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Article

Fermentation ability of specific strains of *Lactiplantibacillus plantarum* using mango as base material

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Abstract

The fermentation ability of two potentially probiotic bacteria, *Lactiplantibacillus plantarum* Lp6 and Lp32, was evaluated on juice from mango *cv* Ataulfo as a substrate. To do this, the strains (10^{10} CFU ml^{-1}) were added in three levels of inoculum (1, 2, and 3%, v/v) in mango juice and incubated for 36 h at 37 °C. Samples were taken (0, 18, and 36 h) to assess bacterial growth, total soluble solids, individual sugars, pH, and titratable acidity (% lactic acid). Additionally, the effect of fermentation on the color and sugars (glucose, fructose, and sucrose) of the juice was determined. Both strains have fermentation ability and a cell growth of four logarithmic cycles after 12 h of fermentation. No differences in color were observed between fermented and unfermented juice. Lp6 and Lp32 bacteria can be used as starter cultures for the production of mango-based functional beverages.

Keywords:

Mangifera indica, fermentation, lactic acid bacteria, quality.

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Introduction

Lactic acid bacteria (LAB) are used in the food and beverage industries as starter cultures or adjunct cultures, including some with probiotic properties for the production of fermented beverages, which can increase the shelf life, nutritional value, and sensory properties of the beverage.

During the fermentation process, bacteria are able to convert carbohydrates into organic acids such as lactic acid, which, during fermentation, is influenced by temperature, nutrients and the strain used, such as *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Oenococcus* and *Lactobacillus*; from the last genus cited, the most widely used species is *Lactiplantibacillus plantarum* due to its ecological adaptability (Punia *et al*., 2022). Yang *et al*. (2018) indicated that strains of *L. plantarum* are successfully used for the formulation of fruit and vegetable beverages.

In this regard, Cele *et al*. (2022) reported the use of *L. plantarum* for the fermentation of juice from mango *cv* 'Sabre', 'Peach' and 'Tommy Atkins'. Their results showed that the strain of *L. plantarum* used improved the content of volatile compounds, ascorbic acid, phenolic content, and antioxidants, which could have a beneficial effect on human health, which in turn places it as a substrate for the development of functional beverages (García *et al*., 2020; Lan *et al*., 2023).

Functional beverages are one of the fastest-growing categories of functional foods due to consumer interest in healthier beverages (Ahmed *et al*., 2023; Isas *et al*., 2023). For this reason, fermentable substrates are sought to obtain value-added products. Of particular interest is the mango *cv* Ataulfo (*Mangifera indica* L.), which contains significant amounts of bioactive compounds, such as carotenoids, fiber, polyphenols, minerals, vitamins, and is also sweet in taste, with low acidity, intense aroma, and its high sugar content can act as a substrate for the growth of fermentative bacteria (Palafox *et al*., 2012; Quirós *et al*., 2017; Kesa *et al*., 2021).

Although there are studies that have shown that fruit juices of pineapple (Toan *et al*., 2019), blueberries (Li *et al*., 2021), pomegranate (Kumar *et al*., 2015) and peaches (Managa *et al*., 2021), there is limited information on the use of mango, Ataulfo variety, as a matrix for the fermentation of lactic acid bacteria. The work aimed to evaluate the fermentation ability (cell growth and percentage of lactic acid) of two potentially probiotic bacteria, *Lactiplantibacillus plantarum* Lp6 and Lp32, using juice from mango *cv* Ataulfo as a substrate.

Materials and methods

Preparation of a mango-based beverage

Fruits of mango *cv* Ataulfo in commercial maturity were obtained from Chiapas, Mexico; the pulp of the fruit was collected manually (°Brix 16-17, pH 4.2-4.5), vacuum-packed and stored at -20 °C. The thawed pulp was ground in an Oster domestic blender (model: BLST4126R, Newell Brands, Mexico) to make the fermentable matrix with mango pulp/water in a 50:50 ratio, adjusted to 13 °Brix with sucrose. Mango juice (200 ml) was pasteurized using a water bath at 85 °C (Thermo Scientific, Precision CIR 35, MA, USA) for 5 min and cooled with an ice bath. The prepared juice was kept refrigerated (4 ±1 °C) until use.

Mango juice inoculation

Fermentation was carried out with the Lp6 and Lp32 strains of the strain collection of the CIAD Dairy Products Laboratory (García *et al*., 2022; Santiago *et al*., 2023). The bacteria were propagated in MRS broth (Man, Rogosa, and Sharpe; Condalab, St. Forja, Madrid, Spain) using three consecutive subcultures (12, 10, and 8 h) at 37 °C under anaerobic conditions. Bacteria were recovered from the last subculture by centrifugation (4 500 × g, 10 min, 4 °C), washed twice with phosphate-buffered saline (PBS; 0.1 M, pH 7.2), and adjusted to an initial concentration of 10^{10} CFU ml^{-1}. Fermentation was carried out with 1, 2 and 3% v/v inoculum and incubated at 37 °C under anaerobic conditions for 36 h. Samples were taken at 0, 18 and 36 h of fermentation in triplicate. Fermentation efficiency

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with 1% inoculum was determined with different fermentation times (0, 6, 12, 24, and 48 h) and color and sugars were analyzed.

pH determination

Changes in pH values were recorded with a digital pH potentiometer (Thermo Scientific, Orion Versa Star, MA, USA); the electrode was inserted into the samples (10 ml, temperature 35 °C) and the reading was recorded (AOAC, 1990; Cele *et al*., 2022).

Determination of titratable acidity

Titratable acidity was determined following the procedures described in AOAC (2000); Chen *et al*. (2023) with slight modifications. Five milliliters of sample were mixed with 50 ml of deionized water and placed in an automatic titrator (Mettler Toledo, T50, CDMX, Mexico), using 1 N sodium hydroxide standard solution until the pH was 8.0. The result was expressed as (%) lactic acid:

$$[\%\ \text{titratable acidity} = \frac{(\text{ml of NAOH used})(\text{N from NaOH})(0.09)}{\text{ml of titrated juice}} 1 \quad 09 = \text{milliequivalent of lactic acid}$$

Determination of total soluble solids (TSS

The TSS in the samples were performed in a digital refractometer (Mettler Toledo, RE40D, CDMX, Mexico) at 20 °C. The refractometer was calibrated with distilled water. The value of TSS (° Brix) was determined in triplicate by placing a drop of sample in the equipment.

Determination of sugars

Glucose, fructose, and sucrose in the samples were analyzed with the Megazyme kit (Megazyme, International Ireland Ltd Wicklow, Ireland), measuring the glucose concentration before and after the hydrolysis of sucrose by the β-fructosidase (invertase) enzyme. For D-fructose, it is determined after D-glucose, with isomerization caused by the phosphoglucose isomerase (PGI) enzyme. All measurements were made on a spectrophotometer at 340 nm (Cary 60 UV-Vis spectrophotometer, Agilent Technologies, Santa Clara, CA, USA) and reported as a percentage.

Cell growth

The cell growth of the bacteria was determined using the plate emptying method (Sanders, 2012) with MRS agar (Merck Millipore®, Darmstadt, Germany). One milliliter of each sample was added to a tube with 9 ml of peptone water, then serial dilutions were performed; 1 ml of the dilution was placed on the plates and the culture medium was added to the point of gelling. All plates were incubated in anaerobic conditions at 37 °C for 24-48 h and the results were expressed as Log colony-forming units (CFU) ml^{-1}.

Color determination

The color of the samples was determined with a spectrophotometer (Konica CM-700, Minolta Inc., Japan) and the color coordinates L^*, a^*, b^*, chromaticity (C) and hue (h) angle were calculated using the OnColor QC program version 5 (Siller *et al*., 1994; Minolta, 1994).

Statistical analysis

An A*B (A: % inoculum and B: time, h) factorial design with units repeated over time was used for the inoculum level. Factor A: 0, 1, 2 and 3% inoculum and factor B: fermentation time (0, 18, and 38 h). To determine the effect of the strains, an A*B (A: type of strain and B: time, h) factorial design with units repeated over time was performed. Factor A, 0, Lp6 and Lp32 and factor B, fermentation (0, 6, 24, and 48 h). For the variables of pH, titratable acidity, °Brix and sugars, a design of repeated measures over time was used. The differences in the parameters evaluated were determined by

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an Anova with significance of 5% and a Tukey test in the MINITAB 19 statistical package (Minitab Inc., State College, PA, USA). Data were reported as means.

Results and discussion

The results for TSS (°Brix), pH and acidity (% lactic acid) are shown in Table 1 A decrease in TSS content was observed in samples with the three inoculum levels during the fermentation process; no difference (p> 0.05) was recorded in inoculum level and time for the Lp6 strain, while for the Lp32 strain, only 2% inoculum at 36 h was significantly lower. This behavior could indicate that the bacteria consumed the sugars (added and from the fruit) to generate energy and continue their growth (Punia *et al*., 2022; Cele *et al*., 2022).

Table 1. Changes recorded during fermentation by the Lp6 and Lp32 strains in the mango-based matrix.

Inoculum (%)	Time (h)	°Brix	pH	Lactic acid (%)	°Brix	pH	Lactic acid (%)
0	0	13.01*^{A}	4.29^{A}	0.23^{E}	12.97^{A}	4.23^{A}	0.32^{EF}
	18	12.96^{A}	4.24^{A}	0.25^{E}	12.95^{A}	4.27^{A}	0.31^{F}
	36	12.96^{A}	4.26^{A}	0.25^{E}	12.96^{A}	4.21^{A}	0.311^{F}
1	0	13^{A}	4.29^{A}	0.25^{E}	12.95^{A}	4.31^{A}	0.32^{EF}
	18	12.9^{A}	3.5^{BC}	0.52^{C}	12.88^{AB}	3.58^{B}	0.66^{D}
	36	12.82^{A}	3.4^{C}	0.99^{A}	12.76^{AB}	3.31^{C}	0.89^{B}
2	0	12.93^{A}	4.28 ^{A}	0.24^{E}	12.96^{A}	4.26^{A}	0.34^{E}
	18	12.87^{A}	3.59^{BC}	0.38^{D}	12.86^{AB}	3.74^{B}	0.69^{C}
	36	12.84^{A}	3.43 ^{C}	0.73^{B}	12.68^{B}	3.32^{C}	0.92^{B}
3	0	12.95^{A}	4.28^{A}	0.25^{E}	12.95^{AB}	4.19^{A}	0.31^{F}
	18	12.84^{A}	3.52^{B}	0.42^{D}	12.78^{AB}	3.74^{B}	0.71^{C}
	36	12.83^{A}	3.43^{C}	0.67^{B}	12.72^{AB}	3.35^{C}	0.95^{A}

*Means of three repetitions. Same literal by response variable is statistically equal (Tukey> 0.05).

The initial pH value of the samples was 4.26, with a decrease of 20% for the three inoculum levels after 36 h of fermentation, showing no difference (p> 0.05) between the inoculum concentration levels, but in the fermentation time (Table 1), where the fermentation process in mango is not dependent on the inoculum concentration level, and 1% can be used.

The decrease in pH is due to the fermentation process, where the LAB use the carbohydrates present in mango as a substrate for the transformation into acidic products (H+) (Punia *et al*., 2022). Mwanzia *et al*. (2022) reported that the pH of mango juice (*cv* Sabre, Peach, and Tommy Atkins) fermented decreased after 72 h of process with three cultures: pH= 3.23 for Abt-5 (*S. thermophilus*, *Bifidobacterium* and *L. acidophilus*), pH= 3.6 for Fiti (*L. rhamnosus*) and pH= 3.7 for *L. delbrueckii* ssp. *bulgaricus*. Cele *et al*. (2022) point out that *L. plantarum* was the one that developed the lowest pH= 3.66.

In mango juice fermented with the Lp6 and Lp32 strains, a difference (p# 0.05) was observed in the percentage of acidity between inoculum levels and fermentation time (Table 1), behaving inversely to pH. The maximum acidity values (0.99%) were recorded in juice fermented by Lp6 at 36 h with 1% inoculum, while for Lp32, it was with 3% inoculum. Kumar *et al*. (2015) report an increase in acidity (0.49 to 0.66%) in a mango beverage with *L. plantarum* NCDC LP 20 at 72 h.

Cell concentration

The initial count for mango juices was 7.7 $\log_{10}$ CFU ml^{-1} in both strains (Lp6 and Lp32), after 18 h of fermentation an increase of 4 $\log_{10}$ CFU ml^{-1} was observed regardless of the initial inoculum concentration (p> 0.05), followed by a decrease (p≤ 0.05) of 1 $\log_{10}$ CFU ml^{-1} at 36 h (Figure 1).

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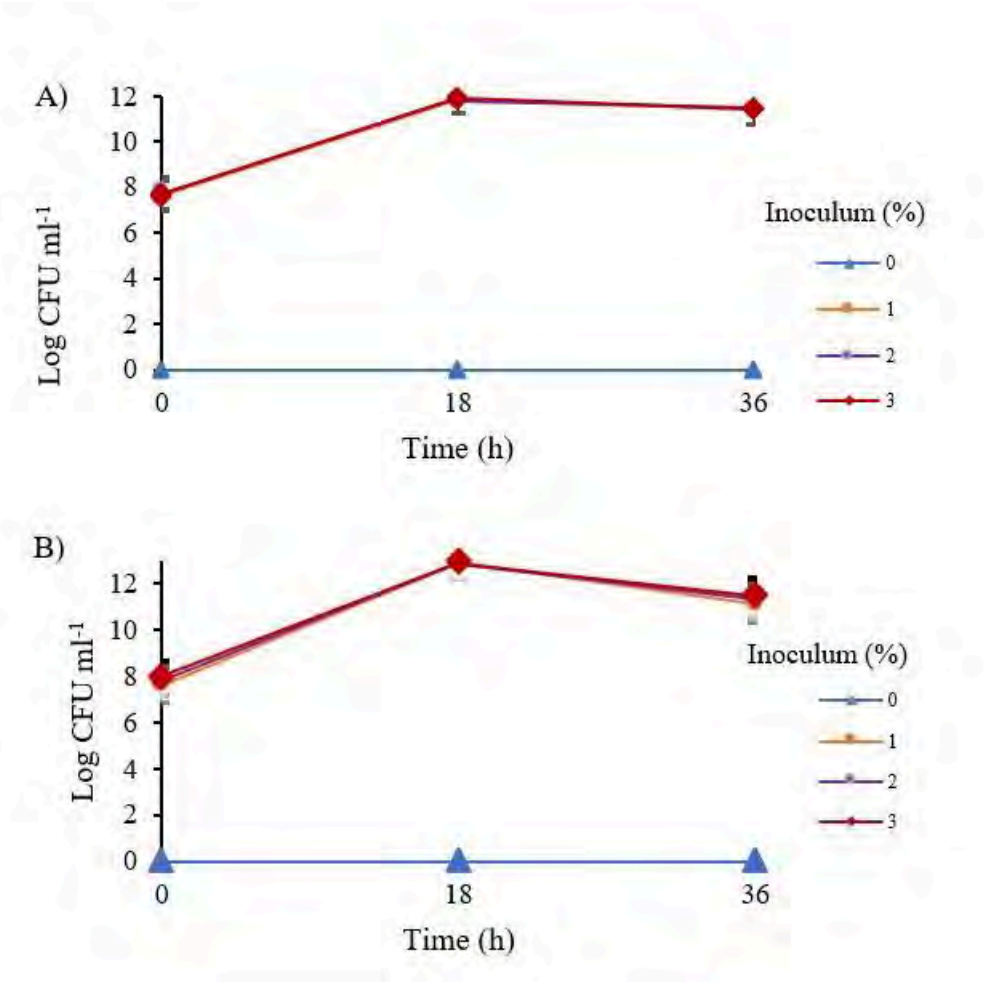
The cell growth observed during fermentation with the Lp6 and Lp32 strains was higher than previously reported in mango beverage fermented with three LAB cultures (Mwanzia *et al*., 2022) and with the strain *L. plantarum* NCDC LP20 (Kumar *et al*., 2015). The increase in cell concentration could indicate that the strains adapted to the mango-based matrix, while the decrease recorded at 36 h could be due to the fact the strains are in competition for nutrients and due to the antimicrobial effect of organic acids (lactic acid) produced by bacteria (Coban, 2020).

Figure 1. Cell concentration of Lp6 (A) and Lp32 (B) strains during the fermentation time of mango juice added at different inoculum levels.



LAB are microorganisms capable of hydrolyzing proteins and their sustainable growth depends on the production of specific proteinases, peptidases, and peptides (Parra, 2010), the low amount of protein in mangoes limits their growth; however, their metabolic capacity of proteins, sugars and lipids favor the sensory properties of fermented foods (Palafox *et al*., 2012; Punia *et al*., 2022). As no difference ($p > 0.05$) was found between the three inoculum levels for the variables of cell concentration, °Brix and pH, it was decided to use the inoculum level of 1% to corroborate their fermentation ability and include new study variables (color and sugars).

Fermentation of mango pulp juice with 1% Lp6 and Lp32

In the TSS (°Brix) content in the samples, a significant decrease ($p \leq 0.5$) was found between the Lp6 and Lp32 strains compared to the control (juice without LAB) (Table 2). Nevertheless, the changes

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in °Brix were numerically minimal but significant because the °Brix were expressed as dissolved solids of sugars and acids; in this sense, the total sugars were statistically different ($p \leq 0.5$) at 48 h of fermentation with the strains, related to the degradation of glucose by the LAB, with no difference ($p > 0.5$) in glucose and fructose. When glucose degraded, lactic acid increased and pH decreased, with differences ($p\# 0.5$) between the strains at 48 h of fermentation.

Table 2. Changes recorded during fermentation of the mango-based matrix by the Lp6 and Lp32 strains.

Strain	Time (h)	°Brix	Total sugars	Gluc g 100 g^{-1}	Fruc g 100 g^{-1}	Suc g 100 g^{-1}	pH	Acidity
Control	0	13*^{A}	11.1^{A}	0.59^{B}	1.5^{A}	9^{A}	4.19^{A}	0.32^{E}
	6	13^{A}	11.2^{AB}	0.64^{B}	1.4^{A}	8.1^{A}	4.19^{A}	0.32^{E}
	12	13^{A}	-	-	-	-	4.19^{A}	0.32^{E}
	24	13^{A}	10.6^{B}	0.97^{A}	1.7^{A}	7.9^{A}	4.18^{A}	0.32^{E}
	48	13^{A}	9.5^{AB}	1.1^{A}	1.9^{A}	6.6^{BC}	4.12^{B}	0.33^{E}
Lp6	0	12.97^{AB}	10^{AB}	0.53^{B}	1.3^{AB}	8.1^{A}	4.19^{A}	0.36^{E}
	6	12.87^{A}	9.1 ^{C}	0.55^{B}	1.2^{B}	7.4^{A}	4.11^{C}	0.36^{DE}
	12	12.81^{A}	-	-	-	-	4.73^{D}	0.5^{C}
	24	12.81^{B}	9.1^{C}	0.79^{AB}	1.2^{B}	6.9^{B}	3.57^{E}	0.54^{C}
	48	12.78^{BC}	7.5^{D}	0.91^{A}	1.5^{A}	5.1^{C}	3.57^{E}	0.82^{B}
Lp32	0	12. 96^{AB}	10.1^{AB}	0.59^{B}	1.5^{A}	8.2^{A}	4.18^{A}	0.32^{A}
	6	12.87^{A}	9.1^{C}	0.5^{B}	1.3^{AB}	7.4^{A}	4.09^{B}	0.35^{E}
	12	12.81^{A}	-	-	-	-	3.71^{D}	0.49^{CD}
	24	12.78^{B}	8.6^{CD}	0.74^{AB}	1.6^{A}	6.3^{C}	3.37^{F}	0.72^{B}
	48	12.71^{C}	8.4^{CD}	1.1^{A}	1.8^{A}	5.5^{C}	3.2 ^{G}	0.98 ^{A}

Gluc= glucose; Fruc= fructose; Suc= sucrose. Acidity (% lactic acid). *Average of three repetitions. Same letter by column is statistically equal (Tukey> 0.05).

Mango mainly contains three sugars: sucrose, fructose, and glucose, substrates that bacteria such as Lp6 and Lp32 may be using in the production of lactic acid, demonstrating that both strains can be used to obtain a fermented mango-based beverage (Punia *et al*., 2022).

Growth of bacteria inoculated at 1%

The initial count of the Lp6 and Lp32 strains was 7.7 $\log_{10}$ CFU ml^{-1}, with maximum cell growth of 4.5 $\log_{10}$ CFU ml^{-1} at 12 h of fermentation. After reaching the stationary phase of the bacteria, a decrease occurred at 24 and 48 h of fermentation, while the sample without inoculum remained without bacterial growth. The bacterial behavior may be due to the decrease in pH and increase in the percentage of acidity, which causes the inhibition of bacterial growth, reducing LAB, in addition to the accumulation of metabolites (propionic, acetic, lactic acid and bacteriocins), which could develop a toxic system, the cytoplasm acidifies, energy consumption increases to maintain the pH, and enzymatic reactions are inhibited (Punia *et al*., 2022; Cele *et al*., 2022).

Color

Table 3 shows that the fermentation process by the Lp6 and Lp32 strains did not affect the color of the mango juice, expressed in luminosity (40.8 to 42.8), a* value (0.1-0.4) and hue angle (°Hue) or true color (87.2-89.5), placing the beverage in a yellow color, according to the Minolta (1994) color circle.

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Table 3. Changes in the color variables of the mango beverage fermented with Lp6 and Lp32 strains.

	Time (h)	Luminosity	a* value	b* value	Chromaticity	°Hue
Control	0	42.8*^{A}	0.3^{A}	16^{C}	16^{C}	89^{A}
	6	42.4^{A}	0.4^{A}	17.6^{A}	17.6^{A}	87.2^{A}
	12	42^{A}	0.4^{A}	17.6^{A}	17.6^{A}	88.4^{A}
	24	41.4^{A}	0.4^{A}	14.6^{BC}	14.6^{BC}	88.6^{A}
	48	42.3^{A}	0.3^{A}	14.2^{BC}	14.2^{BC}	88.6^{A}
Lp6	0	42.7^{A}	0.3^{A}	17.1 ^{AB}	17.1 ^{AB}	88.8^{A}
	6	41^{A}	0.3^{A}	16.8^{B}	16.8^{B}	88.7^{A}
	12	40.3^{A}	0.3^{A}	16.7 ^{B}	16.7 ^{B}	88.8^{A}
	24	41^{A}	0.1^{A}	17.6^{A}	17.6^{A}	89.3^{A}
	48	41.8^{A}	0.1^{A}	14.4^{BC}	14.4^{BC}	89.7^{A}
Lp32	0	40.8^{A}	0.1^{A}	14.7 ^{BC}	14.7 ^{BC}	89.5^{A}
	6	41.2^{A}	0.4^{A}	15.1^{C}	15.1^{BC}	89.4^{A}
	12	41^{A}	0.3^{A}	15.6^{AB}	15.6^{BC}	89^{A}
	24	41.2^{A}	0.3^{A}	15.1^{BC}	15.1^{BC}	89.4^{A}
	48	40.8^{A}	0.1^{A}	14.7^{A}	14.7^{BC}	89.5^{A}

*Average of three repetitions. Same letter by response variable is statistically the same (Tukey> 0.05).

Nonetheless, a decrease ($p \leq 0.5$) in the value of b* and chromaticity was observed between the hours of fermentation, but not between the samples with the strain and the control at 48 h of fermentation. This suggests that the fermentation process does not affect the color of the juice. In addition, the color changes in the juice confirm that the pasteurization process was sufficient to reduce the enzymatic activity associated with pigment oxidation or other biochemical processes that affect the visual quality of mango juice (Managa *et al*., 2021; Quiros *et al*., 2017). Cele *et al*. (2022) reported a reduction in color values (L*, a*, and chroma) in beverages of mango *cv* Sabre and Peach fermented with the *Lactiplantibacillus plantarum* strain, but not when using Tommy Atkins mango. The authors point out that the increased acidity of fermented juices helps protect against enzymatic browning, in this sense, the mangoes studied presented different amounts of sugars and organic acids; they also comment that food products with dark colors due to the enzymatic effect are less likely to be accepted by consumers.

Conclusions

The strains of *Lactiplantibacillus plantarum* Lp6 and Lp32 can be used for their fermentative ability (in reference to cell growth and percentage of lactic acid) for the production of a functional beverage based on mango *cv* Ataulfo, with good characteristics in terms of color, acidity, sugars, as well as other sensory properties offered by lactic acid bacteria.

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