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Functional fermented cherimoya (*Annona cherimola* Mill.) juice using autochthonous lactic acid bacteria

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ABSTRACT

The biochemical and functional properties of fermented *Annona cherimola* Mill. (cherimoya) juice using five lactic acid bacteria (LAB) isolated from autochthonous fruits from Northwestern Argentina were studied in this work. Fermentation was carried out at 30 °C for 48 h followed by a 21 day-storage period at 4 °C. The assayed LAB grew well during fermentation (final count of 10^8 CFU/mL, ΔpH ca. 1 U) and survived after the storage period. All strains consumed fructose and glucose present in cherimoya juice as energy sources, with the consequent synthesis of lactic and/or acetic acids as final metabolic products. However, only two of the five evaluated strains were capable to produce fermented cherimoya juices with a perceptible color change. Due to lactic acid fermentation, a moderate reduction in the total phenolic content (between 13% and 43%) was observed in the majority of the samples, although no change in the antioxidant capacity was detected. The fermented cherimoya juices showed a weak antiplatelet activity when adenosine diphosphate agonist was used. The findings of this study evidenced the potential use of *Annona cherimola* Mill. fermented juice as a novel matrix for the formulation of stable functional beverages with appealing nutritional and functional properties.

1. Introduction

Cherimoya (*Annona cherimola* Mill.) is an exotic sub-tropical fruit belonging to the Annonaceae family, native from the Andes mountains. In addition to its delicious taste and sweet aroma, this fruit also contributes to human nutrition with vitamins, carbohydrates, minerals, fibers, and other bioactive compounds (Barreca et al., 2011). Moreover, *Annona* fruits contain a considerable amount of polyphenolic compounds (Santos et al., 2016), which help preventing diseases associated with oxidative stress such as cancer, atherosclerosis, and neurodegenerative affections (La Vecchia, Altieri, & Tavani, 2001; Mannino et al., 2020; Zibadi et al., 2007). Additionally, cherimoya has been used over years in traditional medicine as antimicrobial, insecticide, and for treatment of intestinal disorders or skin diseases (Amoo, Emenike, & Akpambang, 2008). Recently, it has been determined that cherimoya leaves extract exhibit anti-proliferative activity against different cancer cell lines (Ammoury et al., 2019; Mannino et al., 2020).

Consumption of functional foods has gained a growing interest since it provides health benefits beyond the basic nutritional features of fresh foods. Nowadays, fruit-based fermented juices are by far one of the most active functional food category (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014), especially due to the current tendency to veganism and vegetarianism among consumers and also to lactose intolerance caused by dairy products.

Lactic acid bacteria have been traditionally and extensively used as natural food biopreservatives due to their ability to produce a rapid

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acidification through the synthesis of organic acids preventing future harmful microorganism contamination and thus, extending the shelf life of food products.

The use of carefully selected LAB strains as starter cultures for fruit and vegetable fermentation not only preserve food quality but also can increase *in situ* expression of desired sensorial attributes and enhanced their nutritional properties to promote human health care (Demir, Bahçeci, & Acar, 2006; Di Cagno, Coda, De Angelis, & Gobbetti, 2013; Filannino et al., 2020; Leroy, Verluyten, Messens, & De Vuyst, 2002).

Nowadays, LAB with specific characteristics have been isolated from a variety of traditional fermented products and from diverse natural sources such as fruits and flowers to be further used as starter cultures to obtain controlled and uniform fermented foods free of artificial preservatives (Ruiz Rodríguez et al., 2019). Autochthonous bacteria represent an optimal alternative over allochthonous strains due to a better niche-specific adaptation. In addition, LAB exhibit specific capabilities that allow them to resist the biochemical characteristics of a particular fruit matrix, such as low pH, high content of phenolic compounds and other intrinsic properties.

To the best of our knowledge, cherimoya juice has not been previously used as substrate for LAB fermentation. The aim of this work was to evaluate the ability of fruit-origin LAB to grow during cherimoya juice fermentation and to extend its shelf life as well as to improve the final biochemical and functional properties.

2. Materials and methods

2.1. Strains and growth conditions

Five LAB strains, belonging to the species *Lactobacillus brevis* (2 strains), *L. plantarum* (1), *L. rhamnosus* (1), and *Fructobacillus tropaeoli* (1), were obtained from the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA, Tucumán, Argentina). Strains were previously isolated from fruits growing in the Northwest region of Argentina (fig, guava, and cherimoya) and were genetically and technologically characterized (Ruiz Rodríguez et al., 2019) (Table 1). *L. brevis* CRL 2050 and *F. tropaeoli* CRL 2039 were the only strains isolated from cherimoya (considered as autochthonous) while the remaining LAB were selected based on their high acidifying capacity and their ability to produce aroma and organic acid compounds in culture media (Ruiz Rodríguez et al., 2019). The strains were stored at −20 °C in Yeast Extract Milk medium containing: skim milk (100 g/L), yeast extract (5 g/L), glucose (10 g/L), and 10% (v/v) glycerol. Cultures of *L. brevis, L. plantarum,* and *L. rhamnosus* were activated in MRS broth (Biokar Diagnostics, Beauvais, France) for 24 h at 30 °C, whereas *F. tropaeoli* was activated in MRS broth supplemented with 20 g/L of fructose (fMRS) at the same conditions. Then, all active cultures were grown for 16 h at 30 °C in fMRS broth, and further harvested by centrifugation (DCS-16 RV Centrifuge, Presvac, Buenos Aires, Argentina) at 8,000 × *g* for 15 min, washed twice in physiological saline solution (NaCl 0.90%, w/v), and re-suspended in the same solution. Each strain was individually added (2%, v/v, inoculum) to 30% (w/v) cherimoya juice (initial cell number corresponding to ca. 10^7 CFU/mL).

Table 1
LAB strains used for cherimoya juice fermentation and their source.

Strain	Isolation source
Lactobacillus brevis CRL 2050	Cherimoya: *Annona cherimola* Mill.
L. brevis CRL 2051	Guava: *Psidium guajava*
L. plantarum CRL 2030	Guava: *Psidium guajava*
L. rhamnosus CRL 2049	Fig: *Ficus carica*
Fructobacillus tropaeoli CRL 2039	Cherimoya: *Annona cherimola* Mill.

2.2. Cherimoya juice formulation

Ripe cherimoya fruits (13–14 °Brix) were obtained from a local market, washed twice with tap water, and carefully peeled removing the husk and seeds to obtain the pulp. Then, 30 g of the pulp were mashed with an electric hand blender (Moulinex, Buenos Aires, Argentina) and tap water was added until a final volume of 100 mL to obtain a 30% (w/v) juice concentration. Finally, cherimoya juice (ChJ) was pasteurized at 65 °C for 30 min in water bath, and immediately cooled on ice. In order to prevent natural cherimoya browning caused by the polyphenol oxidase enzyme, pasteurized ChJ was supplemented with ascorbic acid 0.05% (v/v), sterilized by using a 0.22 μm filter membrane (Millipore, São Paulo, Brazil). A 10% (w/v) ascorbic acid stock solution was used and different concentrations ranging from 0.01 to 1%, v/v, were previously tested to get the minimal amount necessary to avoid cherimoya browning during processing without affecting bacterial growth.

2.3. Cherimoya juice fermentation

Each strain culture was separately inoculated at 2% (v/v) in 50 mL of ChJ; fermentation was carried out statically at 30 °C for 48 h. Pasteurized ChJ without inoculum was incubated under the same conditions and used as control (non-fermented cherimoya juice, NFChJ). After fermentation, juices were maintained at low temperature (4 °C) for 21 days.

2.4. Cell count and pH determination

Bacterial growth and acidification capacity were evaluated at 0, 4, 8, 16, 24 and 48 h during fermentation. For LAB count, 100 μL of fermented cherimoya juices (FChJ) were resuspended in 0.10% (w/v) sterile peptone-water solution and serial dilutions were plated in MRS agar for all *Lactobacillus* strains while in fMRS agar for *F. tropaeoli*. Plates were incubated for 48–72 h at 30 °C. Microbiological quality of pasteurized ChJ (both NFChJ and FChJ) was evaluated in Mac Conkey agar (Britania, Buenos Aires, Argentina) (37 °C and 45 °C, 48 h) and Yeast and Mould agar (Britania) (20–25 °C, 5–7 days) to quantify coliforms, and yeasts and moulds, respectively. Results were expressed as log colony-forming units per milliliter (log CFU/mL). The pH was measured by using a digital pH-meter (Sartorius, Goettingen, Germany).

When the 48 h-fermentation process was finished, juices were immediately placed at 4 °C for 21 days. Samples were taken the last day of cold storage to determine bacterial count and pH as previously described, and Brix degrees, sugar and organic acid concentration were quantified by using the methods in the following sections.

2.5. Sugar and organic acid analysis

Samples of NFChJ and FChJ at 0, 24 and 48 h of fermentation were withdrawn to determine carbohydrate and organic acid concentrations by high-performance liquid chromatography (HPLC) according to Ortiz, Raya, and Mozzi (2015). Firstly, ChJ samples were deproteinized using a method modified by Vrancken, Rimaux, De Vuyst, and Leroy (2008) as follows: 1 mL of each sample was centrifuged (260D Brushless Microcentrifuge, Denville Scientific Inc., Metuchen, NJ, USA) at 12,000 × *g* for 10 min, subsequently, 50 μL of Carrez A reagent [$K_4(Fe(CN)_6).3H_2O$ 3,60% w/v] (Sigma-Aldrich Chemical Co., St. Louis, USA), 50 μL of Carrez B reagent [$ZnSO_4{\cdot}7H_2O$ 7,20% w/v] (Sigma-Aldrich Chemical Co.), 100 μL of NaOH 0.10 M, and 200 μL of ultra-pure Milli-Q water were added to 600 μL of cell-free culture supernatant (1 mL final volume). Then, samples were mixed 1 min at room temperature (20 ± 2 °C) by using a Vortex shaker, centrifuged (14,000 × *g*, 5 min), and the supernatants were collected. Afterwards, 50 mg of Amberlite IR-120 (H^+) exchange resin (BDH Laboratory Reagents, Philadelphia, USA) and 50 mg of Amberlite IR-45 (OH^-) exchange resin (BDH Laboratory Reagents) were added to the respective supernatants and mixed again with a

Vortex shaker for 1 min at room temperature. Finally, samples were centrifuged (14,000 × *g*, 5 min) and the resulting supernatants were kept at −20 °C until use.

HPLC system was equipped with Smartline Pump 100, refractive index detector K-2301 and Smartline Autosampler 3800 (Knauer GmbH & Co., Berlin, Germany). Carbohydrates and organic acids separation was carried in a Rezex ROA-Organic Acid H + column (300 × 7.8 mm) (Phenomenex Laboratories Inc., Torrance, CA, USA) at 45 °C using 5 mM H_2SO_4 as mobile phase with an isocratic elution mode (flow rate = 0.60 mL/min). The injection volume was 20 μL. Data acquisition and processing was performed by using Eurochrom Basic Edition v3.05 Software. External standards were analyzed as references (Sigma-Aldrich Chemical Co.).

In addition, Brix degrees of fermented juices were measured with a hand Brix refractometer (Alla France, Chemillé, France), and expressed as °Brix.

2.6. Quantification of total phenolic content

Total phenolic compounds were determined in 0 and 48 h fermented samples by the method described by Singleton, Orthofer, and Lamuela-Raventós (1999), with some modifications. Briefly, 10 μL of FChJ or NFChJ were diluted to a final volume of 1 mL with distilled water, and 100 μL of Folin-Ciocalteau reagent (PanReac AppliChem GmbH., Darmstadt, Germany) (diluted 1:2 in distilled water) were added. The obtained reaction mixtures were kept 2 min in darkness at room temperature. Then, 400 μL of Na_2CO_3 15.90% (w/v) were added and samples were incubated at room temperature in darkness for 20 min. A 96-well microplate (Corning Costar®, Corning Inc., NY, USA) was filled with 200 μL of each sample and the absorbance was read at 765 nm in a microplate reader spectrophotometer (Tecan Austria GmbH., Grödig, Austria). The obtained values were correlated with a predetermined gallic acid standard calibration curve ($R^2 = 0.98$) and expressed as mg of gallic acid equivalent (GAE) per 100 mL of ChJ (mg GAE/100 mL).

2.7. Antioxidant activity

The total antioxidant capacity of FChJ and NFChJ at 0 and 48 h of fermentation was determined by applying an improved DPPH radical scavenging activity, the ABTS radical cation decolorization method, and the inhibition ORAC assay.

2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging capacity was evaluated according to Mekni, Azez, Tekaya, Mechri, and Hammami (2013). Firstly, 10 μL of each sample was dissolved to a final volume of 100 μL in HPLC grade absolute methanol (Sintorgan S.A., Buenos Aires, Argentina). Then, 500 μL of DPPH 0.10 mM (in absolute methanol) (Sigma-Aldrich Chemical Co.) and 400 μL Tris HCl 0.10 M (pH 7.60) were added. The reaction mixtures were kept at room temperature in darkness for 30 min. Afterwards, microplate wells were filled with 200 μL of each mixture and the absorbance at 517 nm was measured by using a microplate reader spectrophotometer (Tecan Austria GmbH.). The DPPH radical scavenging activity (RSA) was calculated using the following equation:

$$RSA(\%) = \left(\frac{A_b - A_s}{A_b}\right) \times 100$$

where A_b is the absorbance of the blank reaction (containing all reagents except for the ChJ) and A_s is the absorbance of the sample (obtained by subtracting the optical density of the sample with DPPH from the sample without DPPH).

2.7.2. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC method was performed according to Re et al. (1999), based on the capacity of a sample to inhibit the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) compared with a reference antioxidant standard (Trolox®). Briefly, $ABTS^{\bullet+}$ was generated by reacting ABTS stock solution (7 mM) with $K_2S_2O_8$ 45 mM (final concentration) and allowed the mixture to stand in the dark at room temperature for 16 h before use (time required for radical formation). Then, a solution named TR was prepared by mixing 1 mL of the $ABTS^{\bullet+}$ solution with 12 mL of phosphate buffer saline (PBS) 5 mM. Finally, 10 μL of each sample were mixed with 100 μL of TR solution and the absorbance was measured by spectrophotometry (Spectrometer UV/Vis/NIR LAMBDA, Perkin Elmer, MA, USA) at 734 nm at different time intervals (6, 12 and 18 min). The obtained values were correlated with a predetermined Trolox® standard calibration curve ($R^2 = 0.99$) and expressed as μmol of Trolox® equivalents (TE) per 100 mL of sample (μmol TE/100 mL). All chemicals employed in this assay were purchased from Sigma-Aldrich Chemical Co.

2.7.3. Oxygen radical absorbance capacity (ORAC) assay

The methodology was carried out according to Chirinos et al. (2008) by employing a microplate reader with fluorescence detector (Victor Nivo, Perkin Elmer, MA, USA). The 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) was used as a peroxyl radical generator, Trolox® was used as standard, and fluorescein as fluorescent probe. Briefly, 25 μL of blank, Trolox® standard or the evaluated sample, were mixed with 250 μL of fluorescein (55 nM) and incubated at 37 °C for 10 min before automatic injection of 25 μL of AAPH solution (153 nM). Fluorescence was measured at 485 nm ($\lambda_{excitation}$) and at 520 nm ($\lambda_{emission}$) every min for 50 min. ORAC values were obtained from a Trolox® standard calibration curve by correlating the area under the decay curves, and final results were expressed as μmol TE/100 mL. All chemicals employed in this assay were purchased from Sigma-Aldrich Chemical Co.

2.8. Inhibition of platelet aggregation

2.8.1. Extracts preparation

Inhibition of platelet aggregation was studied in NFChJ (0 and 48 h incubation) and FChJ (after 48 h) aqueous extracts reconstituted from lyophilized powders, but also their methanolic and ethanolic extracts were used. All lyophilized samples (aqueous, ethanolic and methanolic extracts) were dissolved in PBS and tested at 1 mg/mL in this assay. Extracts of ChJ were obtained by solid–liquid extraction performed for 10 min in an ultrasonic bath (As 3120 ultrasonic bath, Auto Science, China) at 37 °C in H_2O and solvent/H_2O mixture (7:3, v/v). Then, supernatants were removed after centrifugation (1,200 × *g*, 5 min, 37 °C) (Digital Centrifuge, Eppendorf 5804, NY, USA), and the obtained extracts were dried (≤60 °C) and stored at −80 °C until use.

2.8.2. Human platelet suspensions

Platelet suspensions were prepared as previously reported by Sepúlveda et al. (2019) from six healthy volunteers who previously signed an informed consent. Blood samples were collected in citrate tubes (3.2%; 9:1, v/v) (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) and centrifuged (DCS-16 RV Centrifuge, Presvac) at 240 × *g* for 10 min to obtain platelet rich plasma (PRP), and then at 1,200 × *g* for 10 min to obtain platelet poor plasma (PPP). PPP was used to adjust the platelet concentration in the PRP, and also as turbidity control in the turbidimetric method. Afterwards, platelet count was performed on a blood count analyzer (Bayer Advia 60 Hematology System, Tarrytown, NY, USA) and the PRP was adjusted to a concentration between 200 × 10^9 platelets/L.

2.8.3. Platelet aggregation assay

This study was performed according to the methodology of Born and Cross (1963) with some modifications. Platelet aggregation (PA) was measured by employing a lumi-aggregometer (Chrono-Log, Haverton, PA, USA). Briefly, 480 μL of PRP (200 × 10^9 platelets/L) was

preincubated with 20 μL of PBS (negative control) or the tested extract at 37 °C for 3 min. Aggregation of platelets was induced by the addition of the adenosine diphosphate (ADP) agonist (4 μM) for 6 min. Measurements were made in six volunteers and the results were expressed as a percentage of platelet aggregation in figures, and discussed as inhibition of platelet aggregation (IPA), calculated by using the following equation:

$$IPA(\%) = 100 - \frac{\mathrm{PA}_{\mathrm{sample}}\ (\%) \times 100}{\mathrm{PA}_{\mathrm{PBS}}(\%)}$$

2.9. Colorimetric determination after refrigerated storage

The color of the juices was measured at the beginning and after 21 days of refrigerated storage by using a colorimeter (Konica Minolta CM-5, Osaka, Japan). The L^*a^*b color space analysis was employed, where L^* represents lightness (white-black), and a^* and b^* the chromaticity coordinates (red-green and yellow-blue, respectively). The resulting values were reported as total color difference (ΔE^*) calculated with the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

ΔL^*, Δa^* and Δb^* represent the differences between initial and final values of L^*, a^* and b^*, respectively, of each evaluated sample.

2.10. Statistical analysis

All assays were performed by triplicate and results were expressed as mean ± standard deviation (SD). Data were submitted to ANOVA general linear model, and pair-comparison of means was performed by Tukey's *post hoc* test. Student *t*-test was performed to compare paired samples. Statistical analysis was carried out by using Minitab® 17.1.0 software (Minitab, State College, PA, USA) and GraphPad Prism® 8.0 software (GraphPad Inc., San Diego CA, USA). The $P < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Cell growth and pH during fermentation

Vegetables and fruits could serve as good media for the growth of LAB, due to their wide variety of nutrients which could maintain a noticeable cell viability over time (Filannino, Bai, Di Cagno, Gobbetti, & Gänzle, 2015; Kimoto-Nira, Moriya, Nogata, Sekiyama, & Toguchi, 2019; Mustafa et al., 2019). As it is known, *Lactobacillus* species are widely employed to ferment plant and fruit derived matrices, presenting a great metabolic versatility during fermentation. Bacterial growth kinetics data is often useful to define the parameters of the process such as fermentation time, adequacy of substrate, and lactic acid production (Di Cagno et al., 2009).

In ChJ, all strains were able to grow at 30 °C, starting from an initial inoculum of ca. 10^7 CFU/mL and reaching a final cell count of 10^8 CFU/mL at the end of fermentation time (Fig. 1a). In general, fruit juices are stressful substrates for microorganisms, due to their low pH, high sugar content, presence of phenolic compounds, and malic and/or citric acids (Ricci et al., 2019a). Despite the fact that all the evaluated LABs grew in ChJ, a different microbial adaptation to this matrix was noticed among them. *F. tropaeoli* CRL 2039, *L. plantarum* CRL 2030, and *L. brevis* CRL 2051 were the best adapted strains, which increased their cell density about 1.20 Log CFU/mL and was significantly higher ($P < 0.05$) than the increase observed for *L. rhamnosus* CRL 2049 and *L. brevis* CRL 2050 (0.24 and 0.26 Log CFU/mL, respectively) after 48 h. For these two strains, the ChJ matrix represented a more unfavorable environment by limiting their growth capacity, especially for *L. rhamnosus* CRL 2049, which showed a prolonged lag growth phase at the beginning of fermentation (0–4 h) followed by a cell count decrease. Afterwards, this strains improved its matrix adaptability and showed a slight cell growth increase. Accordingly with these observations, *L. rhamnosus* CRL 2049, *F. tropaeoli* CRL 2039 and *L. brevis* CRL 2050 reached their maximum cell density at 24 h of fermentation whereas the highest cell count for *L. plantarum* CRL 2030 and *L. brevis* CRL 2051 was detected after 48 h.

L. plantarum strains are frequently used as starter cultures to ferment vegetables and fruits (Di Cagno et al., 2008; Filannino et al., 2020; Rodríguez et al., 2009) due to their high tolerance to acidic environments and marked growth capacity. This was previously reported by Reddy, Min, and Wee (2015) when mango juice was inoculated with a probiotic *L. plantarum* strain, obtaining a high viability value ca. 10^7 CFU/mL after incubation at 30 °C for 72 h. Similarly, Russo et al. (2015) observed a cell count up to 10^8 CFU/g in fresh-cut melon pieces fermented with the strain *L. plantarum* B2; also, a high viability was reached for *L. plantarum* ATCC14917 (near 10^8 CFU/mL) in apple juice (Li et al., 2019). These findings are coincident with the marked growth observed for *L. plantarum* CRL 2030 in ChJ in this work.

Five *L. rhamnosus* strains isolated from Italian cheeses were individually inoculated for elderberry juice fermentation with the consequent increase of 2 Log units in cell density after incubation at 37 °C 48 h (Ricci et al., 2019b). Moreover, Nazzaro, Fratianni, Sada, and Orlando (2008) formulated a healthy carrot juice using *L. rhamnosus* DSM 20 711

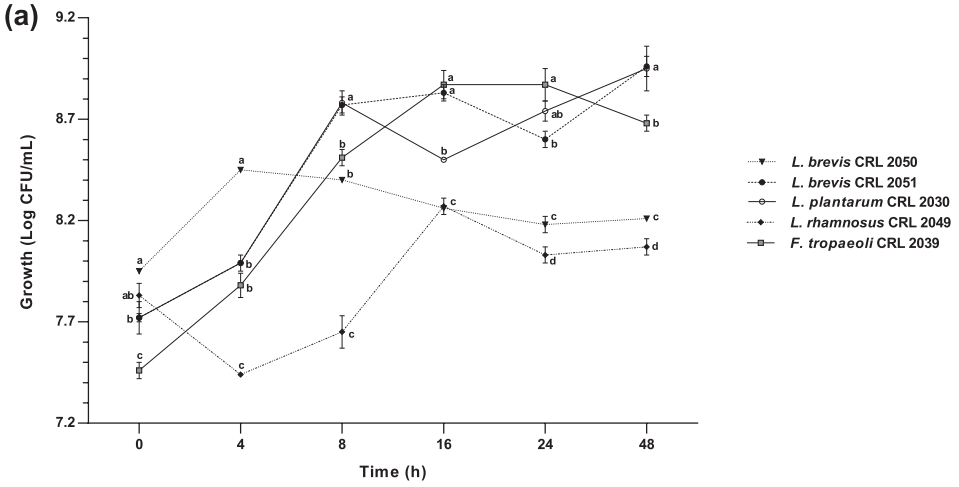

Fig. 1a. Growth kinetics of LAB strains during ChJ fermentation at 30 °C for 48 h. Different letters (a-d) indicate significant differences ($P < 0.05$) among samples at the same time.

strain as starter culture and reported a cell growth of about 3 Log units at the end of the process. Comparing with these studies, *L. rhamnosus* CRL 2049 showed lower growth rate (<1 Log unit) during ChJ fermentation; however, a high final cell count (ca. 10^8 CFU/mL) was observed. The bacterial growth kinetics could present differences among diverse fruit juices, which are attributed to several factors such as phytochemical composition of the matrix, strains source and adaptation as well as fermentation conditions.

Two fruit-origin *L. brevis* strains were used to ferment *Portulaca oleracea* L. (Di Cagno et al., 2019) and white cabbage juices (Jaiswal & Abu-Ghannam, 2013). A cell count increase of more than 1 Log unit was observed for both strains, in accordance with our findings with *L. brevis* CRL 2051 growth. The use of autochthonous LAB strains as starter cultures ensures better growth kinetics and adaptation than allochthonous bacteria as it was previously demonstrated in fruit and vegetable fermentations (Di Cagno et al., 2009; Filannino et al., 2020). Despite *L. brevis* CRL 2050 was isolated from cherimoya flower, it demonstrated a weak growth capacity in pulp juice (0.26 Log CFU/mL) as compared to the other ones.

Fructophilic lactic acid bacteria are found in fructose-rich niches as flowers and fruits and they have great potential for food and feed applications (Endo, 2012). In this work, *F. tropaeoli* CRL 2039 increased its viability up to 1 Log unit, similarly to the results reported by Martínez, Barrientos, Mozzi, and Pescuma (2019) for *F. tropaeoli* CRL 2034, which was inoculated in a milk-based mango-orange juice. At present, no other studies have been reported on the use of *Fructobacillus* strains as starter cultures for fruit juice fermentation.

Neither coliforms or yeasts and moulds were detected in pasteurized ChJ (NFChJ and FChJ), proving that samples presented a suitable microbiological quality before strain inoculation (data not shown). Indeed, juice pasteurization and lactic acid fermentation appropriately avoided matrix spoilage by undesirable microorganisms (Di Cagno et al., 2013; Mantzourani et al., 2018).

Cherimoya juices had an initial pH value between 4.35 and 4.53, and after 48 h of fermentation all strains were capable to decrease about 1 pH unit (Fig. 1b). The acidifying capacity was not significantly different among strains after 48 h of fermentation. The highest acidifying capacity was shown by *F. tropaeoli* CRL 2039 ($\Delta pH = 1.23$) while the lowest by *L. brevis* CRL 2050 ($\Delta pH = 0.93$). As expected, the three strains which grew better showed the lowest pH values during fermentation.

3.2. Sugar consumption and organic acid production

Cherimoya fruit is rich in fermentable carbohydrates, especially glucose and fructose. In NFChJ 30% (w/v), the initial amount of glucose and fructose was 13.31 and 13.83 g/L, respectively, values that remained constant during fermentation time (Table 2). The studied strains used glucose and fructose differently as energy sources; *F. tropaeoli* CRL 2039 and *L. brevis* CRL 2050 preferably consumed glucose (4.60 and 1.81 g/L, respectively) and only traces of fructose (0.09 and 0.14 g/L, respectively) during 48 h of fermentation. Conversely, *L. brevis* CRL 2051 consumed fructose (1.17 g/L) in higher amounts than glucose (0.40 g/L). Finally, the strains *L. plantarum* CRL 2030 and *L. rhamnosus* CRL 2049 used glucose and fructose similarly as carbon sources (0.81 and 1.08 g/L, and 1.36 and 1.04 g/L, respectively).

Concerning organic acid production, only *L. brevis* CRL 2050 and *F. tropaeoli* CRL 2039 produced both lactic (0.87 and 1.36 g/L, respectively) and acetic acids (0.70 and 1.26 g/L, respectively) after 48 h of fermentation (Table 2). By contrast, the remaining strains *L. brevis* CRL 2051, *L. plantarum* CRL 2030 and *L. rhamnosus* CRL 2049 produced only lactic acid as main metabolic end product, with concentrations of 2.14, 2.08 and 1.44 g/L, respectively.

Some authors have already reported that preference of carbohydrate intake by LAB is a strain-specific property, and that the posterior synthesis of lactic and/or acetic acid depends on the carbon substrate and fermentation conditions (Tomita, Saito, Nakamura, Sekiyama, & Kikuchi, 2017; Ricci et al., 2019a; Nguyen et al., 2019). White cabbage juice fermentation conducted by single culture of *L. rhamnosus*, *L. plantarum* and *L. brevis* led to a simultaneous consumption of glucose and fructose and also to a synthesis of both lactic and acetic acids (Jaiswal & Abu-Ghannam, 2013). Similar findings were reported by Mantzourani et al. (2018) when a probiotic *L. plantarum* ATCC 14917 strain was used to ferment a cornelian cherry juice. Oppositely, Filannino et al. (2015) observed that several *L. plantarum* strains produced lactic acid as the only metabolic end-product in a fermented cherry juice, like our fermentations with *L. plantarum* CRL 2030 in FChJ.

Fructobacillus genus are regarded as "obligate'' fructophilic LAB (FLAB) which preferentially use fructose as source of energy over other carbohydrates. Some FLAB can metabolize D-glucose, but external electron acceptors as pyruvate, oxygen, phenolic acids, citrate, malate, as well as D-fructose, are necessary to metabolize it and enhance bacterial growth (Endo & Dicks, 2014; Endo, Futagawa-Endo, & Dicks, 2009; Filannino, Di Cagno, Addante, Pontonio, & Gobbetti, 2016). In this work, *F. tropaeoli* CRL 2039 consumed mainly glucose over fructose as energy source. Similarly, Endo et al. (2009) reported that *F. fructosus* and *F. pseudoficulneus* showed better growth on D-glucose with pyruvate or oxygen rather than on D-fructose, due to an effective production of ATP (2 mol ATP from 1 mol D-glucose in the presence of oxygen, and 1.5 mol ATP from 1 mol D-glucose in the presence of pyruvate). In ChJ matrix, citrate or phenolic acids could act as the external electron acceptors which allow the preferential intake of glucose with the consequent bacterial growth.

Brix degree measurements resulted in an initial value of 4 °Brix for the NFChJ, remaining constant during the fermentation (Table 2). A

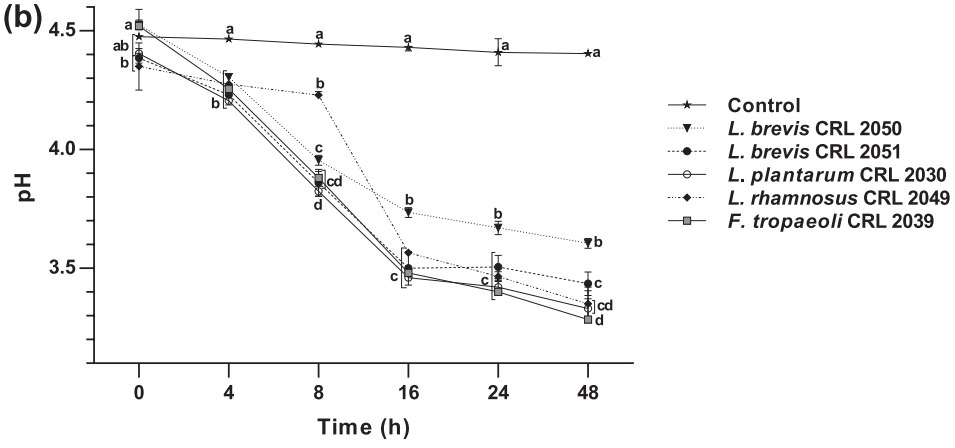

Fig. 1b. pH values throughout lactic acid fermentation of ChJ. Different letters (a-d) indicate significant differences ($P < 0.05$) among samples at the same time.

Table 2
Brix degrees, carbohydrate and organic acid concentration in NFChJ and FChJ with LAB during the fermentation process (30 °C for 48 h).

	NFChJ		FChJ									
	Control		***L. brevis* CRL 2050**		***L. brevis* CRL 2051**		***L. plantarum* CRL 2030**		***L. rhamnosus* CRL 2049**		***F. tropaeoli* CRL 2039**	
	t_{0h}	t_{48h}	t_{24h}	t_{48h}	t_{24h}	t_{48h}	t_{24h}	t_{48h}	t_{24h}	t_{48h}	t_{24h}	t_{48h}
Glucose (g/L)	13.31 ± 2.94^{Aa}	13.28 ± 2.89^{Aa}	12.56 ± 1.98^{Aa}	11.50 ± 2.22^{Ab}	12.86 ± 0.05^{Aa}	12.91 ± 0.30^{Aa}	12.57 ± 0.62^{Aa}	12.50 ± 0.52^{Aa}	12.56 ± 1.51^{Aa}	11.95 ± 0.97^{Aa}	10.45 ± 2.73^{Aa}	8.71 ± 2.00^{Ab}
Fructose (g/L)	13.83 ± 3.07^{Aa}	13.78 ± 3.02^{Ab}	13.90 ± 1.89^{Aa}	13.69 ± 2.33^{Aa}	13.12 ± 0.57^{Aa}	12.66 ± 0.52^{Ab}	12.87 ± 1.00^{Aa}	12.75 ± 0.32^{Aa}	13.30 ± 2.38^{Aa}	12.79 ± 2.35^{Ab}	13.90 ± 2.83^{Aa}	13.74 ± 2.66^{Aa}
Lactic acid (g/L)	ND[1]	ND	0.58 ± 0.00^{Ab}	0.87 ± 0.11^{Aa}	1.27 ± 0.16^{Ab}	2.14 ± 0.41^{Aa}	1.32 ± 0.38^{Ab}	2.08 ± 0.74^{Aa}	0.92 ± 0.32^{Ab}	1.44 ± 0.59^{Aa}	0.99 ± 0.28^{Aa}	1.36 ± 0.53^{Aa}
Acetic acid (g/L)	ND	ND	0.45 ± 0.02^{Bb}	0.70 ± 0.01^{Aa}	ND	ND	ND	ND	ND	ND	0.87 ± 0.11^{Ab}	1.26 ± 0.25^{Aa}
Brix degrees (°Brix)	4.00 ± 0.01^{Aa}	4.00 ± 0.00^{Aa}	3.00 ± 0.00^{Ba}	3.00 ± 0.00^{Ba}	3.00 ± 0.03^{Ba}	3.00 ± 0.00^{Ba}	3.20 ± 0.01^{Ba}	3.20 ± 0.00^{Ba}	3.20 ± 0.00^{Ba}	3.20 ± 0.04^{Ba}	3.20 ± 0.01^{Ba}	3.20 ± 0.02^{Ba}

[1] ND = not detected. Different capital letters (A-B) indicate significant differences ($P < 0.05$) among samples at the same time. Different lower cases letters (a-b) indicate significant differences between time of the same sample.

slight decrease to 3.20 or 3.00 °Brix was determined in all FChJ after 24–48 h at 30 °C due to carbohydrate consumption by LAB and consequent lower sugar concentration.

3.3. Total phenolic content quantification

Consumption of phenolic compounds through diet has many health benefits for humans, especially their chemopreventive effect against carcinogenesis and mutagenesis, mainly due to their strong antioxidant activities (Rodríguez et al., 2009). Additionally, these compounds also influence multiple sensorial characteristics of food such as flavor, astringency, color, and aroma (Naczk & Shahidi, 2003; Rodríguez et al., 2009).

In fruits and vegetables, phenolic acid concentration can vary widely due to genetic, stage of maturity, cultivation practices, and processing factors such as fermentation and storage conditions (Curiel et al., 2015; Da Silva et al., 2014; Deng, West, & Jensen, 2010; Rivera-Tovar, Mariotti-Celis, & Pérez-Correa, 2019). High levels of polyphenols can affect microbial metabolism and it has been observed that several LAB are capable of bioconverting these molecules into less toxic forms, as an efficient detoxification mechanism (Filannino, Di Cagno, & Gobbetti, 2018).

Total phenolic content (TPC) of ChJ was quantified at the beginning (0 h) and the end (48 h) of the fermentation period (Table 3). In general, TPC decreased in the majority of the evaluated samples during fermentation. Regarding NFChJ, the initial TPC varied from 181.18 to 116.75 mg GAE/100 mL after incubating at 30 °C for 48 h; this degradation may be attributed to fruit heat-tolerant enzymes such as polyphenol oxidase and peroxidase, which can remain active at high levels after heat fruit treatments (Tomás-Barberán & Espín, 2001) or fruit juice (Murtaza et al., 2020).

In FChJ, the concentration of phenolic compounds showed a tendency to decrease, except for FChJ with *L. plantarum* CRL 2030 which had remarkably similar TPC over time (ca. 120 mg GAE/100 mL). NFChJ and also ChJ fermented by *L. brevis* CRL 2051 showed similar TPC reduction percentages of about 33–35%. FChJ with *L. rhamnosus* CRL 2049 showed the highest TPC reduction (42%) whereas a slight drop occurred in ChJ with *F. tropaeoli* CRL 2039 and *L. brevis* CRL 2050 (ca. 13%), both strains isolated from cherimoya. The lowest TPC decrease determined for autochthonous strains is possibly due to an adaptation to tolerate the phenolic compounds of this matrix (Filannino et al., 2018; Yu, Leveau, & Marco, 2020).

During fruit fermentation, LAB metabolism can lead to an increment of TPC by improving the bioavailability of phenolic compounds through enzymes, which hydrolyze complex molecules into free and simpler forms (Gan, Shah, Wang, Lui, & Corke, 2017; Hur, Lee, Kim, Choi, & Kim, 2014) or release insoluble bounded phenols from plant cell wall through deglycosylation (Benincasa, Muccilli, Amenta, Perri, & Romeo, 2015; Landete, Curiel, Rodríguez, de las Rivas, & Muñoz, 2014). Additionally, the mentioned mechanisms can enhance the antioxidant

Table 3
Total phenolic content and antioxidant capacity of NFChJ and FChJ during lactic acid fermentation.

	NFChJ		FChJ									
	Control		***L. brevis* CRL 2050**		***L. brevis* CRL 2051**		***L. plantarum* CRL 2030**		***L. rhamnosus* CRL 2049**		***F. tropaeoli* CRL 2039**	
	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}
TPC[1] (mg GAE/100 mL)	181.18 ± 0.35^{Ba}	116.75 ± 0.25^{Cb}	157.55 ± 2.55^{Ca}	136.32 ± 1.87^{Ab}	151.43 ± 0.91^{CDa}	102.30 ± 0.18^{Db}	120.73 ± 1.34^{Ea}	120.23 ± 0.63^{Ca}	202.98 ± 3.71^{Aa}	116.41 ± 2.80^{Cb}	144.54 ± 0.71 ^{Da}	126.16 ± 0.73^{Bb}
RSA[1] (%)	88.81 ± 0.83^{BCa}	87.57 ± 0.79 ^{Aa}	87.92 ± 0.32^{BCb}	89.82 ± 0.26 ^{Aa}	88.52 ± 0.25^{BCa}	89.44 ± 0.40^{Aa}	86.16 ± 1.75^{Ca}	88.14 ± 0.54^{Aa}	93.60 ± 2.86^{ABa}	88.33 ± 0.16^{Aa}	97.78 ± 0.83^{Aa}	86.23 ± 3.71^{Ab}
TEAC[1] (μmol TE/100 mL)	51.75 ± 1.85^{Aa}	41.31 ± 0.39^{Db}	41.08 ± 0.37^{Eb}	43.49 ± 0.66^{BCa}	41.80 ± 0.26^{DEa}	41.70 ± 0.87^{CDa}	43.55 ± 0.62^{CDa}	44.52 ± 0.52^{Ba}	47.08 ± 0.24^{Ba}	47.93 ± 0.24^{Aa}	44.60 ± 0.63^{Ca}	46.58 ± 0.96^{Aa}
ORAC[1] (μmol TE/100 mL)	350.17 ± 7.91^{Aa}	325.33 ± 4.27^{Aa}	338.90 ± 5.91^{ABa}	328.01 ± 3.48^{Aa}	323.27 ± 1.47^{Ba}	325.17 ± 6.14^{Aa}	332.89 ± 5.12^{Ba}	342.95 ± 5.23^{Aa}	322.31 ± 7.39^{Ba}	331.70 ± 6.99^{Aa}	336.26 ± 6.91^{ABa}	329.40 ± 11.48^{Aa}

[1] TPC = total phenolic content; RSA = DPPH radical scavenging activity; TEAC = Trolox equivalent antioxidant capacity; ORAC = Oxygen radical absorbance capacity. Different capital letters (A-E) indicate significant differences ($P < 0.05$) among samples at the same time. Different lower cases letters (a-b) indicate significant differences between time of the same sample.

properties of foods. A higher TPC was reported for apple (Li et al., 2019), pomegranate (Mantzourani et al., 2019) and blueberry pomace juices (Yan et al., 2019) after lactic acid fermentation. Oppositely, a significant TPC decrease can also occur during fermentation caused by the matrix pH, which can modify the structure, conformation, and stability of these compounds (Kwaw et al., 2017; Yan et al., 2019). Certain concentrations of phenolics can produce bacteriostasis, and only LAB which have the capacity to degrade these substrates may keep growing, with the consequent decrease in TPC (Hervert-Hernández, Pintado, Rotger, & Goñi, 2009; Rodríguez et al., 2009). Other authors (Hashemi et al., 2017; Li et al., 2019; Mustafa et al., 2019; Zhang, Ma, He, Lu, & Ren, 2018) reported that lactic acid fermentations of date plum, apple, sweet lemon, and pomegranate juices resulted in TPC losses, in agreement with our results.

3.4. Antioxidant activity

The antioxidant activity of ChJ during lactic acid fermentation were determined by several methods based on the samples capacity of scavenging different radicals as DPPH (RSA), ABTS (TEAC assay), and AAPH (ORAC assay). Results are shown in Table 3.

Regarding DPPH radical scavenging activity, NFChJ exhibited a value near 88% during incubation time, similar to most of the FChJ samples. However, a higher value was determined for the juices fermented by *L. rhamnosus* CRL 2049 and *F. tropaeoli* CRL 2039 (93–98%), perhaps by a higher antioxidant capacity of these strains.

The TEAC value in NFChJ showed a significantly decrease after incubation at 30 °C 48 h (51.75 to 41.31 μmol TE/100 mL) whereas in FChJ the TEAC value remained constant or slightly higher after 48 h of fermentation.

In addition, slight variations in ORAC values for ChJ over time was noticed. A 7% loss in NFChJ was detected whereas a 2–3% loss was registered in juices fermented by *L. brevis* CRL 2050 and *F. tropaeoli* CRL 2039. In the other FChJ, ORAC values were similar or higher (3%) after fermentation.

The antioxidant activity can be affected by the fermentation process. A significantly increase of DPPH RSA and TEAC values in pomegranate fermented juice was reported by Pontonio et al. (2019). In addition, in alcoholic fermentation of orange juice, although TPC was not affected by the process, variations in the antioxidant activity were detected by using different methods. These authors reported that TEAC values remained constant whereas ORAC and DPPH RSA significantly increased after fermentation (Escudero-López et al., 2016). Similarly, we determined changes in the antioxidant activity, showing that DPPH RSA remained constant in most of our samples but the TEAC and ORAC values showed a tendency to increase after fermentation, depending on the LAB culture used.

A strong parallelism between the TPC and the antioxidant capacity of fermented vegetables and fruit juices has been proposed by some authors (Di Cagno et al., 2019; Kwaw et al., 2018; Nguyen et al., 2019). However, Li et al. (2019) reported lower levels of phenolic compounds but higher antioxidant capacity in fermented than unfermented apple juice. Our findings showed that although a reduction in TPC after ChJ fermentation was noticed, the antioxidant activity remained practically unchanged or even slightly higher.

3.5. Anti-platelet activity assay

A preliminary study was performed to evaluate the potential anti-platelet capacity of ChJ against the ADP agonist (4 μM). The obtained results are shown in Fig. 2. The inhibition percentages of the aqueous extracts, estimated from percentages of platelet aggregation, ranged between 31% and 66% for FChJ, and about 70% for NFChJ. Juices fermented by *L. rhamnosus* CRL 2049, *L. plantarum* CRL 2030 and *F. tropaeoli* CRL 2039 and also NFChJ, showed an anti-platelet activity at 1 mg/mL against ADP. Conversely, juices inoculated with both *L. brevis*

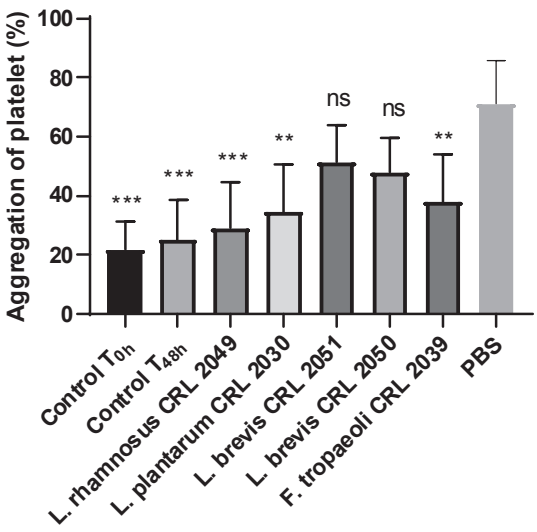

Fig. 2. Percentage of platelet aggregation of ChJ aqueous extracts. Graph represents the mean ± standard deviation of n = 6 experiments. Asterisks denotes statistically significant differences between percentages of platelet aggregation of ChJ extracts and negative control (PBS) at **P < 0.01 and ***P < 0.001, and ns = not statistical differences.

strains did not show significant differences compared to PBS control (maximum PA), and differences were not either statistically significant between FChJ and NFChJ.

The ethanolic (Fig. 3a) and methanolic (Fig. 3b) extracts did not inhibit PA at 1 mg/mL against ADP, except for the NFChJ ethanolic extract (0 and 48 h, Fig. 3a), which showed an anti-platelet effect statistically significant comparing with control.

Polyphenols of plant-derived foods are associated with an anti-platelet activity, decreasing the risk of cardiovascular diseases (Yao et al., 2017; Khan et al., 2018; Ed Nignpense, Chinkwo, Blanchard, & Santhakumar, 2020). Although the anti-platelet capacity of several fruit bioactive compounds has been investigated (Alarcón et al., 2015; Fuentes et al., 2012), the present work reports this property in non-fermented and fermented *Annona cherimola* Mill. juices for the first time. Our results can hardly be compared with others due to the scarce bibliography regarding the anti-platelet activity on pulp in *Annona* genus as the available reports are focused especially on their leaves and stem extracts. Chang, Wei, Teng, and Wu (1998) tested the inhibition of platelet aggregation capacity of a methanolic extract of *Annona purpurea* leaves by using thrombin, arachidonic acid and collagen as pro-aggregating agents. Some compounds of the extract exhibited a significant inhibition. Similarly, Wu, Chang, Ko, and Teng (1995) and Yang, Chang, Wu, Wang, and Wu (2002) detected this property in several compounds from *Annona montana* and *Annona squamosa* stems. A new alkaloid named romucosine was isolated by the first time from the methanolic extract of *Annona cherimola* stem (Chen, Chang, Pan, & Wu, 2001) and belongs to a family of aporphinoid alkaloids, which had potent inhibitory activity over rabbit PA induced by ADP and other agonists (Wu, Chang, Chao, & Teng, 1998). Regarding studies using fruit pulps, Alañón et al. (2019) reported that human PA triggered by ADP was not significantly inhibited by a methanolic extract of mango pulp due to its low content of magniferin. When seed extract was tested, a strong inhibition (72%) was observed because magniferin concentration was higher in seeds than in pulp.

Fruit processing can also affect the anti-platelet activity. In a recent study, a stronger inhibitory effect on rabbit platelet aggregation induced by ADP was determined after processing tomato and grapefruit into juice, the ethanolic extracts reached an inhibitory effect of 64.6 and 45.2%, respectively (Chen et al., 2019). Comparing with our results, a lower inhibitory effect was found in the ethanolic extract of NFChJ

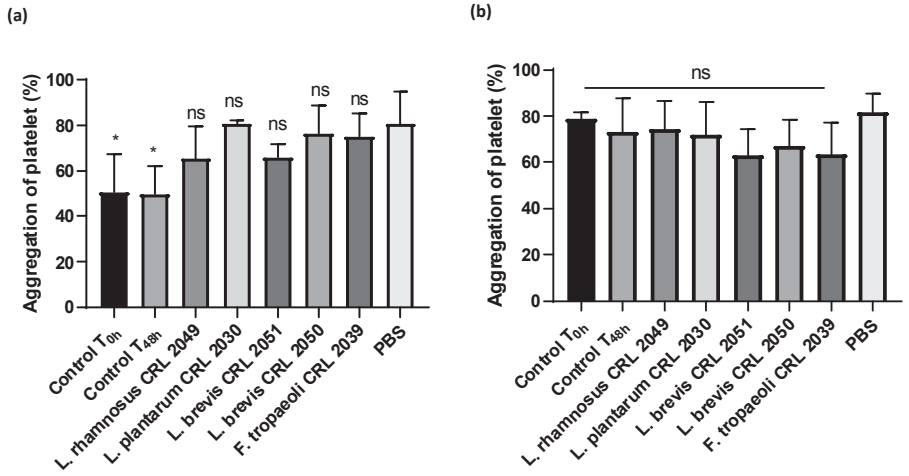

Fig. 3. Percentage of platelet aggregation of NFChJ and FChJ ethanolic (a) and methanolic (b) extracts. Asterisks denotes statistically significant differences between percentages of ChJ extracts and negative control (PBS) at *P < 0.05, and ns = not statistical differences.

(32.5%) by using ADP as agent. Further studies are required to identify the compounds which cause this weak anti-platelet effect in the *Annona cherimola* Mill. juice. In addition, it may be interesting to assess the anti-platelet effect of its seed extracts.

3.6. Cell survival and biochemical properties after refrigerated storage of FChJ

The survival capacity of the evaluated strains in all FChJ samples were analyzed at the last day of the cold storage (Table 4). All lactobacilli strains presented a similar cell density respect to the initial time (ca. 10^8 CFU/mL), demonstrating their ability to survive in ChJ throughout a 21-day shelf life period at 4 °C. In accordance to our findings, other authors also reported a high cell viability (more than 10^7 CFU/mL) for *L. plantarum, L. brevis* and *L. rhamnosus* strains in different fruit-based substrates after cold storage (Di Cagno et al., 2019; Nazzaro et al., 2008; Reddy et al., 2015; Russo et al., 2015).

In contrast to what we observed for all lactobacilli, *F. tropaeoli* CRL 2039 did not show a sufficient viability at day 21 (<10^4 CFU/mL) and a suitable cell count of 10^7 CFU/mL was only detected until day 10 of the storage period (data not shown). Oppositely, Martínez et al. (2019) informed that a *F. tropaeoli* strain presented a high survival (ca. 10^7 CFU/mL) in a milk-based mango-orange juice after 4 weeks of cold storage.

On the other hand, growth of microorganisms was not detected in Mac Conkey and Yeast and Mould agar at day 21, demonstrating that ChJ was preserved from spoilage.

Additionally, the pH value remained constant in NFChJ (4.32) and also in all FChJ (among 3.25 to 3.57) after 21 days of cold incubation (Table 4).

Sugar and organic acids were also quantified in all samples at day 1 and 21 of refrigerated storage (Table 4). Glucose concentration

Table 4
Cell survival, biochemical properties and color determination of NFChJ and FChJ throughout refrigerated storage (21 days, 4 °C).

	NFChJ Control		FChJ *L. brevis* CRL 2050		*L. brevis* CRL 2051		*L. plantarum* CRL 2030		*L. rhamnosus* CRL 2049		*F. tropaeoli* CRL 2039	
	t_{1d}	t_{21d}	t_{1d}	t_{21d}	t_{1d}	t_{21d}	t_{1d}	t_{21d}	t_{1d}	t_{21d}	t_{1d}	t_{21d}
Cell count (Log CFU/mL)	ND[1]	ND	8.21 ± 0.01^{Ca}	7.80 ± 0.11^{Cb}	8.96 ± 0.05^{Aa}	8.39 ± 0.04^{Ab}	8.95 ± 0.11^{Aa}	8.43 ± 0.02^{Ab}	8.07 ± 0.04^{Ca}	8.11 ± 0.01^{Ba}	8.68 ± 0.04^{B}	ND
pH	4.40 ± 0.01^{Aa}	4.32 ± 0.01^{Ab}	3.60 ± 0.02^{Ba}	3.57 ± 0.04^{Ba}	3.43 ± 0.05^{BCa}	3.37 ± 0.02^{Cb}	3.33 ± 0.04^{Ca}	3.25 ± 0.03^{Db}	3.35 ± 0.07^{Ca}	3.31 ± 0.04^{CDa}	3.29 ± 0.12^{Ca}	3.37 ± 0.02^{Ca}
Glucose (g/L)	13.28 ± 2.89^{Aa}	13.27 ± 2.82^{Aa}	11.50 ± 2.22^{Aa}	11.09 ± 2.14^{ABb}	12.91 ± 0.30^{Aa}	12.40 ± 0.43^{ABb}	12.50 ± 0.52^{Aa}	12.51 ± 0.42^{ABa}	11.95 ± 0.97^{Aa}	11.32 ± 0.85^{ABb}	8.71 ± 2.00^{Aa}	8.41 ± 1.70^{Ba}
Fructose (g/L)	13.78 ± 3.02^{Aa}	13.74 ± 3.00^{Aa}	13.69 ± 2.33^{Aa}	13.22 ± 2.26^{Ab}	12.66 ± 0.52^{Aa}	12.54 ± 1.17^{Aa}	12.75 ± 0.32^{Aa}	12.40 ± 0.40^{Ab}	12.79 ± 2.35^{Aa}	11.98 ± 2.49^{Ab}	13.74 ± 2.66^{Aa}	13.48 ± 2.30^{Aa}
Lactic acid (g/L)	ND	ND	0.87 ± 0.11^{Aa}	0.72 ± 0.45^{Aa}	2.14 ± 0.41^{Aa}	2.04 ± 0.49^{Aa}	2.08 ± 0.74^{Ab}	2.22 ± 0.71^{Aa}	1.44 ± 0.59^{Aa}	1.77 ± 0.97^{Aa}	1.36 ± 0.53^{Aa}	1.42 ± 0.43^{Aa}
Acetic acid (g/L)	ND	ND	0.70 ± 0.01^{Aa}	0.66 ± 0.13^{Ba}	ND	ND	ND	ND	ND	ND	1.26 ± 0.25^{Aa}	1.31 ± 0.17^{Aa}
Brix degrees (°Brix)	4.00 ± 0.00^{Aa}	4.00 ± 0.01^{Aa}	3.00 ± 0.00^{Ba}	3.10 ± 0.02^{Ba}	3.00 ± 0.00^{Ba}	3.10 ± 0.05^{Ba}	3.20 ± 0.00^{Ba}	3.10 ± 0.01^{Ba}	3.20 ± 0.04^{Ba}	3.10 ± 0.01^{Ba}	3.20 ± 0.02^{Ba}	3.10 ± 0.03^{Ba}
L*	61.81 ± 0.04^{CDa}	61.92 ± 0.04^{Ea}	62.74 ± 0.45^{Ba}	63.27 ± 0.05^{Ca}	64.15 ± 0.05^{Aa}	63.10 ± 0.06^{CDb}	63.13 ± 0.11^{Bb}	66.10 ± 0.03^{Aa}	61.40 ± 0.03^{Db}	64.91 ± 0.06^{Ba}	62.41 ± 0.08^{BCb}	62.75 ± 0.26 ^{Da}
a*	0.25 ± 0.01^{Eb}	0.44 ± 0.02^{Aa}	0.38 ± 0.01^{CDa}	0.31 ± 0.01^{BCb}	0.58 ± 0.02^{Aa}	0.44 ± 0.01^{Ab}	0.44 ± 0.01^{Ba}	0.34 ± 0.02^{BCb}	0.34 ± 0.01 ^{Da}	0.30 ± 0.01^{Cb}	0.41 ± 0.02^{BCa}	0.37 ± 0.01^{Ba}
b*	12.13 ± 0.03^{Bb}	12.47 ± 0.05^{BCa}	12.02 ± 0.04^{Ba}	12.29 ± 0.27^{Ca}	12.04 ± 0.05^{Ba}	11.67 ± 0.08^{Db}	11.69 ± 0.03^{Ca}	11.27 ± 0.04^{Db}	11.11 ± 0.04^{Db}	13.59 ± 0.06^{Aa}	13.20 ± 0.04^{Aa}	12.78 ± 0.05^{Bb}
Δ*E** [1]	0.40 ± 0.06^{D}		0.66 ± 0.22^{CD}		1.12 ± 0.02^{C}		3.00 ± 0.06^{B}		4.35 ± 0.09^{A}		0.57 ± 0.04^{D}	

[1] ND = not detected; Δ*E**= total color difference. Different capital letters (A-E) indicate significant differences (P < 0.05) among samples at the same time. Different lower cases letters (a-b) indicate significant differences between time of the same sample.

decreased between 0.30 and 0.63 g/L in FChJ, remaining constant only in the juice inoculated with *L. plantarum* CRL 2030. In addition, fructose concentration decreased in all FChJ between 0.12 and 0.81 g/L depending on the strain. In contrast, Russo et al. (2015) reported stable trends for glucose, fructose and Brix degrees over 11 days of refrigerated storage in melon pieces inoculated with *L. fermentum* PBCC11.5. However, when this fruit was fermented by *L. plantarum* B2 strain, a significant higher content for these sugars was determined, probably due to sucrose metabolism of the probiotic bacteria. Despite carbohydrate consumption in FChJ, organic acid concentration and Brix degrees did not show significant variations during cold storage.

3.7. Color determination at cold storage

Lactic acid fermentation may affect the intrinsic color of fruit juices depending on the strain used as starter culture and the matrix nature. The total color difference (ΔE^*) was evaluated at day 1 and 21 of refrigerated storage, by measuring the colorimetric coordinates L^*, a^* and b^* (Table 4). During incubation at 4 °C, L^* parameter remained constant in NFChJ whereas it tended to increase in most of the FChJ samples. Oppositely, coordinate a^* slightly decreased in all FChJ but it was almost 2-fold higher in NFChJ at the end of the storage. Variations in the b^* parameter depended on the sample and was significantly different in the majority of the evaluated ChJ.

The lowest ΔE^* value was found in NFChJ (0.40) while it varied between 0.66 and 4.35 in the FChJ samples. The juices inoculated with *L. brevis* CRL 2050 and *F. tropaeoli* CRL 2039 did not show significant differences with the non-fermented juice, when ΔE^* was compared.

According to several authors, fermented beverages can be affected by color changes during lactic acid fermentation. Pereira, Maciel, and Rodrigues (2011) observed a lightness (L^*) reduction in cashew apple juice due to the higher turbidity caused by bacterial growth. In FChJ, growth turbidity did not negatively affect L^* parameter, as even it tended to increase and led to enhance the brightness of samples. Moreover, Kwaw et al. (2018) reported that a red mulberry juice turned darker when multiple fermentations were conducted by different *Lactobacillus* strains, obtaining ΔE^* values between 1.40 and 3.60.

In our work, only ChJ fermented by the strains of *L. plantarum* and *L. rhamnosus* showed a perceptible color change, considering the minimum noticeable value of ΔE^*=2.30, as reported by Mahy, Van Eycken, and Oosterlinck (1994).

The uses of autochthonous cultures in vegetables and fruits fermentation is recommended due to their adaptation to the matrix (Filannino et al., 2018, 2020; Yu et al., 2020). Our results show that both strains of cherimoya origin (*L. brevis* CRL 2050 and *F. tropaeoli* CRL 2039) exhibited good growing and acidifying properties, slight phenolic degradation and stable antioxidant activity after fermentation of ChJ. In terms of appearance, both FChJ evidenced the lowest ΔE^* after the storage period. Even the strain *F. tropaeoli* CRL 2039 did not remain viable at high cell counts after the storage period, it showed a high glucose consumption as energy source, producing reduced-sugar beverages with potential positive impact on human health. The combination of both autochthonous strains as co-cultures is proposed to potentiate the metabolism of each strain in a synergic way during cherimoya juice fermentation.

4. Conclusions

This work represents a novel study using cherimoya fruit as matrix for the elaboration of new fermented beverages. We demonstrate that LAB, isolated from fruits cultured in Argentina, are able to grow and ferment cherimoya juice and to survive during a cold storage period. Although TPC decreased, the antioxidant capacity of this fruit substrate was preserved by fermentation. Our research provides an alternative product for the exploitation of this exotic fruit, by formulating a juice with reduced sugar content, potential anti-platelet activity, high antioxidant properties, and long shelf-life. Further studies should be performed to evaluate the sensorial characteristics and *in vivo* functional properties of this fermented fruit beverage.

CRediT authorship contribution statement

Ana Sofía Isas: Formal analysis, Methodology, Investigation, Writing - original draft, Visualization. **María Salomé Mariotti-Celis:** Methodology, Investigation, Writing - review & editing. **José Ricardo Pérez-Correa:** Supervision, Writing - review & editing. **Eduardo Fuentes:** Methodology, Formal analysis. **Lyanne Rodríguez:** Methodology, Formal analysis. **Iván Palomo:** Supervision, Visualization, Writing - review & editing. **Fernanda Mozzi:** Supervision, Visualization, Writing - review & editing, Funding acquisition. **Carina Van Nieuwenhove:** Conceptualization, Investigation, Supervision, Writing - review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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