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Assessment of technological and functional features of *Lactiplantibacillus* and *Fructobacillus* strains isolated from *Opuntia ficus-indica* fruits

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ABSTRACT

Opuntia ficus-indica fruits are outstanding for their sensory attributes and multiple health benefits. However, this fruit is highly perishable, and substantial efforts have been carried out to extend its shelf-life. Lactic fermentation of fruits by-products using autochthonous bacteria arises as relevant technology for preserving vegetables and their by-products. In this study, autochthonous *Lactiplantibacillus plantarum* and *Fructobacillus fructosus* strains previously selected from *Opuntia ficus-indica* fruits of Northwestern Argentina were characterized according to their technological and functional properties to select the most suitable for the production of fermented cactus pear products. *L. plantarum* strains showed better acidifying activity, decreasing the pH of the juice by about 2.9 units in 24 h. All the strains studied produced lactic and propionic acids, and *L. plantarum* S-811 and S-TF2 strains and *F. fructosus* S-TF7 strain were the better lactic acid producers, with values around 9.5 g/l. These strains also displayed antimicrobial activities against undesirable pathogen bacteria, showed a safety profile typical of lactic acid bacteria, and the juice fermented with these strains preserves the phenolic compounds content and antioxidant activity of unfermented juice. The obtained results showed the potential of *L. plantarum* S-811 and S-TF2, and *F. fructosus* S-22 for their use as starters for the fermentation of cactus pear by-products, standing out *L. plantarum* S-811 for its potentiality to elaborate a fermented cactus pear beverage. The cactus pear juice fermented by *L. plantarum* S-811 showed physicochemical and microbiological stability that favors juice shelf-life. Besides, fermentation conferred distinctive sensory features to the cactus pear juice without influencing consumers' overall acceptability.

1. Introduction

The cactus pears of *Opuntia ficus-indica* and the juice obtained from this fruit are largely consumed fresh in South American countries. Researches have revealed the positive relation between consumption of a diet rich in fruits and vegetables, and the reduction of the risks of development of some chronic or age-related pathologies [1,2]. Cactus pears are not an exception, and the consumption of these fruits is strongly recommended in the human diet due to their nutritional contribution and biofunctional properties [3]. The health benefits of this fruit are believed to stem from its recognized antioxidant properties related to its content of vitamin C, phenolic compounds, and betalain pigments, and the presence of complex polysaccharides in its pulp that can act as fermentation substrates for the growth of beneficial gut microbiota [3,4].

O. ficus-indica's plants can grow in arid and semiarid regions with large thermal amplitudes, explaining its wide worldwide distribution [3]. These features make this crop an agricultural alternative in areas where water is a scarce resource. Indeed, in arid regions of northwestern Argentina, there is a vernacular consumption mainly of wild varieties of this fruit, which usually intakes as fresh fruit, juices, and traditional *arrope* jam [4]. The combination of the high content of sugars and water, plus the low acidity (pH $>$ 4.5) of its pulp, confer this fruit a very pleasant flavor [5]. However, those very acceptable sensory attributes make cactus pears a perfect substrate for the development of spoilage microorganisms. An alternative to overcome this drawback is the manufacturing of processed foods from the fresh fruit. Among these foods, the cactus pear juice emerges as the food with most possibilities of acceptance by the consumers. Different procedures were developed to guarantee the microbiological stability of cactus pear juices, such as heat

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treatment, juice concentration, or acidification [5]. But the final products do not resemble original fresh juices due to loss of organoleptic properties and nutritional quality, as well as the decrease of health-promoting features [5].

Fermentation with lactic acid bacteria (LAB), especially autochthonous ones, has a long and successful history in the preservation of fruits and vegetables [6]. Therefore, lactic fermentation emerges as relevant biotechnology for maintaining or improving the safety, nutritional, sensory, and shelf-life properties of vegetables and by-products thereof [1,6]. Furthermore, the selection of autochthonous starters (adapted for the specific plant matrix) from the microbiota of fruits, may ensure better performance compared to allochthonous strains: prolonged shelf-life and specific nutritional and sensory features [6,7]. In this sense and following a criterion for the selection of autochthonous bacteria starters, seventeen strains of LAB were previously isolated from ripe cactus pears [4]. Of these, four strains identified as *Lactiplantibacillus plantarum* S-811, *L. plantarum* S-TF2, *Fructobacillus fructosus* S-22, and *F. fructosus* S-TF7 were selected based on probiotic features and their effect on functional properties of the fermented juices as potential starters for cactus pear juice fermentation [4]. This study aimed to determine the suitability of the selected strains for the production of a fermented beverage from *O. ficus-indica*'s fruit juice, guaranteeing improved shelf-life, as well as nutritional and functional features. Studies regarding the stability, physicochemical, and sensory attributes of cactus pear juice fermented with the most promising strain were carried out.

2. Materials and methods

2.1. Fruit samples

Cactus pears of the "green cultivar" (*O. ficus-indica*) were aseptically collected in the rural town of Colalao del Valle, in the Northwest of the province of Tucumán (Argentina). Ripe fruits were harvested during January (summer season). Hand-picked fruits were washed in water, frozen, and stored at −20 °C.

2.2. Bacterial strains

Four potential probiotic strains were evaluated. The strains were previously isolated from *O. ficus-indica* cactus pears and identified based on 16S rRNA profile as *Lactiplantibacillus plantarum* S-811, *L. plantarum* S-TF2, *Fructobacillus fructosus* S-22, and *F. fructosus* S-TF7 [4]. The strains were freeze-stored at −80 °C in Man-Rogosa-Sharpe (MRS) broth (Britania, Buenos Aires, Argentina) containing 20% (*v/v*) glycerol.

2.3. Technological properties of strains

Diacetyl production was determined by α-naphthol reaction in alkaline medium. Strains esterase and lipase activities were evaluated in agar plates containing emulsified tributyrin or soybean oil/$CaCl_2$ by the technique described by Tanasupawat et al. (2015) [8]. The production of enzymes was verified by the appearance of a translucent halo around the colonies in the opalescent culture medium. The production of proteolytic enzymes was determined according to Aarti et al. (2017) [9], growing the strains in casein supplemented agar plates. The presence of translucent halos around the colonies is interpreted as a positive proteolytic activity. Urease activity was assessed through the alkalization of urea agar due to the hydrolysis of urea. The production of exopolysaccharides (EPS) (ropy and mucoid phenotypes) was examined in MRS agar supplemented with 10% (*w/v*) sucrose [10]. Strains that presented viscous or mucous appearance in the medium were considered as producers of EPS with mucoid phenotype. Ropiness was evaluated by the presence of ropy after touching the colony with a loop [10]. Tolerance to saline stress was evaluated growing strains in MRS broth supplemented with different concentrations of NaCl (between 1 and 5%, *w/v*). Phenol tolerance assessment was performed growing LAB strains in MRS broth containing 0.2 or 0.5% (*v/v*) phenol.

2.4. Safety assessment of LAB strains

Safety analysis carried out to the studied strains includes gelatinase and hemolytic activities and antibiotic susceptibility. Gelatinase activity was evaluated in nutritious gelatin (15%, *w/v*) broth. To study the hemolytic activity, strains were grown on blood agar plates. Strains were classified in alpha, beta, and gamma hemolytic according to their lysis degree. The antimicrobial sensitivity test was carried out by the diffusion method, using commercial antibiotics discs (Chloramphenicol, 30 μg; Rifampicin, 5 μg; Tetracycline, 30 μg; Ampicillin, 10 μg; Penicillin, 10 U; Streptomycin, 300 μg; Erythromycin, 15 μg; Vancomycin, 30 μg; Gentamicin, 10 μg; and Clindamycin, 2 μg), following the recommendations of the CLSI (Institute of Clinical and Laboratory Standards, Ex NCCLS) (CLSI, 2016). According to the diameter of the halo of microbial growth inhibition, the strains can be classified as sensitive (S), resistant (R) or moderately sensitive (MS), by the susceptibility criteria pre-established by Charteris et al. (1998) for *Lactobacillus* [11].

2.5. Antagonistic activity of LAB strains against pathogenic strains

The antimicrobial activities of the strains of LAB against the pathogen bacteria *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Listeria monocytogenes* CLIP 74910 were evaluated according to Verón et al. (2017) with some modifications [4]. Antimicrobial activities were assayed in cell-free supernatants (CFS) and neutralized CFS, thermal treated CFS, and deproteinized CFS. The CFS were obtained by centrifugation (12,000 *g*, 5 min) of overnight cultures of LAB in MRS broth (37 °C). The neutralized CFS (pH 7) were obtained by the addition of 1 N NaOH. The thermal treatment of CFS was carried out to eliminate hydrogen peroxide by heating the supernatants at 80 °C for 10 min [12]. The deproteinization of CFS was accomplished by Proteinase K (Sigma-Aldrich, St. Louis, MO, USA) treatment (200 μg/ml) for 2 h at 37 °*C. Petri* dishes containing tempered soft agar (0.9%) BHI (20 ml) were inoculated in-depth with the indicator strains (approximately 10^6 CFU/ml). Wells (10 mm in diameter) were made in the agar layer and filled with the CFS, and then the plates were incubated for 24 h at 37 °C. The presence of growth inhibition halos was evaluated, and their diameters (mm) recorded.

2.6. Preparation of cactus pear juice

Cactus pear juice was prepared as previously described [4]. Briefly, the fruits were manually peeled and homogenized using a blender (Philips) to obtain the juice, that was stored at −20 °C until use. Before inoculation of LAB, the juice was centrifuged (15,000 *g*, 20 min) for removal of insoluble material and then pasteurized by heating at 64 °C for 30 min (pasteurized cactus pear juice). Pasteurized juice was used in all experiments. Non-pasteurized cactus pear juice was also used to study the effect of fermentation on the stability and physicochemical attributes that were evaluated in raw and pasteurized cactus pear juice.

2.7. Cactus pear juice fermentation

Before juice fermentation, each strain was cultured in MRS broth at 37 °C for 18 h (stationary growth phase) and washed in sterile saline solution (0.9%, *w/v*, NaCl; 12,000 *g*, 5 min). Cells were re-suspended to the original volume in sterile saline solution and used to inoculate pasteurized cactus pear juices (2%, *v*/v; equivalent to 2.7 10^4, 1.7 10^4, 1.2 10^4, and 1.1 10^4 CFU/ml, respectively for S-811, S-TF2, S-22, and S-TF7 strains), followed by incubation (24 h) at 37 °C. Each strain was used as the single autochthonous starter for the fermentation of the cactus pear juice. The samples were collected at 6 h intervals up to 24 h of culture and stored at −20 °C. Enumeration of LAB was carried out by

plating onto MRS agar at 37 °C for 24 h. Pasteurized cactus pear juice (PJ) not inoculated with the LAB and subject to the same treatment was used as the control.

For the study of stability, physicochemical and sensory attributes and safety of fermented cactus pear juice using the selected strain, the juice was fermented following the same procedure until reaching a pH value of 3.7 (approximately 7 h of growth and a cell count of 1.2×10^9 CFU/ml). The effectiveness of the fermentation process to guarantee the stability of cactus pear juice was also evaluated in raw cactus pear juice (RJ), which was prepared following the same procedure used to ferment the pasteurized cactus pear juice. Cactus pear juices not inoculated with the starter strain were used as controls.

2.8. Determination in fermented cactus pear juices of soluble solids (°Brix), pH, and kinetics of growth and acidification

Soluble solids were measured using an optical refractometer with automatic temperature compensation (Alla France, Chemillé, France). The pH of the fermented juices was measured by a glass probe digital pH meter (ADWA, Romania). For the determination of growing kinetics and cell counts (CFU/ml), the strains were cultivated in both MRS broth and cactus pear juice, as described previously in Verón et al. (2017) [4]. The parameters of acidification and growth were determined from the changes of pH and CFU/ml on time from linear first-order curves, dpH/dt = f(t) and dLogCFU/dt = f(t), respectively.

2.9. Analysis of nutrients and phytochemicals in fermented cactus fruit juices

Total neutral and reducing sugars were assayed applying the phenol-sulphuric acid and Somogyi-Nelson methods, respectively, according to Torres et al. (2011) [12]. Protein quantification was carried out by the assay of Bradford as previously described by Torres et al. (2011) [12]. Total phenolics content was measured by the Folin-Ciocalteau method [13]. Results were expressed in micrograms of gallic acid equivalents per milliliter of juice (μg of GAE/ml). Betalain contents were spectrophotometrically determined as previously described [14].

2.10. Quantification of organic acids and ethanol

The juices samples (pasteurized cactus pear juice and pasteurized cactus pear juice fermented with autochthonous LAB) were analyzed using a Knauer Wellchrom HPLC system (KNAUER Wissenschaftliche Geräte, GmbH, Germany) equipped with a Smartline Pump 100 and RI detector K-2301. Organic acids and ethanol were determined by using Rezex ROA-Organic Acid H+ (300 × 7.8 mm) (Phenomenex, USA). Column elution was carried out at 45 °C, with a flow rate of 0.6 ml/min, using 5 mM H_2SO_4 as mobile phase. The quotient of fermentation (QF) was determined as the molar ratio between lactic and acetic acid.

2.11. "In situ" antimicrobial activity of fermented cactus pear juices

The antimicrobial activity of fermented pasteurized cactus pear juices was assayed against pathogen bacteria *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Listeria monocytogenes* CLIP 74910. Pasteurized cactus pear juices fermented with each studied LAB were centrifuged (12,000 *g*, 5 min) and filter-sterilized using a 0.22 μm pore size membrane filter (Millipore). Each sterilized fermented juice was inoculated at 1% (*v*/v) with suspensions of each pathogen bacteria (10^6 CFU/ml; overnight cultures in BHI broth at 37 °C), and then incubated for 1 h at 37 °C. Then, the number of CFU/mL of the juices was count culturing aliquots of these juices in BHI agar plates for 24 h at 37 °C. Suspensions of pathogen bacteria were cultured in BHI agar plates as controls. Finally, the survival percentage was calculated.

2.12. Measurement of antioxidant activity of fermented cactus pear juices

The antioxidant capacity was determined by the ABTS cation radical assay and the hydroxyl radical scavenging assay following the methodology described by Orqueda et al. (2017) [15]. For ABTS assay, different dilutions of juices (equivalent to 2 to 25 μg GAE/ml total phenolic compounds) were added to $ABTS^{\bullet+}$ solution (1 ml) and mixed thoroughly. Absorbance was recorded at 734 nm, 6 min after initial mixing. Results were expressed as SC_{50}, which represents the concentration of the total phenolics (mg GAE/l) necessary to scavenge 50% $ABTS^{\bullet+}$. The hydroxyl radical scavenging was evaluated through the deoxyribose degradation assay [15]. The assay was carried out evaluating different dilutions of juices (equivalent to 0.5 to 25 μg GAE/ml). The hydroxyl radical scavenging activity was expressed as SC_{50} (μg GAE/ml), which represents the phenolics concentration required to inhibit by 50% the degradation of 2-deoxy-D-ribose by the hydroxyl radicals.

2.13. Evaluation of the stability, physicochemical and sensory attributes, and safety of cactus pear juice fermented with the selected strain

2.13.1. Microbiological analysis

The effectiveness of the fermentation process to guarantee the microbiological safety of both fermented pasteurized cactus pear juice (FPJ) and fermented raw cactus pear juice (FRJ) was evaluated. The microbial quality of cactus pear juices (RJ, PJ, FPJ, and FRJ) was investigated during the fermentation, and during the storage period (60 days) by using the standard plate method. Juice samples were taken every 1 h during the fermentation (approximately 7 h of growth; up to a pH value of 3.7), and appropriate serial dilutions of them were made in sterile saline solution (0.9%, *w/v*). The dilutions were plated and incubated as follows: LAB were cultured in MRS agar (Britania) at 37 °C for 48 h; Enterobacteriaceae in Mac Conkey agar (Britania) at 27 °C for 48 h and fungi and yeasts on Fungi and Yeast agar (Britania) at 28 °C for seven days. The microbiological stability of juices during 60 days of storage at 4 °C was analyzed at 15, 30, 45, and 60 days of storage according to the previously described methodology. Microbial counts were performed by triplicate and expressed as log CFU/ml.

2.13.2. Physicochemical parameters of fermented cactus pear juices

The evaluation of physicochemical parameters (color, soluble solids, browning index, cloud index, and alcohol insoluble solids) of fermented cactus pear juices was carried out in triplicate in fruit juices stored for 0, 15, 30, 45, and 60 days at 4 °C.

Color parameters were measured with a Chroma-Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan) using the CIELab system. The results were expressed as L*, a*, and b* chromaticity coordinates, where L* indicates lightness, a* represents redness, and b* represents yellowness. These coordinates were used to calculate Chroma (C = [a*2 + b*2]1/2) and Hue angle (h° = tg-1 (b*/a*)). The total difference in color (ΔE) of FRJ and FPJ was calculate using the control juices (FJ and PJ) as references with the following formula: $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ [15]. The browning index was measured according to the method described by Cruz-Cansino et al. (2015) [16]. Ten (10) ml of cactus pear juices were centrifuged (3400 rpm; 10 min) to remove insoluble particles. Then, 5 ml of ethanol 96° was added to 5 ml of supernatant and centrifuged under the same conditions. The browning index of the supernatant was obtained by measuring the absorbance at 420 nm (Biotek ELx 808). The cloud index was determined according to the methodology described by Cruz-Cansino et al. (2015) [16]. Five (5) ml of cactus pear juice were centrifuged at 3400 rpm for 10 min at room temperature, and then the cloud index of the beverages was measured as turbidity at 660 nm (Biotek ELx 808). The alcohol-insoluble solids (AIS) were determined following the methodology developed by Baccouche et al. (2013) [17]. Two (2) parts of ethanol 96° were added to one (1) of

cactus pear juice, and the mix was left to rest for 15 min until the insoluble solids precipitate. The suspension was centrifuged for 10 min at 3400 rpm, and the supernatant was discarded. The weight of the precipitated sediment represents the AIS, and it was measured after drying the precipitate at 50 °C until the weight remained constant.

2.13.3. Sensory evaluation of cactus pear juice fermented with the selected strain

The sensory evaluation was carried out to PJ and FPJ by using two different methodologies: (i) paired comparison and (ii) verbal hedonic scale. (i) For the paired comparison test, a non-trained panel was used, made up of 59 assessors between 25 and 68 years old (25 males and 34 females). All participants were required to be healthy at the moment of the study. Sensory evaluation was approved by the INBIOFIVś Human Ethics Committee (Approval No: 2018–03; date of approval 14-11-2018). Panelists received samples of both cactus pear juices coded with random numbers served in transparent glasses at 4 °C. The characteristics analyzed were color, smell, taste, flavor, and texture. (ii) The hedonic test was carried out according to Prado et al. (2015) [18] using a non-trained panel of 118 people (50 males and 68 females between 25 and 69 years old). Evaluations of each juice were made separately, and the attributes overall liking (acceptability), color, appearance, aroma, flavor, texture, and aftertaste were analyzed. Panelists rated the attributes of the samples on a 7-point hedonic scale (1, dislike very much; 2, dislike moderately; 3, dislike slightly; 4, neither like nor dislike; 5, like slightly; 6, like moderately; and 7, like very much). Sensory tests were performed in individual booths in the morning (9:00 a.m. - 11:30 a.m.) under white light. The samples were served at 4 °C in transparent glasses. To verify the acceptability of JP and JPF fruit juices an acceptability factor (AF) was calculated concerning each attribute analyzed, using the following equation: AF = A × 100 × B-1; where A is the average value obtained for each attribute and B is the maximum value for each attribute [18].

Differences among responses were statistically evaluated at a confidence level of 95%.

2.13.4. Toxicity assessment of cactus pear juice fermented with the selected strain

2.13.4.1. Cytotoxic assay. The acute cytotoxic activity of cactus pear juices was predicted using the *Artemia Salina* (*A. salina*) test as previously described by Orqueda et al. (2020) [19]. *A. salina* larvae (nauplii) were exposed (24 h at 25 °C) to different concentrations of cactus pear juices (equivalent to 8.0–80.0 μg EAG/ml). Potassium dichromate (10–40 μg / ml) and seawater were used as positive and negative controls, respectively. After treatment, the number of dead nauplii in each juice concentration was counted, and the LC_{50} (concentration that kills 50% of the *A. salina* larvae) was calculated.

2.13.4.2. Mutagenic activity. The mutagenicity effects of cactus pear juices were evaluated by the Ames test using two *Salmonella* Typhimurium strains (TA98 and TA100) [19]. Briefly, the bacterial strains were exposed to different concentrations of cactus pear juice (20–160 μg EAG/plate). The revertant colonies (His+) in minimal medium (without histidine) were counted and compared to the number of revertant colonies in the controls. Sterile distilled water was used as the negative control and 4-nitro-*o*-phenylenediamine (4-NPD, 1 mg/ml) as the positive control. Results were expressed as the number of revertants/plate and Mutagenicity Ratio (MR) (ratio between the number of induced revertants (IR) and the number of spontaneous revertants (SR; revertants in the control plate); MR = IR/SR). Juices were considered non-mutagenic if the MR was less than two (2) and mutagenic if the MR was higher than 2 [19].

2.14. Statistical analysis

Results are expressed as the mean ± standard deviation or standard error of two or more experiments with duplicate determinations. The statistical analyses were performed in SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The results were analyzed using a one-way analysis of variance (ANOVA) with Tukey's test as a post-hoc test. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

Preserving cactus pears from decay and enhancing the health-promoting properties of their by-products' is crucial to support and expanding this traditional crop in Argentina. In previous research, we selected four potentially probiotic autochthonous bacteria from cactus pear fruits, based on their probiotic features and the effect thereof on the functional properties of the fermented juices [4] Some probiotic features previously tested in these strains were gastrointestinal stress tolerance, cell surface properties, and feruloyl esterase activity [4]. These isolates were identified by partial sequencing of 16S rRNA as *Lactiplantibacillus plantarum* S-811, *L. plantarum* S-TF2, *Fructobacillus fructosus* S-22, and *F. fructosus* S-TF7 [4]. In the current investigation, we further study the technological properties of these autochthonous bacteria and their value for the lactic acid fermentation of *Opuntia ficus-indica* juice to improve its shelf life and functional properties.

3.1. Technological properties

In the selection of microorganisms with the potential to be used for the preparation of fermented foods, different beneficial properties of the microorganisms should be evaluated. They should confer to fermented foods desirable aspects, such as characteristic flavors, aromas, and textures, or provide nutritional and health benefits to people who consume them [20]. The study of the technological properties of the pre-selected *L. plantarum* and *F. fructosus* strains showed that the four strains were able to produce diacetyl (Table S1 in the Supplementary Material), which contributes to fermented drinks with caramel and creamy flavor appreciated in some types of beers and fermented fruit juices [21–23]. None *Fructobacillus* strains showed lipase, protease, amylase or urease activities in the assayed conditions (Table S1). However, *L. plantarum* strains S-811 and S-TF2 displayed esterase and amylase activities (Table S1). LAB are an important source of microbial hydrolases, including carboxylesterases and lipases. In particular, esterases from *L. plantarum* species were extensively investigated [24]. From a technological and functional point of view, these enzymes play a fundamental role [25]. Esterases can hydrolyze and produce esters, having fundamental consequences on the flavor of fermented foods, but also, esterases are involved in the bioconversion of phenolic compounds in vegetable fermented foods impacting their antioxidant potential and bioavailability [25]. Regarding amylases, these enzymes could be related to the released of oligosaccharides from food starch. The studied strains were neither able to produce EPS, except for *L. plantarum* S-811, which showed a mucoid phenotype in MRS agar supplemented with sucrose (Table S1). *L. plantarum* is recognized as an EPS producing species, a secondary metabolite of LAB with diverse applications in food and pharmaceutical industries [26].

Another beneficial feature in probiotics is the tolerance to saline stress, particularly that produced by NaCl, an osmotically active agent that at high concentrations can drastically affect microorganisms and prevent their cellular processes. In our study, all strains studied were defiant to NaCl and were able to grow at 1–5% NaCl concentration (Table S1). *L. plantarum* S-811 and *L. plantarum* S-TF2 showed good salt-tolerance, and their growth was slightly influenced by NaCl up to 5% (*v*/v) concentration. These results agree with many studies that demonstrated the salt-tolerance of *L. plantarum* that can grow without difficulty in NaCl concentrations between 5 and 7% [27]. Concerning *F. fructose* S-

22 and *F. fructose* S-TF7, 1% NaCl had not impacted their growth, but at 5% NaCl, their growth decreased by approximately 30 and 50%, respectively. Almost no research about the tolerance of *Fructobacillus* spp. to osmotic stress was available. Recent work reported a scarce growth of papaya's autochthonous *F. tropaeoli* 77 strain in NaCl 5%, a lactic acid bacterium with the potential to improve vegetable-based food shelf life [28].

The selected *L. plantarum* and *F. fructosus* strains were also screened for their aptitude to endure the phenolic environment (Table S1). Phenol is a toxic metabolite of gut bacteria produced through the deamination of aromatic amino acids [29]. Various reports demonstrated the differences regarding phenol tolerance displayed by probiotic LAB, which showed a varied ability to tolerate phenol between 0.2 and 0.6% [29,30]. In this study, all the examined LAB strains were competent to tolerate both 0.2% and 0.5% phenol concentrations, indicating their ability to resist the impact of bacteriostatic phenol in the intestine [29]. As expected, 0.5% phenol treatment resulted more toxic for the studied strains where showed between 17 and 24% relative growth. However, the LAB strains had good tolerance to 0.2% phenol, displaying relative growth between 81 and 94%. These results are consistent with the performance of other probiotic LAB to withstand phenol toxicity, which showed similar or less tolerance to phenol, like probiotics *L. plantarum* TA4, *L. plantarum* R17, *Limosilactobacillus fermentum* RV02, *Lacticaseibacillus paracasei* CCMA 0504, or *L. plantarum* CCMA 0743 isolated from several fermented foods or beverages [29,31,32].

3.2. Safety evaluation

Despite LAB being considered safe microorganisms, recognized as GRAS (Generally regarded as safe; FDA) and QPS (Qualified Presumption of Safety; EFSA) microorganisms, it is necessary to determine their safety if new bacterial isolates want to be used as probiotics. Among the tests to be carried out are the study of gelatinase activity, hemolytic activity, and antibiotic susceptibility (Table 1) [33].

3.2.1. Hemolysins production and gelatinase activity

Many pathogenic microorganisms can produce cytotoxins, which may cause lysis of a wide variety of cells, including red blood cells, by generating pores in the cytoplasmic membrane. This characteristic is strain-dependent and therefore is necessary to evaluate to ensure the safe use of microorganisms. In agreement with many reports for LAB with probiotic potentiality, the selected LAB were nonhemolytic (γ-hemolytic) (Table 1) [34]. Moreover, the four strains under study exhibited negative activity for the harmful enzyme gelatinase, which is known as potential virulence factor in bacteria (Table 1).

3.2.2. Antibiotic susceptibility

The QPS program included the evaluation of antibiotic susceptibility to declare probiotics as GRAS. The susceptibility study to antimicrobial agents of the four selected strains is shown in Table 1. It was shown that the *L. plantarum* S-811 and *L. plantarum* S-TF2 strains have a resistance profile to antibiotics similar to those previously described for these species [34,35]. Other studies on various species of *Lactobacillus* and *S. thermophilus* demonstrated frequent resistance of these LAB to antibiotics of the aminoglycoside group (gentamicin, kanamycin, and streptomycin) [36]. Zhang et al. (2018) [37] showed that streptomycin resistance in *L. plantarum* is intrinsic. Besides, *Lactobacillus* species are also intrinsically resistant to the vancomycin glycopeptide antibiotic, and this type of constitutive resistance is not considered transferable to other species [38]. To date, knowledge about the susceptibility to antibiotics of the genus *Fructobacillus* is scarce. Sakandar et al. (2019) [39] isolated several *Fructobacillus* species, including *F. fructosus* species from various fruits and flowers of China. In the studies conducted on these strains, authors did not observe resistance to any of the antibiotics tested (ciprofloxacin, ceftriaxone), except for moderate sensitivity to novobiocin (an aminocoumarin antibiotic) and gentamicin. The results

Table 1
Safety evaluation of cactus pear autochthonous LAB.

Autochthonous LAB strain	Gelatinase activity	Hemolysis	Antibiotic susceptibility[a]									
			Ampicillin	Penicillin	Vancomycin	Gentamicin	Streptomycin	Tetracycline	Chloramphenicol	Erythromycin	Clindamycin	Rifampin
L. plantarum S-811	–	γ	S	MS	R	R	R	MS	S	S	MS	MS
L. plantarum S-TF2	–	γ	S	MS	R	R	MS	MS	S	S	S	S
F. fructosus S-TF7	–	γ	S	S	R	R	MS	MS	S	S	S	S
F. fructosus S-22	–	γ	S	S	R	R	S	R	S	S	R	S

Gelatinase activity, hemolytic activity, and antibiotic susceptibility profile of selected LAB. (−) negative activity; (**γ**) absence of lysis of red blood cells.

[a] Antibiotic susceptibility: (**S**), Susceptible; (**MS**), Moderately Susceptible; (**R**), Resistant. Inhibition zone diameters (mm): Ampicillin, S (≤12), MS (13–15), R (≥16); Penicillin, S (≤19), MS (20–27), R (≥28); Vancomycin, S (≤14), MS (15–16), R (≥17); Gentamicin, S (≤12), R (≥13); Streptomycin, S (≤11), MS (12–14, R (≥15); Tetracycline, S (≤14), MS (15–18), R (≥19); Chloramphenicol, S (≤13), MS (14–17), R (≥18); Erythromycin, S (≤13), MS (14–17), R (≥18); Clindamycin, S (≤8), MS (9–11), R (≥12); Rifampin, S (≤14), MS (15–17), R (≥18).

obtained with the strains under study suggest that their consumption, especially of the *L. plantarum* strains, would not represent a risk to human health due to antibiotic resistance. However, genetic studies should be conducted to confirm the intrinsic nature of resistance genes.

3.3. Cactus pear juice fermentation by indigenous Lactiplantibacillus and Fructobacillus strains

Cactus pear juice was fermented with the four strains: *L. plantarum* S-811, *L. plantarum* S-TF2, *F. fructosus* S-TF7, and *F. fructosus* S-22. Growth kinetics, as well as changes in total soluble solids (°Brix), sugars, total

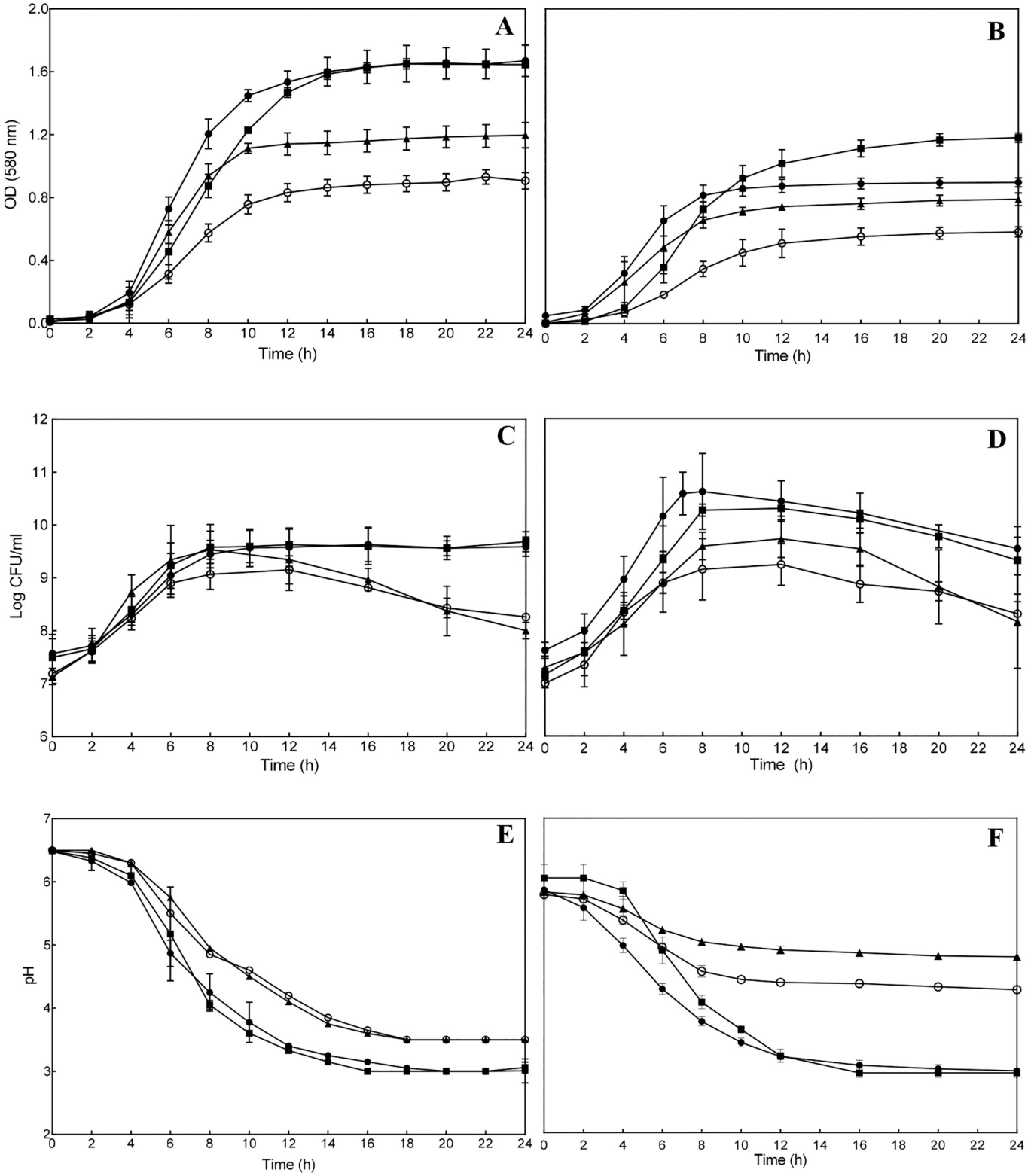

Fig. 1. Time courses of optical density (OD_{580nm}), Log CFU/ml, and pH of *L. plantarum* S-811 (●), *L. plantarum* S-TF2 (■), *F. fructosus* S-TF7 (▲), and *F. fructosus* S-22 (○) in MRS broth (graphs A, C, and E) and pasteurized cactus pear juice (graphs B, D, and F) at 37 °C.

phenolics, and betalains contents, production of organic acids and ethanol, and antioxidant activities, were analyzed in fermented cactus pear juices.

3.3.1. Growth kinetics in cactus pear juice

As previously shown, the four selected microorganisms were able to grow in cactus pear juice [4]. In this study, we analyzed the growth kinetics both in MRS broth and cactus pear juice (Fig. 1A-1F; Table 2). In MRS broth *L. plantarum* S-811 and *F. fructosus* S-TF7 showed the highest specific growth rate (μ; h^{-1}). Whereas, in cactus pear juice, *L. plantarum* S-811 and *L. plantarum* S-TF2 had the highest μ and reached higher cell counts (CFU/ml) (Table 2). *L. plantarum* S-811 fermented the cactus pear juice faster than the other strains (0.186 h^{-1}). This strain reached after about 7 h the stationary growth phase and decreased the pH of the juice to values ranging from 3.75 to 4.00, features that are rather attractive from a technological point of view (Fig. 1). In all fermented juices a decrease in the growth rate was observed in comparison to the growth rate measured in MRS broth. However, at the end of 24 h of growth, all strains reached similar cell counts in both juice and MRS broth. Reddy et al. (2015) [40] reported similar behavior for *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *L. plantarum*, and *Lacticaseibacillus casei*, in the fermentation of mango juice.

3.3.2. Sugars, total phenolics, and betalains contents in fermented cactus pear juices

The content of sugars was determined after 24 h of fermentation. Total and reducing sugars decreased between 15% and 25% at 24 h of growth. Besides, a significant decrease between 27% and 42% of the glucose content was observed for the four LAB strains at 24 h of growth (Table 3). Reddy et al. (2015) [40], observed that strains of *L. plantarum*, *Lactobacillus acidophilus*, *Lacticaseibacillus casei,* and *Lactobacillus delbrueckii* similarly reduce the levels of reducing sugars during the fermentation of mango juice. In general, the consumption of sugars in fruit juices fermented with LAB varies considerably according to the strain used, the composition of sugars in the fruit juice, and the time of fermentation [41,42].

As was expected for green varieties of cactus pears, the concentrations of indicaxanthin and betanin were low (1.6 and 1.5 g/l, respectively; data not shown). Besides, matching previous reports [43,44], the fermentation of cactus pear juice with the studied strains decreased between 10 and 25% betalains concentrations. Regarding phenolic compounds, after 24 h of fermentation, there were no significant changes in the concentration of total phenolics in the fermented juices with *L. plantarum* S-TF2 and *F. fructosus* S-22 (Table 3). The juice fermented with *L. plantarum* S-811 showed a slight increase ($P < 0.05$), higher than 10%, reaching a value of 798 μg GAE/ml after 24 h. This increase in phenolic compound content can be related to the enzymatic release of simple phenolics from fibers or polymeric phenolics due to feruloyl esterase or tannase enzymes reported in *L. plantarum* strains [45,46]. In the juice fermented with *F. fructosus* S-TF7, there was a significant decrease ($P < 0.05$) in the concentration of phenolics throughout the fermentation. However, this decrease was <10%. Panda et al. (2017) [47] observed in the fermentation of cactus pear juice by the collection strain *Limosilactobacillus fermentum*-ATCC 9338, a 10% decrease in the content of phenolics in fermented juice. Although, previous studies reported an increase in the concentration of phenolic compounds during the lactic fermentation of different plant matrix. Mango or sapota juices, fermented with *L. plantarum* NCDC LP 20, or fermented cactus pear with *Leuc. mesenteroides,* significantly increased the concentration of phenolic compared to unfermented foods [5,48]. The fermentation of vegetable substrates by LAB depends on the ability of these microorganisms to quickly adapt and metabolize the nutrients and various compounds available therein, including phenolic compounds [49]. This adaptation is specific for each species and bacterial strain and varies considerably according to the plant matrix.

Table 3
Nutritional and functional attributes of fermented cactus pear juices.

Compound or attribute	Cactus pear Juice				
	PJ	FPJ-*L. plantarum* S-811	FPJ-*L. plantarum* S-TF2	FPJ-*F. fructosus* S-TF7	FPJ-*F. fructosus* S-22
Total sugars (mg/ml)	126.7 ± 3.4[a]	106.1 ± 2.2[c]	99.4 ± 5.8[b]	86.1 ± 5.8[c]	105.6 ± 6.9[b]
Reducing sugars (mg/ml)	97.7 ± 2.4[a]	82.8 ± 7.9[a]	73.4 ± 9.6[b]	74.8 ± 7.2[b]	75.9 ± 11.4[b]
Glucose (mg/ml)	71.4 ± 1.1[a]	41.4 ± 0.7[d]	52.2 ± 0.8[c]	42.9 ± 0.7[d]	47.3 ± 0.8[d]
°Brix	11.7 ± 0.5[a]	10.2 ± 0.1[b]	12.4 ± 0.1[a]	11.9 ± 0.1[a]	11.9 ± 0.1[a]
Organic acids (g/l) [mM]					
Lactic acid	0.0	9.4 ± 0.1 [104.8][e]	9.6 ± 0.1 [106.2][e]	9.5 ± 0.1 [105.7][e]	4.3 ± 0.1 [47.4][f]
Acetic acid	0.0	0.1 ± 0.0 [2.2][a]	0.3 ± 0.0 [4.7][b]	0.1 ± 0.0 [1.2][a]	2.7 ± 0.0 [44.6][c]
Lactic a./ Acetic a.	0.0	25.8	16.1	23.6	1.7
Propionic acid	0.0	0.2 ± 0.0 [2.7][d]	0.1 ± 0.0 [2.0][d]	0.2 ± 0.0 [2.2][d]	0.2 ± 0.0 [2.3][d]
Ethanol (g/l) [mM]	0.0	0.0	11.2 ± 0.0 [242.9]	0.0	0.0
Total phenolics (μgGAE/ml)	711.2 ± 9.5[ab]	798.7 ± 3.9[c]	725.1 ± 15.9[a]	627.5 ± 7.5[d]	703.6 ± 2.5[b]
ABTS (SC_{50} μgGAE/ml)	3.7 ± 0.9[a]	3.8 ± 1.2[a]	3.6 ± 0.5[a]	3.6 ± 0.9[a]	3.3 ± 0.5[a]
Hydroxyl radical (SC_{50} μgGAE/ml)	1.2 ± 0.1[a]	2.4 ± 0.1[b]	1.9 ± 0.1[c]	2.1 ± 0.1[bc]	1.5 ± 0.1[a]

PJ: pasteurized cactus pear juice; FPJ: pasteurized cactus pear juice fermented with autochthonous LAB. Sugars, organic acids, ethanol, total phenolics, and antioxidant activity were evaluated in not fermented juice and juices fermented at 37 °C for 24 h using autochthonous LAB. Antioxidant activity was measured as the ability to scavenge the ABTS cation radical and the hydroxyl radical. Results were expressed as SC_{50}, which represents the total phenolics concentration (μg of GAE/ml) required to scavenge 50% ABTS cation radical or necessary to inhibit by 50% the degradation of 2-deoxy-D-ribose by the hydroxyl radicals, respectively. The values are presented as the mean ± standard deviation of triplicates. For the same particular compound or attribute, different letters indicate significant differences ($P < 0.05$) between the juices.

Table 2
Kinetic parameters of cactus pear autochthonous LAB in MRS broth and cactus pear juice.

Parameter	μ (h^{-1})		ΔpH (24 h)		CFU/ml (24 h)		λ (h)	
Medium	MRS	JUICE[a]	MRS	JUICE[a]	MRS	JUICE[a]	MRS	JUICE[a]
L. plantarum S-811	0.267	0.186	3.48	2.88	3.89×10^9	3.52×10^9	2	2
L. plantarum S-TF2	0.210	0.184	3.43	2.90	4.84×10^9	2.10×10^9	2	3
F. fructosus S-TF7	0.220	0.112	3.00	1.03	1.01×10^8	1.45×10^8	2	2
F. fructosus S-22	0.130	0.082	3.00	1.51	1.82×10^8	2.05×10^8	2	3

[a] Pasteurized cactus pear juice fermented using the selected LAB. (**μ**) Specific growth rate; (**ΔpH**) difference between the pH of the unfermented juice or MRS and the pH of the juice or MRS at the end of the fermentation (24 h); (λ), duration of the lag phase in cactus pear juice and in MRS broth.

3.3.3. Production of organic acids and ethanol in fermented juices

The determination of organic acids and ethanol in fermented cactus pear juices was carried out at 24 h of fermentation. Organic short-chain acids are recognized as safe natural antimicrobials and are known for their ability as food preservatives, inhibiting the development of pathogenic and spoilage bacteria [50]. Also, these acids contribute to the taste of fermented foods. The presence of lactic acid in fermented beverages gives a bittersweet taste, and is detected from a concentration of 0.93 g/l [51]. Under the conditions assayed, this value of lactic acid was surpassed after the fermentation with the four studied microorganisms (Table 3). No significant difference was observed in the production of lactic acid from *L. plantarum* S-811, *L. plantarum* S-TF2, and *F. fructosus* S-TF7 with values around 9.5 g/l. Regarding acetic acid, *F. fructosus* S-22 produced the highest content of this acid (2.68 g/l) (Table 3). The concentration at which acetic acid can be sensory detected is 0.4 g/l [51]. Considering this result and its tolerance to NaCl (Table S1), *F. fructosus* S-22 could be used for the production of pickled cactus pears. High concentrations of acetic acid usually present as an unwanted feature in fermented beverages, since acetic acid has an aroma and taste that is unpleasant in drinks, but not in pickles or pickled vegetables. The production of propionic acid did not show a significant difference between the four microorganisms used, between 0.15 and 0.2 g/l. These values were higher than that produced by *Limosilactobacillus reuteri* NCIMB 701359, a probiotic with potential for use in the prevention and treatment of colon cancer, due to the production of propionic acid [52]. Respecting the production of ethanol in fermented cactus pear juice, the strain *L. plantarum* S-TF2 was the only capable of producing ethanol (11.19 g/l), so it could be used for the production of an alcoholic beverage based on cactus pears. As was expected, due to the absence of the alcohol/acetaldehyde dehydrogenase gene in *Fructobacillus* spp. [53], the strains of *F. fructosus* (S-22 and S-TF7) did not produce ethanol as the final product.

3.3.4. Antioxidant activity of fermented juices

The effect of lactic fermentation on the antioxidant activity of food depends on the LAB strain used, the chemical composition of the substrate, as well as the process used to carry out fermentation [54]. In the present study, none of the studied LAB showed significant changes in the scavenging of the radical cation ABTS concerning unfermented juice (Table 3). Regarding the hydroxyl radical scavenging activity, unfermented and fermented cactus pear juices showed scavenging capacities (SC_{50} between 1.20 and 2.50 μg GAE/ml) (Table 3). Although the SC_{50} values of fermented juices, except for the juice fermented with *F. fructosus* S-22, were higher than those of unfermented juice, they retained 50% or more of the scavenging capacity of the hydroxyl radical.

3.3.5. Antimicrobial activity of LAB and fermented cactus pear juices

Antimicrobial activity towards pathogenic bacteria is an important property in probiotic bacteria. LAB can produce diverse antimicrobial metabolites, such as organic acids, hydrogen peroxide, and antimicrobial peptides [20].

3.3.5.1. Antimicrobial activity of cell-free supernatants. The antimicrobial activity of the cell-free culture supernatants of the four strains was checked using the well-diffusion method agar against strains of *Escherichia coli*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Listeria monocytogenes* (Table 4). To gain insight into the nature of the antimicrobial activities, experiments using non-neutralized and neutralized, heated, and protease treated supernatants were performed. These assays showed that only the non-neutralized supernatants of *L. plantarum* S-811 and *L. plantarum* S-TF2 were capable of inhibiting the growth of the pathogens analyzed. The highest inhibition was observed by *L. plantarum* S-TF2 against *P. aeruginosa*. The loss of activity of supernatants after their neutralization and the absence of effects of thermal and protease treatments on the antimicrobial activity of CFS allowed to estimate that the inhibition occurs due to acidic compounds present in the supernatants, discarding the intervention of hydrogen peroxide or peptides in antimicrobial activity. Regarding *Fructobacillus* species, although previous reports have shown that strain *F. fructosus* MCC 3996 can inhibit bacterial pathogens by non-proteic substances [41], none of the evaluated supernatants of *F. fructosus* S-TF7 and *F. fructosus* S-22 could inhibit any pathogen evaluated.

Table 4
Antimicrobial activity of autochthonous LAB strains and fermented cactus pear juices against undesirable bacteria.

Pathogen strain	Zone of inhibition (mm)[a]				Inhibition in FPJ (%)[b]			
	S-811	S-TF2	S-TF7	S-22	S-811	S-TF2	S-TF7	S-22
Escherichia coli	8	8	–	–	2.1	0.2	0	4.4
Listeria monocytogenes	9	6	–	–	2.5	3.5	0.4	0.6
Salmonella Typhimurium	7	7	–	–	33.4	31.0	0	40.9
Staphylococcus. aureus	5	6	–	–	0	0	0	0
Pseudomonas aeruginosa	8	10	–	–	100	100	0	0

FPJ: pasteurized cactus pear juice fermented with autochthonous LAB.
[a] Diameter (mm) of the zone of inhibition obtained with non-neutralized cell-free supernatants (CFS) of autochthonous LAB strains in MRS broth. Neutralized CFS did not show inhibitory activity against any pathogen strain. Thermal and protease treatments did not show effects on the antimicrobial activity of CFS.
[b] Percentage reduction in cell counts of pathogenic bacteria after exposed to fermented juices without neutralizing for 1 h ("In situ" antimicrobial activity). Controls were carried out by exposing of pathogenic bacteria to unfermented cactus pear juices and then cultivated in BHI agar plates for 24 h at 37 °C. No inhibition of any of the pathogens tested was observed in the unfermented juice.
S-811: *L. plantarum* S-811; S-TF2: *L. plantarum* S-TF2; S-TF7: *F. fructosus* S-TF7; S-22: *F. fructosus* S-22.

3.3.5.2. Antimicrobial activity of fermented cactus pear juices. The antimicrobial activity of fermented juices was studied in situ, inoculating pathogen bacteria in each juice. No inhibition of any of the pathogens tested was observed in the unfermented juice. Concerning fermented juices, only the juice fermented with *F. fructosus* S-TF7 was not able to inhibit the growth of any pathogen after the incubation for 1 h in the juice (Table 4). The other fermented juices showed similar antimicrobial activity *against Ps. aeruginosa* and *S.* Typhimurium. These results prove that antimicrobial activity observed in juices fermented with *L. plantarum* S-811, *L. plantarum* S-TF2, and *F. fructosus* S-22 is strictly related to the LAB activity, discarding an intrinsic function of cactus pear juice related to active compounds naturally present in the fruit matrix. Despite the supernatants of *L. plantarum* S-811 and S-TF2 showed some antimicrobial activity against *E. coli*, *L. monocytogenes*, and *St. aureus*, their fermented juices did not be able to affect the survival of any of these pathogens. However, they were capable to 100% inhibit the growth of *Ps. aeruginosa* and in a 30–40% of *S.* Typhimurium. Similarly, various studies reported the inhibition of pathogen bacteria by fermented plant juices [55–57]. Such is the case of sweet lemon juice fermented using a *L. plantarum* strain, which showed increased inhibitory activity against *E. coli* and *S.* Typhimurium [58].

3.4. Cactus pear juice fermentation by L. plantarum S-811. Effects on the stability, physicochemical, and sensory attributes

Based on the technological and functional attributes of *L. plantarum* S-811, which enables juice preservation and biofunctional properties, this strain was selected as the most promising for fermentation of cactus pear juice with probiotic features.

3.4.1. Microbiological status of the juice

The presence of Gram-positive bacteria, enterobacteria, fungi, and yeasts was evaluated during the fermentation and storage of cactus juices. Fig. 2.A shows the colony counts (log CFU/ml) carried out in the different culture media and pH of raw and pasteurized cactus pear juices fermented with *L. plantarum* S-811 (FRJ-*L. plantarum* S-811 and FPJ-*L. plantarum* S-811) during 7 h of fermentation. The growth of the *L. plantarum* S-811 strain was similar in both juices (pasteurized and fresh) (Fig. 2.A). In the FPJ-*L. plantarum* S-811, no development of contaminating microorganisms was detected during the 7 h of fermentation, only the growth of the inoculated lactobacilli (MRS medium) was observed. Contaminating yeast and enterobacteria were detected in the RJ. However, after 2 h of fermentation with *L. plantarum* S-811, yeasts cells were no longer detected in the started fresh juice, and the number of enterobacteria was gradually decreasing until disappearing after 7 h of fermentation (Fig. 2.A). These results demonstrate that fermentation of cactus pear juice with *L. plantarum* S-811 confers antimicrobial activity to the fermented beverage against these contaminating microorganisms, providing safety to the product. Besides, storage at 4 °C for 60 days (Fig. 2.B) showed that both fermented juices presented microbiological stability. The control raw juice (RJ), in contrast, showed a development of microorganisms that caused its total spoilage before 15 days of storage. Similarly, Panda et al. (2017) reported that fermentation of cactus pear juice using a commercial probiotic, *L. fermentum* - ATCC 9338, allowed to eliminate of unwanted microbial load in the fruit [47]. However, *L. fermentum* - ATCC 9338 lost viability during storage, contrary to *L. plantarum* S-811 that remained viable during the 60 days of storage (Fig. 2.B).

3.4.2. Physicochemical parameters of fermented cactus pear juices

In both fermented juices (FPJ-*L. plantarum* S-811 and FRJ-*L. plantarum* S-811), the luminosity (L*) did not considerably change during fermentation and up to 30 days of storage, but decreased ($P < 0.05$) between 10 and 15% at 60 days of storage (Table 5). Regarding green color, an increase ($P < 0.05$) in a* values (which represents a decrease in green color) was observed after 15 days of storage in both fermented juices. Whereas yellow color (positive values in the blue-yellow scale, b*) did not undergo significant changes during the pasteurization and fermentation process, nor in refrigerated storage for 60 days (Table 5). Also, no significant changes ($P > 0.05$) were observed in the values of the Index C (Chroma, visual intensity of the color) of the juices due to the effect of pasteurization, fermentation, or storage processes. This result indicates that the color remained stable throughout the process, which is a desired characteristic for food quality, contrary to what is observed in cactus pear juices subjected to heat treatment or ultrasound for their preservation [16].

Browning is relevant for the food industry because it is associated with changes in nutritional values and shelf life of food during storage. Browning occurs in many fruits and vegetables through the oxidation of phenolics to quinones catalyzed by the phenol oxidase, followed by condensation of the quinones in insoluble brown polymeric pigments (melanins) [59]. The Browning index increased slightly ($P < 0.05$) with fermentation in both juices (pasteurized and fresh) (between 8 and 14%). However, during storage at 4 °C, there was a significant decrease in this index for the fermented juices, with values lower than the unfermented controls (RJ and PJ) (Table 6). Previous studies showed this parameter varied significantly in juices subjected to thermal and ultrasound treatments for their preservation and can increase after treatment and during storage [16]. However, the fermentation of the juices was effective in preventing browning during storage. Also, a significant decrease in cloud index (turbidity, a negative attribute in clarified juices) was observed after the fermentation in both fruit juices (pasteurized and fresh), and then the turbidity remained stable up to 60 days of storage at 4 °C. The decrease in cloud index in fermented juices may be related to changes in polysaccharides composition due to lactic fermentation or by the precipitation of pectic polysaccharides associated with pH reduction [5]. The obtained results suggest that the visual texture of both juices fermented with *L. plantarum* S-811 remains stable during storage.

3.4.3. Sensory evaluation of fermented cactus pear juice

Sensory analysis of fermented cactus pear juice (FPJ-*L. plantarum* S-811) and non-fermented juice (PJ) were compared. The hedonic survey scoring sensory attributes of overall acceptability, color, appearance, aroma, flavor, texture, and aftertaste showed that overall acceptability and the taste did not significantly ($P > 0.05$) differ between both juices (Fig. 3). Regarding the other attributes evaluated (color, appearance, aroma, texture, and aftertaste), they showed a slight but significant ($P < 0.05$) preference in the non-fermented juice. Despite this, the FPJ-*L. plantarum* S-811 presented positive acceptability by the consumers with scores close to 5 (above the average value). Besides, FPJ-*L. plantarum* S-811 showed acceptability factors (AF) $\geq$ 70% (excluding the aroma and aftertaste, with AF of 67.28 and 68.18, respectively) (Table S2), which represents good acceptability for an attribute in a sensory evaluation [18]. Also, the acceptability of the taste of the FPJ-*L. plantarum* S-811 was similar to that reported by Panda et al. (2017) for

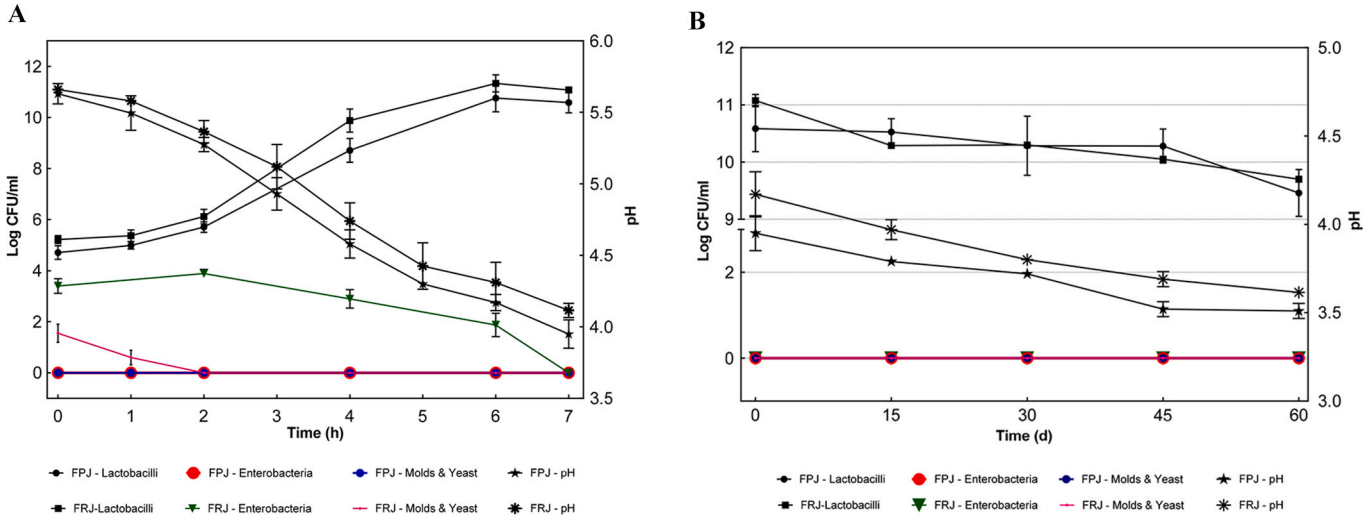

Fig. 2. Microbiological status of raw and pasteurized cactus pear juices fermented with *L. plantarum* S-811 (FRJ and FPJ, respectively) during 7 h of fermentation (A) and during the 60 days of refrigerated (4 °C) storage (B).

Table 5
Quality parameters (°Brix and color profile) evaluated in raw and pasteurized cactus pear juices (RJ and PJ, respectively) fermented with *L. plantarum* S-811 (FRJ-S-811 and FPJ-S-811) during refrigerated storage (4 °C) for 60 days.

Parameter		Storage (days)	RJ	PJ	FRJ-S-811	FPJ-S-811
°Brix		0	14.00 ± 0.20	13.40 ± 0.60	14.20 ± 0.70	13.20 ± 0.85
		15			13.90 ± 0.55	13.40 ± 0.55
		30			14.10 ± 0.25	13.40 ± 0.60
		45			13.70 ± 0.50	12.90 ± 1.00
Color	L*	0	63.58 ± 0.37^{Z}	64.13 ± 0.96^{Z}	60.40 ± 0.37^{Z,Y}	61.80 ± 0.76^{Z,Y}
		15			59.69 ± 1.73^{Y}	59.55 ± 0.45^{Y}
		30			61.14 ± 1.65^{Z,Y}	60.54 ± 0.02^{Z,Y}
		45			58.88 ± 1.07^{Y}	58.97 ± 0.14^{Y}
		60			54.25 ± 0.98^{X}	58.25 ± 1.04^{Y}
	a*	0	−7.15 ± 0.4^{v}	−6.47 ± 0.31^{u,v}	−6.38 ± 0.21^{u,v}	−6.54 ± 0.32^{u,v}
		15			−5.35 ± 0.52^{t,u}	−5.43 ± 0.19^{t,u}
		30			−4.86 ± 0.25^{t}	−4.24 ± 0.53^{s,t}
		45			−3.04 ± 0.27^{r,s}	−3.11 ± 0.12^{r,s}
		60			−2.67 ± 0.31^{r}	−3.21 ± 0.33^{r,s}
	b*	0	28.32 ± 1.39*	29.78 ± 1.08*	30.33 ± 1.03*	30.22 ± 0.93*
		15			30.96 ± 1.43*	30.34 ± 2.11*
		30			29.58 ± 0.86*	30.41 ± 1.87*
		45			30.09 ± 0.73*	29.87 ± 1.25*
		60			29.46 ± 0.23*	29.24 ± 0.91*
	C (Chroma)	0	29.21 ± 1.35•	30.48 ± 1.12•	30.99 ± 0.96•	30.92 ± 0.98•
		15			31.42 ± 1.32•	30.83 ± 2.11•
		30			29.98 ± 0.88•	30.70 ± 1.93•
		45			30.25 ± 0.75•	30.03 ± 1.25•
		60			29.58 ± 0.20•	29.41 ± 0.94•
	h° (Hue angle)	0	−1.32 ± 0.01^{a}	−1.36 ± 0.01^{a,b}	−1.36 ± 0.01^{a,b,c}	−1.36 ± 0.01^{a,b}
		15			−1.40 ± 0.02^{c,d}	−1.39 ± 0.01^{b,c,d}
		30			−1.41 ± 0.01^{d}	−1.43 ± 0.01^{d,e}
		45			−1.47 ± 0.01^{e,f}	−1.47 ± 0.01^{e,f}
		60			−1.48 ± 0.01^{f}	−1.46 ± 0.01^{e,f}
	ΔE	0			3.85 ± 0.14^{h}	2.37 ± 1.71^{h}
		15			5.07 ± 1.26^{h}	4.80 ± 0.34^{h}
		30			3.70 ± 1.64^{h}	4.32 ± 0.79^{h}
		45			6.52 ± 1.36^{g,h}	6.19 ± 0.82^{g,h}
		60			10.48 ± 1.50^{g}	6.78 ± 1.73^{g,h}

Different letters for data corresponding to each parameter evaluated represent significant differences among juices for this studied parameter ($P < 0.05$). Comparisons were made between raw and pasteurized cactus pear juices and the corresponding fermented juices and over storage time within the same sample.

Table 6
Quality parameters (Browning Index, Cloud Index, and Alcohol insoluble solids) evaluated in raw and pasteurized cactus pear juices (RJ and PJ, respectively) fermented with *L. plantarum* S-811 (FRJ-S-811 and FPJ-S-811) during refrigerated storage (4 °C) for 60 days.

Parameter	Storage (days)	RJ	PJ	FRJ-S-811	FPJ-S-811
Browning Index	0	0.350 ± 0.011^{B}	0.343 ± 0.003^{B}	0.379 ± 0.007^{A}	0.401 ± 0.004^{A}
	15			0.337 ± 0.006^{B}	0.270 ± 0.006^{C,D}
	30			0.292 ± 0.008^{C}	0.267 ± 0.012^{C,D}
	45			0.287 ± 0.008^{C}	0.256 ± 0.001^{D}
	60			0.286 ± 0.006^{C}	0.253 ± 0.002^{D}
Cloud Index	0	0.072 ± 0.001^{a}	0.060 ± 0.001^{b}	0.054 ± 0.001^{b,c}	0.053 ± 0.001^{b,c}
	15			0.055 ± 0.001^{b,c}	0.051 ± 0.003^{c}
	30			0.054 ± 0.001^{b,c}	0.056 ± 0.003^{b,c}
	45			0.056 ± 0.001^{b,c}	0.057 ± 0.003^{b,c}
	60			0.056 ± 0.001^{b,c}	0.057 ± 0.001^{b,c}
Alcohol insoluble solids (g/l)	0	10.05 ± 0.70^{j}	10.38 ± 0.04^{j}	6,32 ± 0.11^{n,ñ}	8.51 ± 0.16^{k}
	15			6.12 ± 0.04^{ñ,o}	7.93 ± 0.11^{l}
	30			6.45 ± 0.07^{m,n,ñ}	6.72 ± 0.11^{m}
	45			5.55 ± 0.07^{p}	5.86 ± .,08^{o,p}
	60			6.60 ± 0.14^{m,n}	4.78 ± 0.03^{q}

Different letters for data corresponding to each parameter evaluated represent significant differences among juices for this studied parameter ($P < 0.05$). Comparisons were made between raw and pasteurized cactus pear juices and the corresponding fermented juices and over storage time within the same sample.

cactus pear juice fermented with the reference strain *Lactobacillus fermentum* - ATCC 9338 [47].

Differences in specified attributes, such as color, odor, flavor, taste, and texture, between fermented and non-fermented cactus pear juices were evaluated by performing a paired-comparison test (Table 7). This test showed a clear sensory difference between both juices, which only displayed similarities regarding the perception of fruity aroma and color intensity. In the FPJ-*L. plantarum* S-811 analysis, as a product of lactic fermentation, the acidic aroma, flavor intensity, acidic taste, and thick texture presented a significantly higher frequency ($P < 0.05$). Acidic aroma and taste, a thicker texture, and less bright color were previously observed in coconut water fermented *with L. plantarum* [18]. Like a change in polysaccharides composition due to fermentation may affect the turbidity of cactus pear juices, it may also influence juice texture

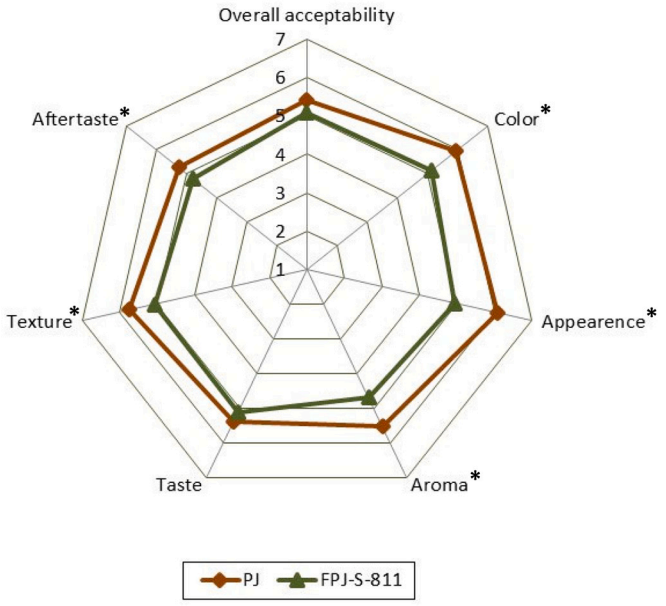

Fig. 3. Sensory analysis of pasteurized cactus pear juice fermented with *L. plantarum* S-811 (FPJ-S-811). Data are the mean (± SD) consumer's liking scores for pasteurized cactus pear juice (PJ) and pasteurized cactus pear juice fermented with *L. plantarum* S-811 (FPJ-S-811). *, Indicate significant differences among PJ and FPJ- S-811 ($P < 0.05$).

Table 7
Consumer responses ($n = 59$) for paired comparison tests of pasteurized juice fermented with *L. plantarum* S-811 (FPJ-S-811) compared to the pasteurized juice (PJ).

Attribute	Descriptors	PJ	FPJ-S-811
Color	Which is more intense?	34^a	25^a
	Which is brighter?	56^a	3^b
Odor	Which is sweeter?	41^a	18^b
	Which is more fruitful?	32^a	27^a
	Which is more acidic?	15^a	44^b
Flavor	Which is more intense?	5^a	54^b
	Which is fresher?	39^a	20^b
Taste	Which is more acidic?	6^a	53^b
	Which is sweeter?	48^a	11^b
Texture	Which is softer?	42^a	7^b
	Which is thicker?	7^a	52^b

Different letters in the same row represent significant differences among PJ and FPJ-S-811 for the studied descriptor ($P < 0.05$).

increasing viscosity and causing the perception of a thicker juice texture [60]. But, in the PJ, the perception of a bright color, sweet aroma, fresh taste, sweet taste, and smooth texture had significantly higher frequencies ($P < 0.05$).

3.4.4. Toxicity assessment of fermented cactus pear juice

The acute toxicity of FPJ-*L. plantarum* S-811 and PJ against *Artemia salina* was studied. As was expected, both juices were non-toxic in the concentration range evaluated. The mutagenicity of the samples was assayed with the Ames test. Similarly, to previously demonstrated in fermented vegetable beverages using lactobacilli, none of the concentrations of the juices evaluated (equivalent to 20 to 161 mg GAE/ml of phenolic compounds) had a mutagenic effect on *S.* Typhimurium (TA98 and TA100 strains) (Table S3) [61].

4. Conclusion

Lactic acid fermentation of cactus pear juice can be a key to the sustainable preservation of this native fruit with restraints to long-term storage. The biotechnological characterization of autochthonous *L. plantarum* and *F. fructosus* strains demonstrated their efficiency to ferment the cactus pear juice contributing to its preservation and the conservation of its functional attributes. These strains showed a safety profile typical of LAB and technological versatility for the production of different cactus pear by-products. *F. fructosus* S-22 stands out for its production of acetic acid and could be used in the elaboration of pickled cactus pears. *L. plantarum* S-TF2 was able to produce ethanol and could be used to elaborate an alcoholic beverage based on cactus pears. *L. plantarum* S-811 is the only of these strains capable of faster ferment and acidify the cactus pear mainly through lactic acid production, without acetic acid and ethanol production, making this strain an ideal candidate for the production of fermented cactus pear juice. *L. plantarum* S-811 strain application for cactus pear juice fermentation resulted in a product with proper biofunctional properties, with physicochemical and microbiological stability that improves its shelf-life. Also, the fermentation of cactus pear juice confers sensory attributes that allow this beverage to differentiate and that contribute to consumer acceptance. More studies are necessary, especially to discern the aroma compounds responsible for the sensorial features of the fermented beverage and to deepen the knowledge concerning its functional properties.

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Ethical Statement - Studies in humans and animals

The sensory evaluation study of the juices was conducted in INBIOFIV, Tucuman, Argentina, in December 2018. Sensory evaluation was approved by the INBIOFIVs´ Human Ethics Committee (Approval No: 2018-03; date of approval 14-11-2018)".

CRediT authorship contribution statement

Hernán E. Verón: Methodology, Investigation, Formal analysis, Visualization. **Luciana Contreras:** Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **María Inés Isla:** Resources, Writing – review & editing, Funding acquisition. **Sebastian Torres:** Conceptualization, Resources, Methodology, Visualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nfs.2023.04.003.

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