Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Prickly pear cactus as a raw material for lactic acid production by *Lactococcus lactis* subsp. *lactis*

Milouda Tamine[1], Aicha Nancib[1]*, Nabil Nancib[1] and Joseph Boudrant[2]

[1]Laboratory of Applied Microbiology, Ferhat Abbas University, Setif 1, Algeria.
[2]Laboratory Reactions and Process Engineering (LRPE), UMR CNRS 7224, University of Lorraine, ENSAIA, Vandoeuvre Cedex, 54505, France.
Email: nancibaicha@yahoo.fr

Received 24 May 2017; Received in revised form 17 June 2017; Accepted 17 June 2017

ABSTRACT

Aims: In recent years, microbial conversion of renewable raw materials has become an important objective in industrial biotechnology. Wastes from *Opuntia ficus indica* (*OFI*) can be considered as potential renewable raw materials in lactic acid production. In this study, the feasibility of lactic acid production using fruits and cladodes of *OFI* as carbohydrate feedstock was investigated.
Methodology and results: Response surface methodology (RSM) based on central composite design (CCD) was used to evaluate the effects of fermentation parameters for lactic acid production from *OFI* fruits by *Lactococcus lactis* subsp. *lactis* strain, isolated from Algerian raw camel milk. Acid hydrolysis of the *OFI* cladodes biomass was performed by dilute H_2SO_4 pretreatment. Lactic acid production from *OFI* fruits was analyzed using response surface methodology. Variables such as inoculum age and reducing sugars concentration were found to significantly influence lactic acid production. Final lactic acid concentration and productivity attained under optimum fermentation conditions were 32.5 g/L and 0.74 g/L.h, respectively. The cladodes of *OFI* are a potential biomass feedstock for lactic acid production. The maximum lactic acid and volumetric productivity were 16.85 g/L and 0.65 g/L.h, respectively.
Conclusion, significance and impact of study: Wastes from *OFI* can be a good feedstock for lactic acid production by *Lactococcus lactis* subsp. *lactis.* The methodology as a whole proved to be quite adequate for the design and optimization of the process. The experimental results also demonstrated the feasibility of using *OFI* cladodes hydrolysate as a substrate for lactic acid production.

Keywords: Opuntia ficus indica, acid hydrolysis, lactic acid, fermentation, response surface methodology

INTRODUCTION

Lactic acid (2-hydroxypropanoic acid), $CH_3CHOHCOOH$ is a natural organic acid with a wide range of applications in food, pharmaceutical and cosmetics industries (Sirisansaneeyakul *et al*., 2007; Zhang *et al*., 2007). It can be obtained either by the action of fermentative microorganisms or chemical synthesis. The chemical synthesis results in a racemic mixture of the two isomers (D(−) lactic acid and L(+) lactic acid), while the fermentation process can yield an optically pure form of lactic acid or racemate, depending on microorganisms, substrates and fermentation conditions employed in the production process (Zhang *et al*., 2007) .

The cost and availability of the substrate are the most important issues relevant to the conversion of carbohydrates to lactic acid. Application of agro-industrial wastes in bioprocesses provides an alternative way to replace the refined and costly raw materials. Hence, research efforts are focused on seeking new and effective nutritional sources and new progressive fermentation techniques, enabling the achievement of both high substrate conversion and high production (Bulut *et al*., 2004). Cheap raw materials, such as molasses (Umar *et al*., 2012), whey (Panesar *et al*., 2010), wheat (Gonzalez *et al*., 2016), date waste (Nancib *et al*., 2015), artichokes (Shi *et al*., 2012), sugarcane bagasse (Van der Pol *et al*., 2016) and even wood (Buyondo and Liu, 2011) have been used for lactic acid production. An alternative to these widely sources is Prickly pear cactus (*Opuntia ficus indica*).

Opuntia ficus indica (*OFI*) is a member of the Cactaceae family and is an important forage crop for livestock in many arid and semi-arid regions of the world. This plant singled out as a relevant health promoting food with a great number of potentially active nutrients, the fairly high sugar content and low acidity of the fruit makes it very susceptible to microbial invasion, thus limiting its

*Corresponding author

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

storage life in the fresh state (Sepulveda and Saenz, 1990; Joubert, 1993). *OFI* are used for manufacturing juices, jams, jellies, pickles, candied nopales, alcoholic beverages and bioethanol (Kuloyo *et al*., 2014; Saenz, 2002; Retamal *et al.*, 1987). In fact, the fruits and cladodes contain large amounts of sugars, and also contain proteins, dietary fiber, phytochemical contents, lipids, mineral elements and some vitamins (Jana, 2012; Alimi *et al*., 2013; Stintzing *et al*., 2001; Kabas *et al*., 2006), making its juice particularly suitable as fermentation substrates. Because of its high adaptation to the harsh desert environment and its different applications, the *Opuntia ficus-indica* (*OFI*) is an important and abundant potential raw material for the Algerian industry. Efforts are currently made to develop the fruit production and to discover new applications in the food industries. Due to the high productivity of biomass, cladodes represent a cheap and suitable substrate for the production of lactic acid. The conversion of this biomass to lactic acid usually requires some form of pre-treatment prior to hydrolysis and fermentation. The present research aimed to investigate the use of the prickly pear wastes as the main raw material for lactic acid production using *Lactococcus lactis* subsp. *lactis*. A central composite experimental design was employed in planning the experiment in order to determine which experiment variables affect lactic acid production potential from *OFI* fruits by using RSM and a predictive polynomial quadratic equation. The ability of this strain in using dilute-acid hydrolysate of *OFI* cladodes for lactic acid production was further tested.

MATERIALS AND METHODS

Raw material

Fruits and cladodes of *OFI* were harvested between July and September from the mountainous zone of Setif, East of Algeria.

Extraction of *OFI* fruit sugars

The wastes from the recovered prickly pear fruits were washed and the glochides on the peel surface were removed under running tap water by rubbing. Fruits were cut longitudinally, the flesh and thick peel were separated, and tap water added at a ratio of two parts of water to one part of fruit pulp. The mixture was heated at 50 °C for 45 min with continuous stirring followed by centrifuging at 4000 rpm for 20 min in order to separate the cellulosic debris, while the supernatant was used essentially as the carbon source in the fermentation medium.

Dilute acid hydrolysis of *OFI* cladodes

The cladodes were washed with running water from the faucet; excess humidity was removed with absorbing paper. The cladodes were cut manually with a knife into small cubes and desiccated for 48 h at 60 °C in a drying oven using cold air current. The dried samples were milled into a powdery form in an analytical grinder for a few minutes and stored at room temperature until further use. Before lactic acid fermentation, the dried cladodes were suspended in 6.8% sulfuric acid at a 10% (w/v) loading, the mixture was heated to 100 °C for 92 min.

Detoxification of acid hydrolysate

To eliminate inhibiting substances from the fermentation medium, the solid residue of acid hydrolysate was separated by centrifugation and the pH of the resulting supernatant was adjusted to 10 using NaOH, filtering, acidifying to pH 5.5 with sulfuric acid, and adding sodium sulfite (1 g/L). The resulting precipitate was centrifuged off and the pH was adjusted to 6.

Media and growth conditions

Lactococcus lactis subsp. *lactis* (*Lactococcus lactis*) used in this study was isolated from Algerian raw camel milk. The strain was stored in a M17 medium with 20% (v/v) glycerol at –20 °C. The inoculum was prepared by transferring glycerol stock culture (1 mL) to an Erlenmeyer flask containing 100 mL of M17 medium and incubated at 33.5 °C for 6 h (time needed for the microorganism to reach the exponential growth phase) on a rotary shaker at 200 rpm. Then, the culture containing the production medium (*OFI* juice) was inoculated.

Table 1: Characteristics of raw *OFI.*

Characteristics	Water (%)	Protein concentration (%)	Total sugars (%)	Reducing sugars (%)	pH (units)
OFI fruit	85.25 ± 1.39	0.7 ± 0.05	15.2 ± 0.25	13.5 ± 0.30	5.5 ± 0.07
OFI cladode	92 ± 1	5.12[a] ± 0.5	8[a] ± 1	3.22[a] ± 0.37	5.13 ± 0.06

[a] % Dry weight

Values represent means ± standard deviation calculated from three determinations

Fermentations were carried out in 250 mL Erlenmeyer flasks containing 100 mL cultivation medium. *OFI* juice (*OFI* fruit juice or *OFI* cladode hydrolysate) was used essentially as the carbon source in the fermentation medium. Table 1 summarizes the characteristics of the raw *OFI* used. The medium for the cultures has the following composition: *OFI* juice; yeast extract, KH_2PO_4 0.5 g/L, K_2HPO_4 0.5 g/L; $MnSO_4 \cdot H_2O$ 0.07 g/L; $MgSO_4 \cdot 7H_2O$ 0.5 g/L and $CH_3COONa \cdot 3H_2O$ 6 g/L (the solution of nutrients were sterilized separately). The flasks

were inoculated with 10% of seed inoculums and incubated at 33.5 °C on a rotary shaker at 200 rpm. The pH of the medium was adjusted to 6. A central composite design (CCD) was used in the optimization of lactic acid production from *OFI* fruit juice. Experiments were replicated three times. All the media were sterilized at 121 °C for 20 min.

Experimental design and statistical analysis

The central composite design (CCD) was used to evaluate the effects of three independent variables: reducing sugars concentration of *OFI* fruit (X_1), yeast extract concentration (X_2) and inoculum age (X_3). For an optimized procedure, the variables selected were prescribed into five levels, coded as –α, -1, 0, +1 and +α, as presented in Table 2.

Table 2: Levels of variables used in the experimental design.

Variables	Codes	Levels				
		-α	-1	0	+1	+α
Reducing sugars *OFI* fruit (g/L)	X_1	33.18	40	50	60	66.82
Yeast extract (g/L)	X_2	6.63	8	10	12	13.36
Inoculum age (h)	X_3	4.63	6	8	10	11.36

α=1.682

The experimental data obtained from the CCD model can be represented with the following equation:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \beta_{ij} X_i X_j \quad \text{[Eq1]}$$

Where *Yi* is the predicted response, X_i and X_j are the independent variables in the model, *k* is the number of independent variables, *βo* is the intercept (constants and regression coefficients of the model), *βi* is the linear coefficient, *βii* is the quadratic coefficient and *βij is*s the interaction coefficient. Multivariate regression analysis with model equation 1 (Eq. 1) was carried out on the data to yield equation 2 (Eq. 2) which was used to optimize the product responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \text{[Eq. 2]}$$

The graphical representation of these equations are called response surfaces, which was used to describe the individual and cumulative effects of the test variables on the response and to determine the mutual interactions between the test variables and their subsequent effect on the response. The probability values (*P-values*) indicate the significance of each of the coefficient, which in turn governs the patterns of interactions between the variables. The quality of fit of the second-order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by the F-test. The coefficients of the equation were determined by employing MINITAB 16 software (Minitab Inc, State College, PA - www.minitab.com) and the analysis of variance (ANOVA) test was performed to assay the statistical significance of the analysis.

Analytical methods

The total sugars in *OFI* juice were determined by the phenol-sulfuric method (Dubois *et al*., 1956) and reducing sugars content were determined by the colorimetric method using the UVVis spectrophotometer, (Spectronic Genesis 20) at 540 nm using 3, 5- dinitrosalicylic acid (DNS reagent) with glucose as standard (Miller, 1959). Glucose was measured using an enzymatic kit (Glucose PAP SL, Elitech). The protein concentrations were determined using the Lowry method with bovine serum albumin as the standard (Lowry *et al*., 1951). The moisture content of the raw *OFI* was estimated according to the AOAC method (2000). Cell growth was monitored spectrophotometrically (Spectronic 20 Genesys) by optical density (OD) measurements at 650 nm, and lactic acid estimation was determined following Taylor method (Taylor, 1996).

RESULTS AND DISCUSSION

Lactic acid production from *OFI* fruit

Optimization of the culture conditions

Table 3 shows the predicted lactic acid value obtained from central composite design with corresponding values observed. The polynomial model for lactic acid concentration is shown below:

$$Y\ (g/L) = 29.98 + 2.01X_1 + 1.16X_2 + 2.56X_3 - 2X_1^2 - 2.29X_2^2 - 1.8X_3^2 + 0.19X_1X_2 + 1.39X_1X_3 + 1.53X_2X_3 \quad \text{[Eq. 3]}$$

The fit validity of the model was checked using the determination coefficient (R^2). The values of the determination coefficients, R^2 and adjusted R^2 which measure the model fitting reliability, were calculated and obtained as 0.91 and 0.82, respectively. This suggests that approximately 91% of the variance is attributed to the variables, which indicate the high significance of the model, where only 9% of the total variations cannot be explained by the model. Confirmation of the adequacy of the regression model was reflected also by the good agreement between the experimental and the predicted values of the response variables as shown in Table 3, where the experimental lactic acid concentration ranged from 19.49 to 32.42 g/L and the corresponding predicted values were 20.13 and 29.98 g/L, respectively.

Pareto chart was used in this work for easier visualization of the main and interaction effects of the

Table 3: Central composite design matrix showing real values along with experimental and predicted values of lactic acid concentration.

	Reducing sugars (g/L)	Yeast extract (g/L)	Inoculum age (h)	Lactic acid concentration (g/L)	
Run	X_1	X_2	X_3	Experimental	Predicted
1	60	12	10	31.87	32.73
2	50	13.36	8	26.64	25.45
3	66.82	10	8	29.94	27.7
4	50	10	8	31.59	29.98
5	60	12	6	20.04	21.75
6	50	10	11.36	29.39	29.19
7	40	12	10	25.54	25.54
8	50	10	8	29.12	29.98
9	40	12	6	19.49	20.13
10	60	8	10	26.09	26.97
11	50	10	8	28.43	29.98
12	50	10	8	27.74	29.98
13	50	6.63	8	22.52	21.54
14	60	8	6	20.59	22.11
15	40	8	10	20.73	20.54
16	40	8	6	20.59	21.25
17	50	10	8	32.42	29.98
18	50	10	4.63	22.52	20.55
19	50	10	8	30.22	29.98
20	33.18	10	8	20.87	20.93

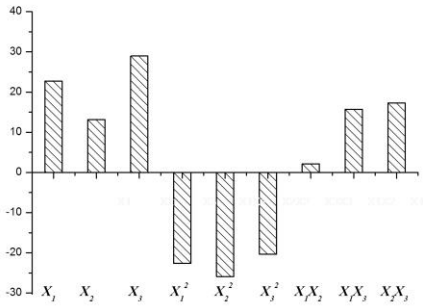

Figure 1: Pareto chart showing the effects of different independent variables on lactic acid concentration

factors to the response variable, that is, lactic acid concentration (Figure 1).

The model identified that within the studied range of experiments the inoculum age (X_3) has the highest positive impact on the fermentation process followed by the reducing sugars (X_1), interactive effect of yeast extract concentration and inoculum age (X_2X_3), interactive effect of reducing sugars concentration and inoculum age (X_1X_3), interactive effect of reducing sugars concentration and yeast extract concentration (X_1X_2), in a decreasing order. While the yeast extract has a slight positive impact on the lactic acid concentration, its quadratic effect (X_2^2) has the highest negative impact on the fermentation process, followed by the negative quadratic effect of reducing sugars concentration (X_1^2) and inoculum age (X_3^2) .

As shown in Table 4, a model *F-value* of 10.87 and a probability value of 0.000 imply significant model fit. The Lack of Fit *F-value* of 1.13 implies that there is insignificant lack of fit. The *P*-value of 0.447 for lack of fit implies that there is only 44.7% chance that the Lack of Fit *F*-value could occur due to noise. From the *P*-values of each model term (Table 4), it can be concluded that, the quadratic coefficients (X_1^2, X_2^2 and X_3^2) and linear

coefficients (X_1 and X_3) are the most significant coefficient ($p < 0.01$). The optimum level of each variable and the effect of their interactions on lactic acid production as a function of two variables were studied by plotting three-dimensional response surface curves (while keeping the other variables at central point). Graphical representation of response surfaces shown in Figures 2A-2C aided the visualization of the effects of the different variables on lactic acid production. The interactive effect of reducing sugars *OFI* fruit concentration and yeast extract concentration on lactic acid production at a constant inoculum age of 8 h is shown in Figure 2A. An increase in the reducing sugars with yeast extract concentration up to the optimum point (near of the central levels) increased the lactic acid to a maximum level and a further increase in the reducing sugars *OFI* fruit with yeast extract concentration caused the trend to be reversed. The effect of reducing sugars *OFI* fruit and inoculum age on the lactic acid production is presented in Figure 2B. An increase in reducing sugars *OFI* fruit with an increase in inoculum age resulted in an increase in lactic acid concentration. The optimal range for lactic acid production was from 45 to 50 g/L of reducing sugars *OFI* fruit and from 6 to 6.8 h of inoculum age. Figure 2C shows the effect of yeast extract concentration and inoculum age on lactic acid production. The trend observed indicates that lactic acid production was favored at high inoculum age with yeast extract near the central level.

Table 4: ANOVA for response surface quadratic model obtained from experimental designs.

Source	Degree of freedom	Sum of squares	Mean Squares	*F*-Value	*P*-Value ($P > F$)
Model	9	349.41	38.82	10.87	0.000
X_1	1	55.35	55.35	15.50	0.003**
X_2	1	18.44	18.44	5.16	0.046*
X_3	1	90.08	90.08	25.23	0.001**
X_1^2	1	39.01	57.68	16.15	0.002**
X_2^2	1	64.99	75.73	21.21	0.001**
X_3^2	1	47.02	47.02	13.17	0.005**
X_1X_2	1	0.29	0.28	0.08	0.782
X_1X_3	1	15.51	15.51	4.34	0.064*
X_2X_3	1	18.72	18.72	5.24	0.045*
Residual Error	10	35.71	3.57		
Lack of fit	5	18.97	3.79	1.13	0.447
Pure Error	5	16.74	3.35		
Total	19	385.12			

** Significant at 1% level

* Significant at 5% level

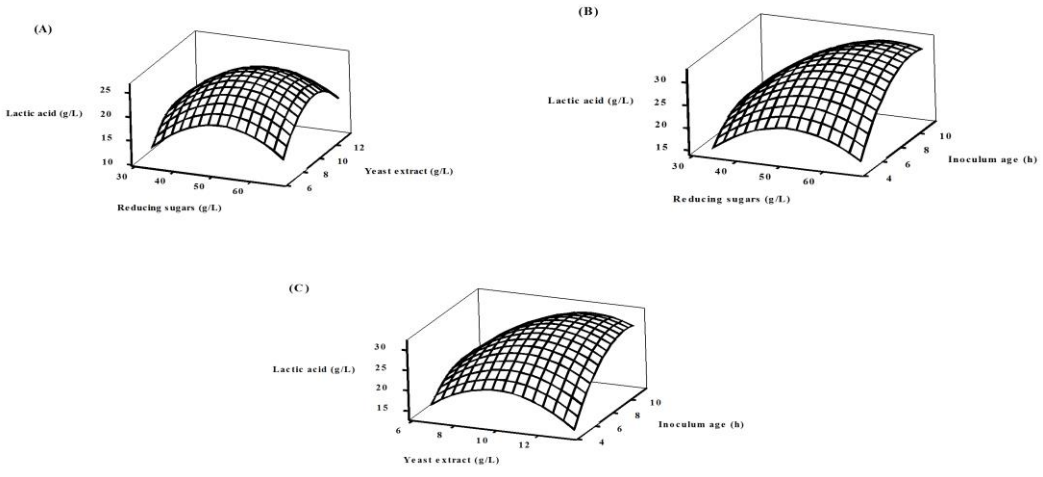

Figure 2: Three-dimensional surface plots showing the effect of different variables on lactic acid production (A) Effect of reducing sugars OFI fruit and yeast extract (B) Effect of reducing sugars OFI fruit and inoculum age (C) Effect of yeast extract and inoculums age.

Validation of the model

To evaluate the validity of the quadratic model, the optimal medium conditions, namely, 60 g/L initial reducing *OFI* fruit juice sugars, 11 g/L yeast extract and 10 h inoculum age were used. The profile of growth, lactic acid production, and fruit *OFI* sugar utilization is shown in Figure 3. Consequently, as shown in this figure, the consumption of *OFI* fruit juice sugars increased with time of fermentation, and about 60% was consumed at the end of fermentation. Maximum yield, ([P]/[S consumed]), was obtained as 96% and the productivity equal to 0.74 g/L.h. The maximum production of lactic acid obtained was 32.5 g/L, which is in good agreement with the predicted value (33 g/L), thus confirming the model's authenticity.

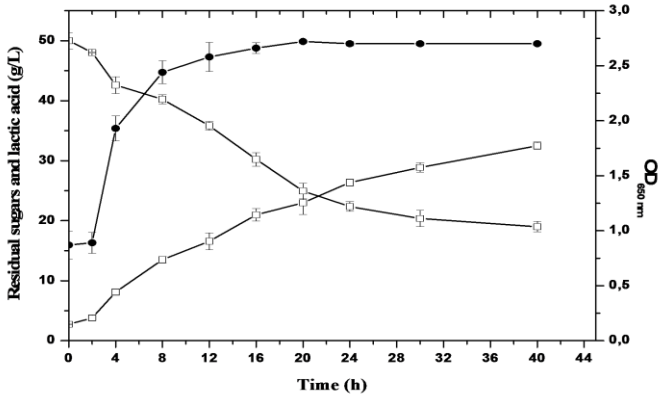

Figure 3: Kinetics of growth (●), sugars consumption (▲), and lactic acid production (□) by *Lactococcus lactis* subsp. *lactis* at optimized conditions. Error bars indicate the standard deviations from three independent experiments

Note that with the initial used medium *OFI* fruit juice as a carbon source without supplementation), hence not optimized (data not shown), the obtained lactic acid concentration was 11.87 g/L with lactic acid yield and a productivity of 0.5 g/g and 0.4 g/L.h, respectively.

These results also confirm the importance of prickly pear fruit juice as a potential carbon source for the production of lactic acid.

A similar result was also obtained in a study by Serna Cock and Rodriguez de Stouvenel (2006) using *Lactococcus lactis* subsp. *lactis* for lactic acid production from sugar cane molasses. A maximum lactic acid concentration of 35 g/L was obtained. In another study by Flores-Albino *et al.* (2012), it was reported that the maximum lactic acid production was 19.5 g/L using a strain of *Lactobacillus* sp. B2. Srivastava *et al.* (2015) obtained with cane molasses a maximum concentration of 55.89 g/L using *Lactobacillus delbrueckii* NCIM 2025. Coelho *et al.* (2010) reported that the cassava wastewater for the production of lactic acid by *Lactobacillus rhamnosus* B 103 produceds lactic acid of 41.65 g/L after 48 h of fermentation. John *et al*. (2008) reported 40 g/L lactic acid production with a productivity of 0.42 g/L.h from cassava bagasse via genetically modified *Lactobacillus delbrueckii.* Nancib *et al*. (2005) reported 24.8 g/L of lactic acid. These last authors utilized date juice as carbon source (50 g/L of glucose) and yeast extract (10 g/L) as nitrogen source for lactic acid production with *Lactobacillus casei* subsp. *rhamnosus* NRRL-B445. During their experiments which were performed in flasks containing date palm waste, maximum concentration of lactic acid with volumetric productivity of 0.62 g/L.h was attained in 40 h. Meziane *et al*. (2013) reported a maximal lactic acid concentration of 15.8 g/L on molasses using *Lactococcus lactis* ssp. and in this latter case, with a much lower yield and productivity (0.10 g/g and 0.11 g/L.h, respectively) compared to our results (0.96 g/g and 0.74 g/L.h).

Lactic acid production from *OFI* cladodes hydrolysate

Since *Lactococcus lactis* could not directly utilize lignocellulose, hydrolysis of *OFI* cladodes by acid treatment was necessary to transform lignocellulosic biomass into fermentable sugars. To determine the optimal condition for hydrolysis, central composite design was applied to investigate the influence of acid concentration and treatment time of *OFI* cladodes on the production of fermentable sugars. The best results were obtained with acid treatment using 6.8% sulfuric acid for 92.4 min (data not shown).

The obstacle for the use of lignocellulosic hydrolysates is the inhibition effect of toxic compounds released during acid hydrolysis process. In addition to the fermentable

sugars, compounds that are toxic to fermentative organisms such as furfural and 5-hydroxymethylfurfural were also produced in this process (Neureiter *et al*., 2004).

To understand the performance of *Lactococcus lactis* in the *OFI* cladodes acid hydrolysate, the latter was used as a raw material for lactic acid production. In all cases, the initial glucose level of hydrolysate was 12 g/L. The experimental results are shown in Table 5. The data shows that *Lactococcus lactis* could convert the sugars in hydrolysate into lactic acid. The maximum lactic acid concentration of 6.29 g/L was achieved at 24 h with lactic acid productivity of 0.26 g/L.h. On the other hand, to improve lactic acid production, hydrolysate of cladodes was detoxified and supplemented with yeast extract and salts.

Table 5: Kinetics patarmeters of lactic acid production on *OFI* juice.

Raw material	Lactic acid (g/L)	Productivity (g/L.h)
OFI fruit juice	32.5 ± 0.61	0.74
Hydrolysate[a]	6.29 ± 0.54	0.22
Hydrolysate[b]	8.97 ± 0.40	0.33
Hydrolysate[c]	13.93 ± 0.50	0.53
Hydrolysate[d]	16.85 ± 0.43	0.65

[a]: Cladode hydrolysate without detoxification
[b]: Detoxified cladode hydrolysate
[c]: Detoxified cladode hydrolysate supplemented with yeast extract
[d]: Detoxified cladode hydrolysate supplemented with yeast extract and salts
Values are the average ± standard deviation of three repeated fermentations

The use of the detoxified acid hydrolysate increased the lactic acid production by 42.6%. Furthermore, supplementation of detoxified acid hydrolysate with 5 g/L yeast extract and salts could notably enhance the titer of lactic acid. As can be seen in Table 5, the lactic acid production and productivity were practically threefold higher than that obtained in un-detoxified acid hydrolysate without any supplementation.

It is clear that supplementation with yeast extract and salts gave better results. Lactic acid bacteria (LAB) are known as fastidious micro organisms that cannot grow on simple mineral media supplemented only with a carbon source (Hébert *et al*., 2004). In addition to carbohydrates, culture media of LAB are usually supplemented with various free amino acids, peptides, nucleic acid derivatives, minerals and vitamins (John *et al*., 2007). The growth factors are usually provided by nitrogen sources. In particular, yeast extract has the greatest effect due to the presence of purines, pyrimidine and vitamins B (Hujanen *et al*., 2001 ; Narayanan *et al*., 2004; Nancib *et al*., 2005; Yu *et al*., 2008). Indeed, these results show a high lactic concentration in comparison to other previously published studies which mention lower maximum lactic acid concentrations: 10.9 g/L from sugarcane bagasses using *Lactococcus lactis* IO-1 obtained by Laopaiboon *et al*. (2010), 10.6 g/L from sugarcane bagasses using *Lactococcus lactis* TISTER-1401 obtained by Jonglertjunya *et al.* (2012) and about 9 g/L from wheat-straw hemicellulose hydrolysates with mixed culture of *Lactobacillus brevis* and *L. pentosus* obtained by Garde *et al*. (2002).

This high lactic acid concentration was probably related to the composition of cladodes (in sugars, vitamins, fatty acids and amino acids) and to the capacity of *Lactococcus lactis* to metabolize several sugars. Most bacteria have the ability to use a great variety of carbon sources as an evolutionary advantage to be able to adapt to their ever-changing habitats. Acid cladode hydrolysate contain not only hexoses but also pentoses (Akanni *et al*., 2014). Hexoses can easily be fermented by LAB, but a few LAB metabolize pentose sugars via the phosphoketolase pathway. Tanaka *et al*. (2002) reported that *Lactococcus lactis* could utilize xylose with a high lactate yield and low acetate production. Passerini *et al*. (2013) investigated the role and adaptation of *Lactococcus lactis* (A12) in sourdough. These authors showed that this strain was able to grow on galactose and L-arabinose. Based on the experimental results of this study, the *OFI* cladodes seemed promising as a lignocellulosic biomass feedstock for lactic acid production.

CONCLUSION

Prickly pear cactus has been revealed as a suitable substrate to lactic acid production by *Lactococcus lactis.* The central composite design and response surface methodology enable the determination of optimal operating conditions suitable for obtaining greater lactic acid production. The validity of the model was proved by fitting the values of the variables in the second order polynomial equation and by actually carrying out the experiment at those predicted values for the three independent variables. Cladodes were also proven to be an economically feasible raw material for lactic acid production.

REFERENCES

Akanni, G. B., du Preez, J. C., Steyn, L. and Kilian, G. C. (2014). Protein enrichment of an Opuntia ficus-indica cladode hydrolysate by cultivation of *Candida utilis* and *Kluyveromyces marxianus*. *Journal of the Science of Food* and *Agriculture* **95, 1094-1102.**

Alimi, H., Hfaeidha, N., Bouonia, Z., Saklyb, M. and Ben Rhoumab, K. (2013). Ameliorative effect of *Opuntia ficus indica* juice on ethanol-induced oxidative stress in rat erythrocytes. *Experimental and Toxicologic Pathology* **65, 391-396.**

AOAC (2000). Official Methods of Analysis, 17th Edn. Association of Official Analytical Chemists, Gaithersburg, USA. Broihier, K. (1999). The Phyto-chemical Renaissance. *Food Processing* **44, 46-48.**

Bulut, S., Elibol, M. and Ozer, D. (2004). Effect of different carbon sources on L(+) -lactic acid production by *Rhizopus oryzae*. *Biochemical Engineering Journal* **21(1), 33-37.**

Buyondo, J. P. and Liu, S. (2011). Lactic acid production by *Lactobacillus pentosus* from wood extract hydrolysates. *Journal* of *Science and Technology for Forest Products and Processes* **1, 38-47.**

Coelho, L. F., de Lima, C. J. B., Bernardo, M. P., Alvarez, G. M. and Contiero, J. (2010). Improvement of L(+)-lactic acid production from cassava wastewater by *Lactobacillus rhamnosus* B 103. *Journal of the Science of Food and Agriculture* **90, 1944-1950.**

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28, 350-356.**

Flores-Albino, B., Arias, L., Gomez, J., Castillo, A., Gimeno, M. and Shirai, K. (2012). Chitin and L(+)-lactic acid production from crab (*Callinectes bellicosus*) wastes by fermentation of *Lactobacillus* sp. B2 using sugar cane molasses as carbon source. *Bioprocess and Biosystems Engineering* **35, 1193-1200.**

Garde, A., Jonson, G., Schmidt, A. S. and Ahring, B. K. (2002). Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. *Bioresource Technology* **81, 217-223.**

Gonzalez, K., Tebbani, S., Lopes, F., Thorigné, A., Givry, S., Dumur, D. and Pareau, D. (2016). Modelling the continuous lactic acid production process from wheat flour. *Applied Microbiology* and *Biotechnology* **100, 147-159.**

Hébert, E. M., Raya, R. R. and Giori, G. S. (2004). Nutritional requirements of *Lactobacillus delbrueckii* subsp. *lactis* in a chemically defined medium. *Current Microbiology* **49, 341-345.**

Hujanen, M., Linko, S., Linko, Y. and Leisola, M. (2001). Optimisation of media and cultivation conditions for L(+)(S)-lactic acid production by *Lactobacillus casei* NRRLB-441. *Applied Microbiology and Biotechnology* **56, 126-130.**

Jana, S. (2012). Nutraceutical and functional properties of cactus pear (*Opuntia* spp.) and its utilization for food applications. *Journal of Engineering Research and Studies* **3, 60-66.**

John, R. P., Gangadharan, D. and Nampoothiri, K. M. (2008). Genome shuffling of *Lactobacillus delbrueckii* mutant and *Bacillus amyloliquefaciens* through protoplasmic fusion for L-lactic acid production from starchy wastes. *Bioresource Technology* **99, 8008-8015.**

John, R. P., Nampoothiri, K. M. and Pandey, A. (2007). Fermentative production of lactic acid from biomass: An over-view on process developments and future perspectives. *Applied Microbiology and Biotechnology* **74, 524-534.**

Jonglertjunya, W., Pranrawang, N., Phookongka, N., Sridangtip, T., Sawedrungreang, W. and Krongtaew, C. (2012). Utilization of sugarcane bagasse for lactic acid production by acid hydrolysis and fermentation using *Lactobacillus* sp. *World Academy of Science, Engineering and Technology* **66, 173-178.**

Joubert, E. (1993). Processing of the fruit of five prickly pear cultivars grown in South Africa. *International Journal of Food Science and Technology* **28, 377-387.**

Kabas, O., Ozmerzi, A. and Akinci, I. (2006). Physical properties of cactus pear (*Opuntia ficus india* L.) grown wild in Turkey. *Journal of Food Engineering* **73, 198-202.**

Kuloyo, O. O., du Preez, J. C., García-Aparicio Mdel, P., Kilian, S. G., Steyn, L. and Görgens, J. (2014). Opuntia ficus indica cladodes as feedstock for ethanol production by *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnology* **30, 3173-3183.**

Laopaiboon, P., Thani, A., Leelavatcharamas, V. and Laopaiboon, L. (2010). Acid hydrolysis of sugarcane bagasse for lactic acid production. *Bioresource Technology* **101, 1036-1043.**

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193, 265-275.**

Meziane, M., Dilmi Bouras, A. and El Hameur, H. (2013). Lactic acid fermentation of a diluted molasses medium by two strains of *Lactococcus lactis* ssp. immobilized on pouzzolane and bone bovine conference: ISITES, At Sakarya, Turquie **774-782**.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31, 426-428.**

Nancib, A., Nancib, N., Boubendir, A. and Boudrant, J. (2015). The use of date waste for lactic acid production by a fed-batch culture using *Lactobacillus casei* subsp*. rhamnosus*. *Brazilian Journal of Microbiology* **46, 893-902.**

Nancib, A., Nancib, N., Meziane-Cherif, D., Boubendir, A., Fick, M. and Boudrant, J. (2005). Joint effect of nitrogen sources and B vitamin supplementation of date juice on lactic acid production by *Lactobacillus casei* subsp. *rhamnosus*. *Bioresource Technology* **96(1), 63-67.**

Narayanan, N., Roychoudhury, P. K. and Srivastava, A. (2004). L(+) lactic acid fermentation and its product polymerization. *Electronic Journal of Biotechnology* **7, 167-179.**

Neureiter, M., Danner, H., Madzingaidzo, L., Miyafuji, H., Thomasser, C., Bvochora, J., Bamusi, S. and

Braun, R. (2004). Lignocellulose feedstocks for the production of lactic acid. *Chemical and Biochemical Engineering Quarterly* **18(1), 55-63.**

Panesar, P. S., Kennedy, J. F., Knill, C. J. and Kosseva, M. (2010). Production of L(+) lactic acid using *Lactobacillus casei* from whey. *Brazilian Archives of Biology and Technology* **53, 219-226.**

Passerini, D., Coddeville, M., Le Bourgeois, P., Loubière, P., Ritzenthaler, P., Fontagné-Faucher, C., Daveran-Mingot, M. L. and Cocaign-Bousquet, M. (2013). The carbohydrate metabolism signature of Lactococcus *lactis* Strain A12 reveals its sourdough ecosystem origin. *Applied and Environmental Microbiology* **79, 5844-5852.**

Retamal, N., Duran, J. M. and Fernandez, J. (1987). Ethanol production by fermentation of fruits and cladodes of prickly pear cactus (*Opuntia ficus-indica* (L.) Miller). *Journal of the Science of Food and Agriculture* **40, 213-218.**

Saenz, C. (2002). Cactus pear fruit and cladodes: A source of functional components for foods. *Acta Hort* **581, 253-263.**

Sepulveda, E. and Saenz, C. (1990). Caracteristicas quimicas y fisicas de pulpa de tuna (Opuntia ficus indica). *Revista de Agroquimica y Tecnologia de Alimentos* **30, 51-555.**

Serna Cock, L. and Rodriguez de Stouvenel, A. (2006). Lactic acid production by a strain of *Lactococcus lactis* subs. *lactis* isolated from sugar cane plants. *Electronic Journal of Biotechnology* **9, 40-45.**

Shi, Z., Wei, P., Zhu, X., Cai, J., Huang, L. and Xu, Z. (2012). Efficient production of l-lactic acid from hydrolysate of Jerusalem artichoke with immobilized cells of *Lactococcus lactis* in fibrous bed bioreactors. *Enzyme* and *Microbial Technology* **51, 263-268.**

Sirisansaneeyakul, S., Luangpipat, T., Vanichsriratana, W., Srinophakun, T., Chen, H. H. and Chisti, Y. (2007). Optimization of lactic acid production by immobilized *Lactococcus lactis* IO-1. *Journal of Industrial Microbiology* and *Biotechnology* **34, 381-391.**

Srivastava, A. K., Tripathi, A. D., Jha, A., Poonia, A. and Sharma, N. (2015). Production, optimization and characterization of lactic acid by *Lactobacillus delbrueckii* NCIM 2025 from utilizing agro-industrial byproduct (cane molasses). *Journal of Food Science and Technology* **52, 3571-3578.**

Stintzing, F. C., Schieber, A. and Carle, R. (2001). Phytochemical and nutritional significance of Cactus pear. *European Food Research* and *Technology* **212, 396-407.**

Tanaka, K., Komiyama, A., Sonomoto, K., Ishizaki, A., Hall, S. J. and Stanbury, P. F. (2002). Two different pathways for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of L-lactate in mixed acid fermentation by the lactic acid bacterium *Lactococcus lactis* IO-1. *Applied Microbiology* and *Biotechnology* **60, 160-167.**

Taylor, A. C. C. K. (1996). A simple colorimetric assay for muramic acid and lactic acid. *Applied Biochemistry and Biotechnology* **56, 49-58.**

Umar, F., Faqir, M. A., Tahir, Z., Sajjad, U. R., Mouhammd Atif, R., Anwaar, A. and Khashif, A. (2012). Optimization of lactic acid production from cheap raw material: Sugarcane molasses. *Pakistan Journal of Botany* **44, 333-338.**

Van der Pol, E. C., Eggink, G. and Weusthuis, R. A. (2016). Production of L(+)-lactic acid from acid pretreated sugarcane bagasse using *Bacillus coagulans* DSM2314 in simultaneous saccharification and fermentation strategy. *Biotechnology for Biofuels* **9, 248.**

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

Zhang, Z. Y., Jin, B. and Kelly, J. M. (2007). Production of lactic acid from renewable materials by *Rhizopus fungi*. *Biochemical Engineering Journal* **35, 251-263.**