cookies. Exact and Earth Sciences, Agrarian Sciences and Engineering, 1, 19-26 (2005).

Pritchard, G., Coolbear, T., The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiol* 12:179-206 (1993).

Poliakoff, M., Fitzpatrick, J. M., Farren, T. R., Anastas, P. T., Green chemistry: Science and politics of change. Science,297, 807- 810 (2002).

Rodrigues, M. I., Iemma, A. F., Experimental Design and Process Optimization, CRC Press, Campinas (2014).

Schwan, R. F., Mendonça, A. T,Silva, J. J,Silva, J. R., Rodrigues, V., Wheals, A. E., Microbiology and physiology of cachaça (aguardente) fermentations. A Van Leeuw J. Microb., 79, 89-96 (2001).

Silva, N., Junqueira, V., Silveira, N. F. A., Taniwaki, M. H., Santos, R. F. S., Gomes, R. A. R. Manual de métodos de análise microbiológica de alimentos e água, 4th edn., São Paulo (2010).

Södergård, A., Stolt, M. Properties of lactic acid based polymers and their correlation with composition. Progr. Polym. Sci., 27, 123-1163 (2002).

Souza, E. R. N., Tebaldi, V. M. R., Piccoli, R. H., Adaptação e adaptação cruzada de *Listeria monocytogenes* aos compostos eugenol e carvacrol. Ver. Bras. Plantas Med., 17, 528-533 (2015).

Statistica. Data Analysis Software System. Disponível em: http://www.stasolft.com (2008).

Tinoi, J., Rakariyatham, N., Deming, N. R. L Simplex optimization of carotenoid production by *Rhodotorula glutinis*using hydrolyzed mung bean waste flour as substrate. Process Biochem., 40, 2551-2557 (2005).

Vijayakumar, J., Aravindan, R., Viruthagiri, T., Recent trends in the production, purification and application of lactic acid. Chem. Biochem. Eng. Q., 22, 245-264 (2008).

Wang, L., Zhao, B., Liu, B., Yang, C., Yu, B., Li, Q., Ma, C., Xu, P., Efficient production of L-lactic acid from cassava powder by *Lactobacillus rhamnosus*. Bioresource Technol., 101, 7895-7901(2010).

Wang, L., Zhao, B., Liu, B., Yang, C., Yu, B., Li, Q., Ma, C., Xu, P., Efficient production of L-lactic acid from cassava powder by *Lactobacillus rhamnosus*. Bioresource Technol., 101:7895-7901(2010).

Wee, Y., Reddy, L. V. A., Ryu, H. W., Fermentative production of L (+)-lactic acid from starch hydrolysate and corn steep liquor as inexpensive nutrients by batch culture of *Enterococcus faecalis* RKY1. J. Chem. Technol. Biotecnol., 83, 1387-1393 (2008).

Wee, Y. J., Kim, J. N.,Ryu, H. W., Biotechnological production of lactic acid and its recent applications. Food Technol. Biotech., 44, 163-172 (2006).

Wee, Y. J., Kim, J. N., Yun, J. S., Ryu, R. W., Utilization of sugar molasses for economical L-(+) lactic acid production by batch fermentation of *Enterococcus faecalis*. Enzyme Microb. Tech., 35, 568-573 (2004).

Wu, D. Recycle technology for potato peel waste processing: A review. Procedia Environ. Sci., 31, 103 - 107, (2016).

Yu, L., Lei, T., Ren, X., Pei, X., Feng, Y., Response surface optimization of L (+)-lactic acid production using corn steep liquor as an alternative nitrogen source by *Lactobacillus rhamnosus* CGMCC 1466. Biochem. Eng. J., 39, 496-502 (2008).

Yuwono, S. D., Kokugan, T., Study of the effects of temperature and pH on lactic acid production from fresh cassava roots in tofu liquid waste by *Streptococcus bovis*. Biochem. Eng. J., 40, 175-183(2008).