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Lactic fermentation enhances *in vitro* carbohydrate-digesting enzyme inhibition in cherimoya (*Annona cherimola*) juice with minor phenolic changes and preserved sensory acceptance

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ABSTRACT

Cherimoya (*Annona cherimola* Miller) is a highly perishable tropical fruit rich in bioactive polyphenols, whose valorization through fermentation could enable the development of a candidate functional beverage. This study evaluated the impact of lactic fermentation on phenolic profile distribution, antioxidant capacity, *in vitro* inhibition of carbohydrate-digesting enzymes, and sensory acceptance of cherimoya juice. Pasteurized cherimoya juice (30%, w/v) was fermented for 48 h at 30 °C with three fruit-origin LAB strains: *Lactiplantibacillus plantarum* CRL2051, *Lpb. plantarum* CRL2030, and *Lacticaseibacillus rhamnosus* CRL2049. All strains grew efficiently in the juice matrix, reaching 8.2–8.9 log CFU/mL, while pH decreased from 4.5 to 3.5–3.7 after fermentation. Total phenolic content (1243–1382 mg GAE/L) and radical scavenging activity showed no major differences between fermented and unfermented juices (DPPH: ~55–58%; ABTS: ~71–75%). However, fermentation significantly enhanced the *in vitro* inhibitory activity of the juices against carbohydrate-digesting enzymes, with lower IC_{50} values, with a stronger effect observed for α-glucosidase than for α-amylase. LC-ESI-QToF analysis tentatively identified 37 phenolic compounds and semi-quantitatively estimated total phenolics at 1070–1203 mg/L, dominated by flavan-3-ols (catechins and procyanidins), representing >96–99% of total quantified phenols. Hedonic sensory evaluation (n = 105) showed no significant differences between unfermented juice and FChJ-CRL2051 across attributes (scores 5.1–6.6), indicating comparable consumer acceptance. Overall, lactic fermentation, particularly with CRL2051, may represent a feasible strategy for developing a fermented cherimoya-based beverage, showing enhanced *in vitro* carbohydrate-digesting enzyme inhibitory activity while largely preserving phenolic composition and sensory quality under the evaluated conditions.

1. Introduction

Cherimoya (*Annona cherimola* Miller) is a worldwide consumed fruit, belonging to Annonaceae family, native to South America. Cherimoya is appreciated by consumers for its distinctive organoleptic properties and nutritional value. It is also a source of diverse polyphenolic compounds with recognized antioxidant properties and associated bioactive potential (Lasota et al., 2024). Due to its high demand, its cultivation has spread to European countries such as Spain and Portugal, as well as to Israel and the United States, among others. This pleasant and soft fruit is a rich source of vitamins, dietary fiber, minerals, essential amino acids, and polyphenols (Arun Jyothi B et al., 2011; Cordeiro et al., 2013; Gentile et al., 2021). Traditionally, it has been used in folk medicine to treat parasitic infections, constipation, and diabetes (Al Kazman et al., 2022). Recent studies have reported several cherimoya-derived extracts and bioactive compounds with interesting biological properties such as

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antibacterial, antiviral, antidiabetic, anti-hyperlipidemic, and anti-cancer activities (Calzada et al., 2017, 2023; Jamkhande et al., 2017; Al Kazman et al., 2022; Ramirez-Santos et al., 2025). Polyphenols are secondary plant metabolites that are considered important molecules for wellness and health, known for their anti-inflammatory, neuro-protective, and cardioprotective effects, as well as for their role in modulating oxidative stress. These bioactive compounds are present in cherimoya pulp, leaves and seeds, and include hydroxycinnamic acids, flavanols, and lignans. This profile of bioactive phytochemicals, together with the use of cherimoya in traditional medicine, reinforces the importance of its consumption. However, despite the global cultivation of this fruit and its health benefits, its rapid deterioration rate remains a significant barrier to its market distribution. In recent years, beverages derived from tropical fruits fermented with lactic acid bacteria (LAB) with potential functional properties have gained attention due to their reported bioactive potential and extended shelf-life (Crespo et al., 2025; Isas et al., 2020; Ruiz Rodríguez et al., 2021).

In addition to their general health-promoting effects, polyphenols have also been linked to the prevention and management of metabolic disorders, particularly type 2 diabetes mellitus. The incidence of this disease is rising at an alarming rate globally due to several factors, such as unbalanced dietary intake, sedentary lifestyle, stress, and other risk factors associated with pregnancy, age, and genetics. Therefore, compounds that inhibit α-amylase and α-glucosidase enzymes, two key enzymes in starch digestion, are currently used to regulate postprandial blood glucose levels. Metformin, GLP-1 receptor agonists, acarbose, and voglibose are commonly used antidiabetic drugs, but they may cause side effects during long-term administration. In this scenario, ongoing research is seeking natural products that could act as potent inhibitors against both enzymes. Polyphenols have gained attention as inhibitors of α-amylase and α-glucosidase enzymes in experimental systems, and are considered promising candidates for supporting modulation of postprandial glucose metabolism (Bahadoran et al., 2013; Ćorković et al., 2022).

In this context, fruit juice fermentation offers a valuable opportunity to produce novel beverages with potential functional-related properties. For instance, it can enhance the bioavailability of phenolic compounds and extend the shelf life beyond that of raw fruit (Dissanayake et al., 2025; Satora et al., 2025). However, a rational selection of indigenous LAB strains as starter cultures is required to enable microbial growth in the often acidic, astringent, and low-pH environments of fruit. In this regard, fruit polyphenols are tolerated and selectively metabolized by some LAB, due to their high metabolic versatility (Ruiz Rodríguez et al., 2021).

Although the health-promoting properties of fermented cherimoya-based products have been recently explored (Isas et al., 2020; Palacios-Castillo et al., 2025), the effect of fermentation on their polyphenolic composition and associated *in vitro* bioactivities remains poorly understood. This study aimed to characterize the phenolic profile of cherimoya juice and assess potential qualitative shifts induced by fermentation with three fruit-origin LAB strains, *Lactiplantibacillus plantarum* CRL2051; *Lpb. plantarum* CRL2030, and *Lacticaseibacillus rhamnosus* CRL2049, after 48 h of fermentation at 30 °C. In addition, the antioxidant capacity and *in vitro* inhibitory effect on α-glucosidase and α-amylase enzymes of the LAB fermented juices were evaluated. Finally, hedonic sensory evaluation was performed with the unfermented and a selected fermented cherimoya juice.

2. Materials and methods

2.1. Chemical reagents

Cherimoya fruits were obtained from the local market of Tucumán (Argentina). All solvents and reagents were of analytical grade unless otherwise stated. Gallic acid, protocatechuic acid, 5-caffeoylquinic acid, caffeic acid, *p*-coumaric acid, catechin, epicatechin, gallocatechin, procyanidin dimer type B, quercetin, rutin, kaempherol, secoisolariciresinol, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), p-nitrophenyl- α-D-glucoside, α-glucosidase from *Saccharomyces cerevisiae* yeast, pancreatic porcine α-amylase, acarbose, and dinitrosalicylic acid were acquired from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). Formic acid and acetonitrile (HPLC grade) were acquired from Panreac (Madrid, Spain). The culture medium, MRS agar, MRS broth, Yeast and Mould agar, and MacConkey agar were acquired from Britania (Buenos Aires, Argentina).

2.2. Strains and growth conditions

Three LAB strains, originally isolated from fruits cultivated in the province of Tucumán (Argentina) and belonging to the Culture Collection of the "Centro de Referencia para Lactobacilos" (CERELA-CONICET, Tucumán, Argentina), were used: *Lactiplantibacillus plantarum* CRL2051; *Lpb. plantarum* CRL2030, and *Lacticaseibacillus rhamnosus* CRL2049. For storage, strains were kept at −20 °C in a medium containing skim milk (100 g/L), yeast extract (5 g/L), glucose (10 g/L), and 10% (v/v) glycerol. Before inoculation, each strain was reactivated in MRS broth (Biokar Diagnostics, Beauvais, France) by incubation for 24 h at 30 °C, followed by subculturing for 16 h in fructose-MRS (fMRS) broth under the same conditions. Then, the cells were harvested by centrifugation (DCS-16 RV Centrifuge, Presvac, Buenos Aires, Argentina) at 8000×*g* for 15 min, washed twice with sterile saline solution (0.9% NaCl, w/v), and resuspended in the same medium. Pasteurized cherimoya juice was inoculated with each strain at 2% (v/v), corresponding to an initial bacterial concentration of approximately 10^7 CFU/mL.

2.3. Cherimoya juice formulation and fermentation

Ripe cherimoya fruits (12–14°Brix) were obtained from a local market, washed twice with tap water, and carefully peeled, removing the husk and seeds to obtain the pulp, which was mixed with tap water to obtain cherimoya juice 30% (w/v, ChJ) (Isas et al., 2020). Finally, the juice was pasteurized at 65 °C for 30 min in a water bath, and immediately cooled on ice. To prevent natural cherimoya browning, caused by polyphenol oxidase enzyme, pasteurized ChJ was supplemented with previously sterilized (0.22 μm filter membrane, Millipore, Sao Paulo, Brazil) ascorbic acid (0.05% w/v). Ascorbic acid was added to prevent enzymatic browning; however, its presence may contribute to Folin–Ciocalteu reactivity and therefore total phenolic content values should be interpreted cautiously. Each strain was individually inoculated at 2% (v/v) in ChJ (10^7 CFU/mL); fermented at 30 °C for 48 h (FChJ-CRL2051, FChJ-CRL2030, and FChJ-CRL2049). Results were compared with unfermented juice (NFChJ) under the same culture conditions (48 h at 30 °C). Stability of the unfermented matrix during this incubation period was inferred from previously validated experimental conditions reported by Isas et al. (2020), rather than re-evaluated in the present study. Under these fermentation conditions, previous characterization of the same matrix and strains demonstrated partial sugar consumption together with accumulation of lactic acid and moderate decreases in pH and titratable acidity, consistent with active LAB metabolism (Isas et al., 2020). These compositional trends provide context for interpreting functional changes observed in the present study. Microbiological quality of pasteurized juices (both NFChJ and FChJ) was evaluated in MacConkey agar (37 °C and 45 °C, 48 h) and Yeast and Mould agar (20–25 °C, 5–7 days) to quantify coliforms, and yeasts and moulds, respectively.

2.4. Microbial cell count and pH determination

The LAB enumeration was carried out after resuspending a sample of fermented or unfermented ChJ in 0.1% (w/v) sterile peptone water solution, then plating it in MRS agar for lactobacilli count. The plates were incubated at 30 °C for 48 h. Results were expressed as Log CFU/mL. The

pH was measured by using a digital pH meter (Sartorius, Goettingen, Germany).

2.5. Total phenolic content quantification

The total phenolic content (TPC) of the juices was quantified following previously described methodologies with slight modifications (Isas et al., 2020, 2023). In summary, 10 μL of fermented or unfermented cherimoya juice were diluted with distilled water to a final volume of 1 mL. Then, 100 μL of Folin–Ciocalteu reagent (PanReac AppliChem GmbH, Darmstadt, Germany), previously diluted 1:2 (v/v) in distilled water, were added. The mixture was kept in the dark at room temperature (25 ± 5 °C) for 2 min. Subsequently, 400 μL of sodium carbonate solution (15.9%, w/v) were added, and the reaction was allowed to proceed for 20 min in darkness at room temperature. Afterwards, 200 μL of each sample were transferred to a 96-well microplate (Corning Costar®, Corning Inc., NY, USA), and the absorbance was measured at 765 nm using a microplate reader Multiskan GO (Thermo Fisher Scientific City, Vantaa, Finland). The phenolic content was calculated using a calibration curve prepared with gallic acid standards (Sigma-Aldrich Chemical Co.), and results were expressed as milligrams of gallic acid equivalents per liter of juice (mg GAEq/L). Because the Folin–Ciocalteu assay detects total reducing capacity rather than phenolics exclusively, and ascorbic acid was present in the juice matrix, TPC values were used primarily for comparative purposes among treatments rather than as absolute indicators of phenolic concentration.

2.6. Antioxidant capacity

The antioxidant capacity of fermented and unfermented cherimoya juices was determined by the ABTS and DPPH radical scavenging assays, following the protocols previously described (Isas et al., 2020, 2023).

The radical scavenging activity (RSA) for DPPH and ABTS radicals was estimated by applying the following equation:

$$\text{RSA (\%)} = [(A_0\text{-}A_s)/A_0] \text{ x } 100$$

Where A_0 is the absorbance of the radical (DPPH/ABTS) solution without a sample, and A_s is the absorbance of the radical solution with a sample.

Because DPPH and ABTS assays reflect overall reducing capacity rather than specific phenolic transformations, these measurements were considered complementary indicators of antioxidant capacity.

2.7. In vitro inhibition of α-glucosidase and α-amylase enzymes

The *in vitro* potential inhibition of carbohydrate-digesting enzymes of the samples was measured by α-glucosidase and α-amylase inhibition assays, using acarbose (0.1- 5 mg/mL) as a positive control for comparative biochemical benchmarking within the experimental system. For α-glucosidase assay, the reaction mixture was prepared in a 96-well microplate. Each well contained 10 μL of the sample solution (0.1-5 mg/mL), 90 μL of sodium phosphate buffer (50 mM, pH 6.9), and 20 μL of α-glucosidase solution (0.1 U/mL). This mixture was incubated at 37 °C for 5 min, then 20 μL of substrate p-nitrophenyl-α-D-glucopyranoside (1 mM, pNPG) was added as substrate and incubated at 37 °C for 15 min. The reaction was stopped by the addition of 80 μL of Na_2CO_3 (100 mM). The absorbance was recorded at 405 nm in a microplate spectrophotometer (Thermo Fisher Scientific City, Vantaa, Finland). Control wells containing buffer instead of sample and sample blanks (without enzyme) were included to correct for possible background absorbance or color interference.

The α-amylase assay was performed according to the method described by (Zhang et al., 2021) with minor changes. Briefly, a reaction mixture was prepared in glass tubes consisting of 125 μL of sample (0.1-5 mg/mL) and 125 μL of α-amylase (0.5 mg/mL), which was incubated at 25 °C for 10 min. Each tube was supplemented with 125 μL of 1% starch solution, incubated again at 25 °C for 10 min. Afterwards, the reaction was stopped by adding 250 μL of dinitrosalicylic acid reagent, followed by heating in a boiling water bath at 95 °C for 5 min. Finally, the reaction mixture was diluted with 5 mL of distilled water, and aliquots were transferred to 96-well plates, measuring the absorbance at 540 nm using a microplate spectrophotometer. A color control and a sample blank were used to subtract any possible interference from the juice matrix.

The inhibition of each enzyme was estimated by using the following equation:

$$\text{\% Inhibition} = [(A_c\text{-}A_s)/A_c] \text{ x } 100$$

Where A_c is the absorbance of the control (after blank subtraction), and A_s is the absorbance of the sample (after blank subtraction).

2.8. Phenolic characterization of cherimoya juices by LC-ESI-QTOF analysis

Lyophilized cherimoya juices (0.015 g) were subjected to successive extraction with 1 mL of methanol/water (50:50 v/v, pH 2) and then with 20 mL of acetone/water (70:30, v/v). The two supernatants were then combined, which corresponded to the extractable phenolic compounds fraction.

Phenolic compounds present in cherimoya juice were analyzed using high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (HPLC-ESI-QToF). The system consisted of an Agilent 1200 LC platform connected to an Agilent 6530A Accurate-Mass Q-ToF mass spectrometer with an ESI Jet Stream ionization source (Agilent Technologies). Chromatographic separation was performed on a reverse-phase InfinityLab Poroshell 120 EC-C18 column (15 cm × 3 mm, 2.7 μm; Agilent), fitted with a corresponding guard column (3 × 5 mm, 2.7 μm) and maintained at 40 °C throughout the run. The sample injection volume was 10 μL. The mobile phases consisted of Milli-Q water (phase A) and acetonitrile (phase B), both acidified with 0.1% formic acid. Separation was carried out at a constant flow rate of 0.5 mL/min under a gradient elution program: starting at 10% B and increasing to 30% B over 10 min, then to 40% B in the next 5 min, and finally reaching 50% B in another 5 min. The system was then returned to the initial conditions over 5 min and held for an additional 5 min to re-equilibrate.

Mass spectrometric detection was carried out in negative electrospray ionization mode, scanning from m/z 100 to 1400. Key source parameters included a drying gas (nitrogen, purity >99.9%) flow of 10 L/min at 325 °C, a sheath gas flow of 6 L/min at 250 °C, and a nebulizer pressure of 25 psi. The capillary and nozzle voltages were set at 3500 V and 500 V, respectively, with a fragmentor voltage of 150 V. Data acquisition and analysis were conducted using the MassHunter Workstation Software (Agilent Technologies). Compound identification was assigned according to the Metabolomics Standards Initiative (MSI) guidelines. Identification level 1 corresponded to compounds confirmed with authentic standards; level 2 to putatively annotated compounds based on accurate mass and MS/MS fragmentation patterns; and level 3 to tentative compound class assignments based on spectral features.

Quantification was based on external calibration curves generated from authentic standards, including gallic acid, protocatechuic acid, 5-caffeoylquinic acid, caffeic acid, *p*-coumaric acid, catechin, epicatechin, gallocatechin, procyanidin B2, quercetin, rutin, kaempferol, and secoisolariciresinol. Each compound was analyzed at five concentration levels ranging from 0.001 to 20 μM. Limits of detection (LOD) and quantification (LOQ) were estimated between 0.02–0.04 μM and 0.08–0.10 μM, respectively. The method showed acceptable intra- and inter-day precision, with coefficients of variation between 3.7% and 7.2%, allowing for comparative quantification within the experimental system. Compounds were quantified as equivalents of their

corresponding standards when available, or as equivalents of the most structurally related compound otherwise. Due to the limited commercial availability of authentic standards for oligomeric procyanidins, several compounds were quantified as equivalents of structurally related standards. Therefore, the quantitative data should be interpreted as semi-quantitative estimates. In particular, isomer-level discrimination among oligomeric procyanidins was not possible in the absence of authentic standards. Since all juice samples shared the same matrix composition and were extracted and analyzed under identical conditions, the LC-ESI-QToF results were primarily used for comparative evaluation of phenolic profiles among treatments rather than for absolute quantification.

2.9. Hedonic sensory analysis

A sensory evaluation involving 105 untrained panelists (55 females and 50 males), aged between 17 and 38 years, was conducted. All participants were informed about the aim of the study, and volunteers signed a full disclosure form before the study. Appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of this research. Only the FChJ-CRL2051-fermented juice was included in sensory evaluation because this strain showed comparatively stronger enzyme inhibitory responses within the experimental dataset. Before the assessment, the panelists were given standardized instructions detailing the nature of the products, the sensory attributes to be assessed and the methodology to be followed. The hedonic evaluation was performed five days after fermentation, once a microbiological safety certificate had been issued. Therefore, the sensory profile obtained reflects the characteristics of the refrigerated product after short-term storage rather than those of freshly fermented juice. Until the time of evaluation, all juice samples were stored in a refrigerator at 4 °C. Possible sensory changes occurring during this storage period were not independently evaluated in the present study. The stability of FChJ-CRL2051 over 21 days at 4 °C has been previously investigated (Isas et, al., 2020).

The samples were coded with randomly assigned three-digit numbers and presented to the panelists simultaneously in a randomized order. Each sample was served in 25 mL portions at a temperature of 4 ± 2 °C in white plastic cups to avoid visual bias. Panelists were instructed to cleanse their palates with drinking water and plain crackers between samples. No information was disclosed regarding the potential health benefits of the fermented juice. Participants assessed the attributes of “colour”, “brightness”, “odour”, “acidity”, “astringency”, “texture”, “taste”, and “overall acceptability” using a 9-point hedonic scale, where 1 indicated “dislike extremely”, and 9 indicated “like extremely”.

2.10. Statistical analysis

All *in vitro* assays were performed in triplicate using three independent fermentation batches; therefore, results should be interpreted as comparative experimental estimates rather than definitive strain-performance indicators. Results were expressed as mean ± standard deviation (SD). Before analysis, the Shapiro-Wilk and Levene tests were performed to assess data normality and homoscedasticity, respectively. Based on these results, either a one-way ANOVA followed by Tukey's HSD pairwise comparisons or a Kruskal-Wallis test followed by a Dunn-Bonferroni multiple comparisons test on ranks was applied to each dependent variable. In all cases, the significance level was set at $\alpha = 0.05$. To assess the relationships between phenolic composition and IC_{50} values for α-amylase and α-glucosidase inhibition, Pearson correlation coefficients and linear regression models were applied as exploratory analyses based on four experimental conditions. All statistical analyses were carried out using IBM SPSS Statistics v.27.0.

Comparisons of sensory parameters between juice samples were conducted using the paired-samples Student's t-test. In all analyses, statistical significance was defined as $p < 0.05$. All statistical procedures were performed using SPSS software, version 29.0 (IBM Corp., Armonk, NY, USA) and a GraphPad Prism version 8.00 (GraphPad software, Inc., La Jolla, California).

3. Results and discussion

Cherimoya is a well-known tropical fruit valued in traditional cultures for its pleasant sweetness and taste, and its reported traditional medicinal uses. Although the scientific literature on the bioactive potential of cherimoya is still relatively limited compared to that of other more extensively studied fruits, its richness in polyphenolic compounds suggests that it may offer relevant antioxidant and additional beneficial bioactive potential. In the context of increasing interest in using diet as a tool to prevent and manage chronic diseases, such as obesity, inflammatory bowel disease, diabetes, among others, phenolic compounds have attracted attention due to their safety and protective effects against oxidative stress and inflammation. While fruits and vegetables are the main dietary sources of polyphenols, current research is increasingly focusing on enhancing their bioactivity through biotechnological processes such as lactic fermentation. In this study, we have characterized the phenolic constituent profile of cherimoya juice by LC-ESI-QToF analysis following MSI identification criteria. In addition, the effect of the fermentation process using two fruit-derived *Lpb. plantarum* CRL2051 and CRL2030 and one *L. rhamnosus* CRL2049 strains on potential shifts in the phenolic profile distribution and antioxidant capacity of the juice was analyzed. This provides insight into the potential of fermented cherimoya juice as a candidate fermented beverage matrix with bioactive potential. The LAB strains used in this study were selected based on their strong capacity to grow in other fruit juice matrices, as previously observed by (Isas et al., 2020), as well as by their technological properties (Ruiz Rodríguez et al., 2019).

3.1. Viable cell enumeration, pH, total phenolic content, and antioxidant capacity of fermented cherimoya juices

The pH values and viable cell counts, the TPC (Folin-Ciocalteau assay), and the antioxidant capacity revealed by ABTS and DPPH scavenging activities of NFChJ and FChJ are listed in Table 1. As expected, pH values were significantly different among fermented and unfermented juices, with a mean value of 4.5 for NFChJ and a range of pH values from 3.5 to 3.7 after 48 h of fermentation by *Lpb. plantarum* CRL2051, *Lpb. plantarum* CRL2030, and *Lacticaseibacillus rhamnosus* CRL2049.

The evaluated strains were able to adapt and tolerate the intrinsic characteristics of the fruit matrix, increasing by more than one Log unit after 48 h of fermentation. This increase in viable counts together with

Table 1
Viable cell count, pH, total phenolic content and antioxidant capacity of fermented and unfermented cherimoya juices*.

	NFChJ	FChJ-CRL2030	FChJ-CRL2051	FChJ-CRL2049
pH	4.52 ± 0.03^{a}	3.55 ± 0.05^{c}	3.50 ± 0.01^{c}	3.74 ± 0.03^{b}
Log CFU/mL	ND	8.78 ± 0.36^{b}	8.94 ± 0.23^{b}	8.19 ± 0.08^{a}
TPC (mg GAE/L)	1332 ± 88^{a}	1243 ± 49^{a}	1292 ± 58^{a}	1382 ± 99^{a}
DPPH RSA (%)	55 ± 1^{a}	56 ± 3^{a}	56 ± 2^{a}	58 ± 3^{a}
ABTS RSA (%)	71 ± 3^{a}	75 ± 5^{a}	74 ± 4^{a}	73 ± 5^{a}

NFChJ: unfermented cherimoya juice; FChJ-CRL2030: fermented cherimoya juice by *Lpb. plantarum* CRL2030; FChJ-CRL2051: fermented cherimoya juice by *Lpb. plantarum* 2051; FChJ-CRL2049: fermented cherimoya juice by *L. rhamnosus* CRL2049; ND: non-detected; RSA: radical scavenging activity; [a-c]: Different superscript letters indicate significant differences among samples (p<0.05). * Values are expressed as mean ± standard deviation (SD).

the observed pH decrease is consistent with previously reported sugar consumption and organic acid production in the same fermentation system, supporting active metabolic transformation of the juice matrix during incubation (Isas et al., 2020). The initial inoculum was approximately 10^7 CFU/mL (data not shown), growing to 8.2-8.9 Log CFU/mL after 48 h at 30 °C, coincident with the acidification of the juices. The capacity of these strains isolated from fruits to adapt and grow in diverse juice matrices was reported by several previous studies, including cherimoya juice (Crespo et al., 2025; Isas et al., 2020, 2023).

The TPC did not significantly vary among NFChJ and FChJ, with values ranging from 1243 to 1382 mg GAE/L under the experimental conditions evaluated. These values should be interpreted cautiously because the Folin–Ciocalteu assay may also reflect the contribution of added ascorbic acid. Therefore, conclusions regarding phenolic stability rely primarily on LC-ESI-QToF compositional trends rather than on Folin-derived values alone. In this regard, increased TPC after lactic fermented blueberry pomace, apple, pomegranate, and murta juices has been reported (Escobar-Beiza et al., 2023; Li, Z. et al., 2018; Mantzourani et al., 2018). In contrast, other studies have determined significant losses of TPC after lactic fermentation of fruit juices (Mustafa et al., 2019; Zhang, Z. et al., 2018). These varied results have been related to the matrix composition, bacterial strain, time of fermentation, and pH conditions, which affect microbial metabolism and consequently the structure and stability of polyphenols. In the present study, interpretation of TPC stability is additionally limited by the presence of ascorbic acid in the juice formulation.

The antioxidant capacity, determined as RSA (%) against DPPH and ABTS radicals, remained similar across samples, with values ranging approximately 55-58% for DPPH, and 71-75% for ABTS, and therefore did not indicate major fermentation-related changes in overall reducing capacity. Although some studies report changes in antioxidant activity after lactic fermentation of fruit matrices, no comparable trends were observed under the present experimental conditions. Accordingly, these assays were interpreted as complementary indicators of overall redox capacity rather than as evidence of specific phenolic preservation. Regarding the correlation between chemical composition and antioxidant capacity, both TPC and antioxidant activity remained largely unchanged after fermentation, suggesting that global reducing capacity was not substantially affected. Because DPPH and ABTS assays reflect overall redox capacity rather than specific phenolic transformations, these results were considered complementary to LC-ESI-QToF profiling and enzyme inhibition analyses.

3.2. Fermentation of cherimoya juice enhances in vitro inhibitory activity against carbohydrate-digesting enzymes

The inhibitory potential of α-glucosidase and α-amylase enzymes by unfermented and fermented cherimoya juices were evaluated at different concentrations to obtain IC_{50} value, normalized to phenolic content (μg GAE/mL), as shown in Fig. 1A and B, respectively. FChJ-CRL2051 exhibited significantly stronger α-glucosidase inhibitory activity than acarbose under the assay conditions used after normalization to phenolic content (583.7 vs. 683.7 μg GAE/mL, $p < 0.05$). However, this comparison represents a biochemical reference within the experimental system and does not imply greater physiological efficacy than acarbose *in vivo*. A similar inhibitory activity to the reference compound under the assay conditions was observed for FChJ-CRL2030 (700.8 μg GAE/mL), and lower for FChJ-CRL2049 (841.5 μg GAE/mL, $p < 0.05$). NFChJ evidenced the lowest inhibitory potential for α-glucosidase (1092.1 μg GAE/mL). Regarding α-amylase inhibition (Fig. 1 B), acarbose was the most potent inhibitor (863.1 μg/mL). The three FChJ exhibited significantly lower inhibition capacity (1200-1500 μgGAE/mL), but significantly higher than NFChJ (~2100 μg GAE/mL). Interestingly, among the three FChJ, FChJ-CRL2051 showed the highest α-amylase inhibitory activity within the experimental dataset obtained from three independent fermentation batches.

Both enzymes are involved in the hydrolysis of complex oligo-/disaccharides into more easily absorbed monosaccharides, such as glucose, which is released into the bloodstream. There is a growing interest in identifying foods and beverages containing compounds capable of inhibiting carbohydrate-digesting enzymes involved in postprandial glucose release that may contribute to modulation of carbohydrate digestion under experimental conditions. Lactic fermentation by *Lpb. plantarum* CRL2051; *Lpb. plantarum* CRL2030, and *L. rhamnosus* CRL2049 significantly increased the inhibitory activity against carbohydrate-digesting enzymes of ChJ through enhanced inhibitory activity on both assayed enzymes compared to NFChJ. The strongest α-glucosidase inhibitory activity under the experimental conditions evaluated was observed for FChJ-CRL2051 after normalization to phenolic content, exceeding that of acarbose within this *in vitro* assay framework. It should be noted that comparisons with acarbose are intended only as assay-specific biochemical benchmarks and cannot be directly extrapolated to real intake conditions, serving sizes, or postprandial physiological responses. Interestingly, several studies have highlighted the enzyme inhibitory activity related to glucose metabolism of cherimoya extracts through both *in vitro* and *in vivo* models (Calzada et al., 2023; Galarce-Bustos et al., 2023; Valdes et al., 2023). Ramírez-Santos et al. (2025) also demonstrated the potential metabolic effects associated with glucose regulation of aqueous cherimoya extracts administered in combination with antidiabetic drugs.

Within this context, LAB fermentation may contribute to increased *in vitro* inhibition of carbohydrate-digesting enzymes associated with glucose release from dietary carbohydrates in fermented cherimoya juices. This effect likely reflects the combined influence of phenolic

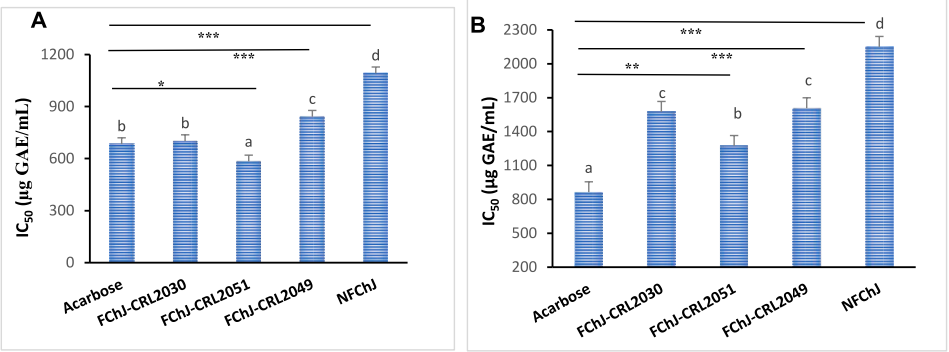


Fig. 1. IC_{50} values for inhibition of (A) α-glucosidase and (B) α-amylase by fermented and unfermented cherimoya juices. Data are presented as mean ± SD (n = 3). [a-d] Different superscript letters indicate significant differences among samples ($p<0.05$). Significance levels for pairwise comparisons are indicated as *$p < 0.05$, **$p < 0.01$*, and ***$p < 0.001$. IC_{50} values were normalized to total phenolic content and are presented as comparative biochemical estimates within the experimental system.

composition, strain-specific metabolism, organic acid production, and changes in sugar availability during fermentation. Accordingly, the fermented juices exhibited a greater reduction in α-glucosidase and α-amylase activities than unfermented ChJ, which is likely related to multiple compositional changes occurring during fermentation, including phenolic redistribution, reductions in simple sugars, and accumulation of organic acids produced by LAB metabolism.

In agreement with these findings, an enhanced inhibitory activity of LAB-fermented matrices against α-glucosidase and α-amylase has been reported (Gui et al., 2025; Liu et al., 2025; Rodríguez-Sánchez et al., 2025), often involving combined effects of organic acids, sugars and phenolic compounds. A recent study reported that simple sugars (glucose, fructose) are negatively correlated with the α-glucosidase and α-amylase inhibition of fermented pear juice (Grondalska & Kolniak-Ostek, 2025). The inclusion of LAB as starter cultures in the development of novel fermented beverages can lead to increase *in vitro* inhibitory activity against carbohydrate-digesting enzymes, by reducing sugar content, increasing organic acids, and modifying phenolic profile after fermentation consistent with our results, as previously reported (Grondalska & Kolniak-Ostek, 2025; Gui et al., 2025; Hashemi & Jafarpour, 2023; Mubeen et al., 2025; Zhong et al., 2021).

3.3. Identification of the phenolic compounds present in Annona cherimola by LC-QToF

A total of 37 phenolic compounds were putatively annotated at MSI identification levels 1-3 in *Annona cherimola* juice using LC-ESI-QToF based on accurate mass measurements, retention time, and MS/MS fragmentation patterns (Table 2). The extracted ion chromatogram (EIC) revealed the overall phenolic profiles of the four juices (Fig. 2), showing a largely conserved phenolic profile after fermentation, with only slight differences in peak intensities, which are discussed below (panel A). Panel B (Fig. 2) was included to improve clarity and highlights the most intense and abundant peaks, which are associated with their corresponding chemical structures shown in panel C of Fig. 2.

The putatively annotated phenolic compounds were classified as hydroxycinnamic acids and their derivatives, simple phenolic acids, flavanols, flavonols and lignans.

Among hydroxycinnamic acids, two isomers of caffeoylquinic acid were putatively annotated at 1.4 and 8.8 min, both showing a molecular ion at m/z 353.0878 and fragment ions at m/z 191 and 179, characteristic of quinic and caffeic acid moieties, respectively. Free caffeic acid was also identified (MSI level 1) at 6.1 min with a molecular ion at m/z 179.0350 and a major fragment at m/z 135. Additionally, two isomers of caffeic acid-*O*-glucoside were found at 3.7 and 4.8 min, both presenting a molecular ion at m/z 341.0878 and fragment ions at m/z 179 and 161. Coumaric acid-*O*-glucoside was detected at 5.4 min with $[M–H]^-$ at m/z

Table 2
LC-QToF identification of phenolic compounds of *Anonna cherimola* juices. MSI identification levels were assigned according to Metabolomics Standards Initiative criteria: level 1, confirmed with authentic standards; level 2, putatively annotated compounds; level 3, tentative compound class assignment.

Identified Compound	RT (min)	Molecular formula	Molecular weight	$[M-H]^-$	Fragment MS^2	MSI Level
HIDROXICINNAMIC ACIDS AND HIDROXICINNAMATES						
Caffeoylquinic acid	1.4	$C_{16}H_{18}O_9$	354.0951	353.0878	179; 191	2
Caffeic acid	6.1	$C_9H_8O_4$	180.0423	179.0350	135	1
Caffeic acid-*O*-glucoside	3.7	$C_{15}H_{18}O_9$	342.0951	341.0878	179; 161	2
Caffeic acid-*O*-glucoside	4.8	$C_{15}H_{18}O_9$	342.0951	341.0878	179; 161	2
Coumaric acid-*O*-glucoside	5.4	$C_{15}H_{18}O_8$	326.1002	325.0929	119; 163	2
Caffeoylquinic acid	8.8	$C_{16}H_{18}O_9$	354.0951	353.0878	179 (173?); 191; 93	2
OTHERS SIMPLE PHENOLIC ACIDS						
Gallic acid	2.0	$C_7H_6O_5$	170.0215	169.0142	125	1
Protocatechuic acid	3.2	$C_7H_6O_4$	154.0266	153.0193	109	1
FLAVANOLS						
Catechin	5.0	$C_{15}H_{14}O_6$	290.0790	289.0718	245	1
Epicatechin	6.6	$C_{15}H_{14}O_6$	290.0790	289.0718	245; 137	1
Epicatechin-*O*-glucoside	4.5	$C_{21}H_{24}O_{15}$	452.1319	451.1246	289; 245	2
Procyanidin dimer type B	3.8	$C_{30}H_{26}O_{12}$	578.1424	577.1351	407; 289; 245; 125	3
Procyanidin dimer type B	4.5	$C_{30}H_{26}O_{12}$	578.1424	577.1351	407; 289; 125	3
Procyanidin dimer type B	5.8	$C_{30}H_{26}O_{12}$	578.1424	577.1351	245; 407; 289; 125	3
Procyanidin dimer type B	7.5	$C_{30}H_{26}O_{12}$	578.1424	577.1351	289; 125	3
Procyanidin dimer type B	9.2	$C_{30}H_{26}O_{12}$	578.1424	577.1351	407; 289; 245	3
Procyanidin trimer type B	4.9	$C_{45}H_{38}O_{18}$	866.2058	865.2029	577; 407; 289	3
Procyanidin trimer type B	5.8	$C_{45}H_{38}O_{18}$	866.2058	865.2029	577; 245	3
Procyanidin trimer type B	7.0	$C_{45}H_{38}O_{18}$	866.2058	865.2029	577; 407; 289; 125	3
Procyanidin trimer type B	7.7	$C_{45}H_{38}O_{18}$	866.2058	865.2029	407; 290; 125	3
Procyanidin trimer type B	8.0	$C_{45}H_{38}O_{18}$	866.2058	865.2029	577; 289	3
Procyanidin trimer type B	8.8	$C_{45}H_{38}O_{18}$	866.2058	865.2029	577; 407; 289	3
Procyanidin trimer type B	9.1	$C_{45}H_{38}O_{18}$	866.2058	865.2029	407; 289; 245; 125	3
Procyanidin tetramer type B	5.1	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	865; 577; 289	3
Procyanidin tetramer type B	6.1	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	866; 289	3
Procyanidin tetramer type B	7.5	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	865; 577; 407; 125	3
Procyanidin tetramer type B	7.9	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	865; 577; 289; 125	3
Procyanidin tetramer type B	8.4	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	865; 577	3
Procyanidin tetramer type B	8.8	$C_{60}H_{50}O_{24}$	1154.2692	1153.2698	577; 289	3
Procyanidin dimer type A	10.6	$C_{30}H_{24}O_{12}$	576.1267	575.1239	289	3
Procyanidin dimer type A	10.7	$C_{30}H_{24}O_{12}$	576.1267	575.1239	289	3
FLAVONOLS						
Calabricoside A	8.0	$C_{32}H_{38}O_{20}$	742.1956	741.1937	301	2
Quercetin-rutinoside	8.8	$C_{27}H_{30}O_{16}$	609.1496	609.1461	300	2
Rutin	9.0	$C_{27}H_{30}O_{16}$	609.1496	609.1461	300	1
Kaempferol	14.2	$C_{15}H_{10}O_6$	286.0477	285.0405	119	1
LIGNANS						
Laricresinol-glucopyranoside	9.2	$C_{26}H_{34}O_{11}$	522.2101	521.2046	151; 289	2
Secoisolariciresinol	12.1	$C_{20}H_{26}O_6$	362.1729	361.1657	165	1

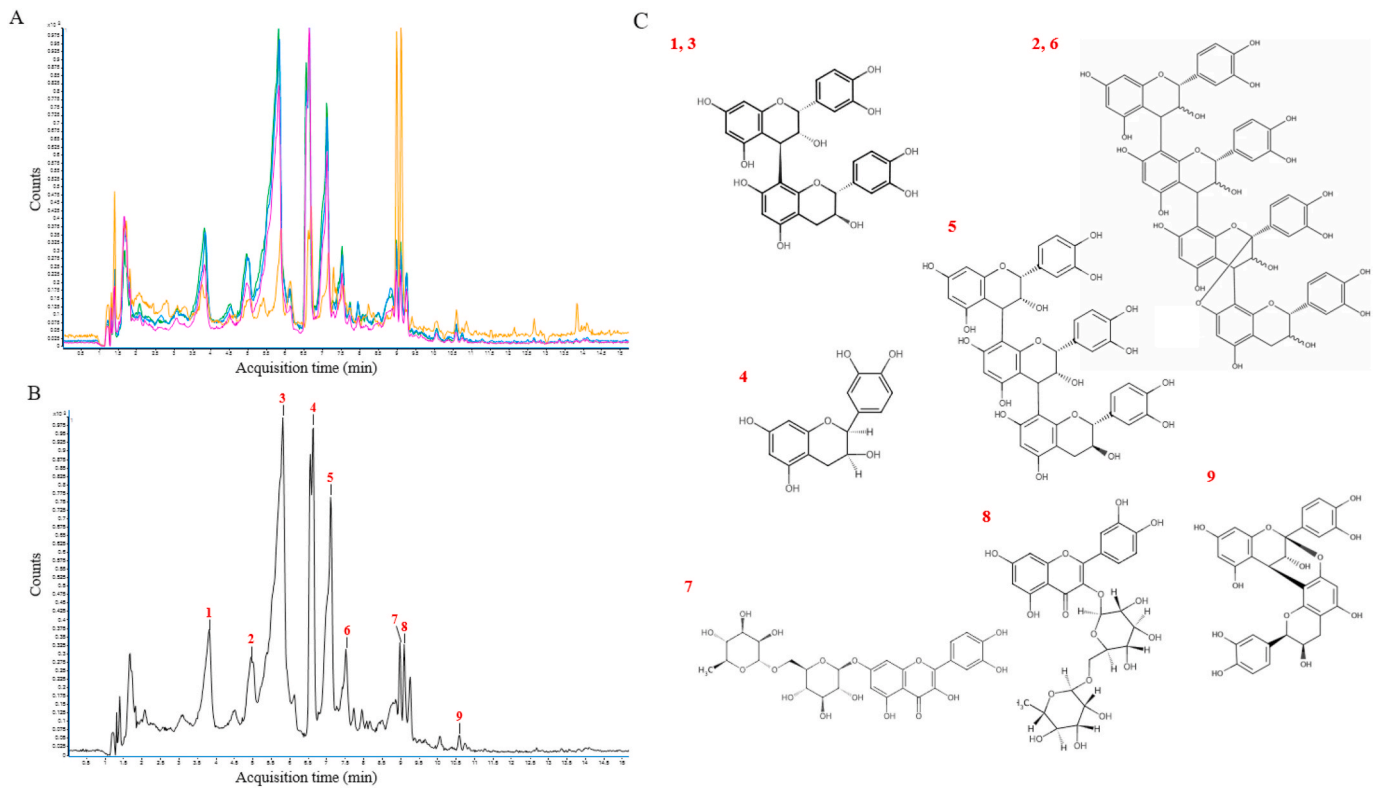


Fig. 2. Extracted ion chromatograms (EICs) obtained in negative ion mode (ESI^-) for cherimoya juice. A. Superimposed EICs of the four variants. B. Representative EIC with the major peaks labelled (1–9). C. Proposed structures of the main putatively annotated phenolic compounds. EICs were generated using the *m/z* values of the annotated compounds, and only selected representative signals are shown for clarity. Peak assignments are as follows: (1,3) Procyanidin dimer type B, (2,6) Procyanidin tetramer type B, (4) Epicatechin, (5) Procyanidin trimer type B, (7) Quercetin rutinoside, (8) Rutin, and (9) Procyanidin dimer type A.

325.0929 and fragments at m/z 163 and 119.

Within the group of simple phenolic acids, gallic acid and protocatechuic acid were identified (MSI level 1) at 2.0 and 3.2 min, respectively. These compounds showed their corresponding specific $[M–H]^-$ ions at m/z 169.0142 and 153.0193, and the respective characteristic fragments at *m/z* 125 and 109.

The most abundant group of polyphenols identified in cherimoya juice corresponded to flavanols, represented by both monomeric and oligomeric forms. Catechin and epicatechin were detected at 5.0 and 6.6 min with a common $[M–H]^-$ ion at *m/z* 289.0718 and typical fragments at m/z 245 and 137. Epicatechin-*O*-glucoside was identified at 4.5 min ($[M–H]^- = 451.1246$), displaying fragments at m/z 289 and 245. Several signals consistent with type B procyanidin dimers ($[M–H]^-$ at *m/z* 577.1351) and trimers ($[M–H]^-$ at *m/z* 865.2029) were tentatively assigned at the compound-class level, based on accurate mass and fragmentation, with MS^2 fragments at m/z 289, 245, 407, and 125, indicative of interflavan linkages. Additionally, six signals consistent with type B procyanidin tetramers were tentatively assigned, based on accurate mass ($[M–H]^-$ at *m/z* 1153.2698) and characteristic MS^2 fragmentation patterns. Signals compatible with type A procyanidin dimers were also tentatively assigned at 10.6 and 10.7 min with a $[M–H]^-$ at *m/z* 575.1239. Isomer-level discrimination among oligomeric procyanidins was not possible in the absence of authentic standards.

Among the flavonol subclass, quercetin derivatives, including rutin (quercetin-3-*O*-rutinoside), were identified (MSI level 1) at 8.8 and 9.0 min, both with $[M–H]^-$ at *m/z* 609.1461 and a major fragment at *m/z* 300. A unique compound, calabricoside A, was putatively annotated at 8.0 min with a $[M–H]^-$ ion at *m/z* 741.1937 and a key fragment at m/z 301. Kaempferol was found at 14.2 min ($[M–H]^-$ at *m/z* 285.0405) with a fragment at m/z 119.

Finally, two lignans were putatively annotated, lariciresinol-glucopyranoside at 9.2 min, with $[M–H]^- = 521.2046$ and fragment ions at *m/z* 151 and 289, and secoisolariciresinol at 12.1 min with a $[M–H]^-$ at *m/z* 361.1657 and a characteristic fragment at *m/z* 165 (Table 2).

The annotated phenolic compounds provide a broader description of the phytochemical composition of *Annona cherimola* juices and supports their potential relevance as candidate matrices for fermented beverage development with bioactive potential. A previous study identified up to 14 polyphenol compounds in cherimoya pulp, with the composition depending on the cherimoya cultivar (Santos et al., 2016). These authors determined the phenolic profile of “Perry Vidal”, “Funchal”, and “Matheus” varieties. In agreement with our results, procyanidins found as dimers, trimers, and tetramers were the most abundant phenolic compounds in the cherimoya mesocarp.

3.4. Quantification of the phenolic content of Annona cherimola by LC-QToF

The quantification of the putatively annotated compounds in cherimoya fruits by LC-QToF showed that they contained approximately 1070-1203 mg of total phenolic compounds per liter based on semi-quantitative LC-ESI-QTOF estimates. It should be noted that the LC-ESI-QToF quantification was performed using available external standards and structurally related equivalents for compounds lacking authentic standards, particularly oligomeric procyanidins. Consequently, the reported concentrations should be considered approximate values suitable for comparing relative differences among samples rather than absolute concentrations. Therefore, LC-ESI-QToF results provide a more specific indication of phenolic profile stability than Folin–Ciocalteu values in the present matrix.

The most abundant group of polyphenols present in cherimoya juices was flavanols, mainly in oligomeric forms, representing more than 99% of the total quantified phenols, except for FChJ-CRL2030, which showed

approximately 96%. These compounds were preferentially present as oligomers, accounting for about 90% compared to approximately 10% of monomeric forms, and were mainly distributed as dimers (35-39%, type A + B), trimers (29-33%) and tetramers (15-20%) of the total flavanols. The next most abundant phenolic group identified in cherimoya juices was hydroxycinnamic acids, representing approximately 0.5% of the total phenolic content, except for FChJ-CRL2051, which showed around 0.3%. This group was dominated by caffeoylquinic acid isomers, accounting for 69-74% of total hydroxycinnamic acids, followed by caffeic acid glucoside.

Flavonols, lignans and simple phenolic acids were present in much lower amounts, with the three groups representing less than 0.2% of the total phenolic compounds.

Although the TPC values estimated by HPLC-ESI-QTOF remained similar among samples, supporting the overall stability of the phenolic composition independently of Folin–Ciocalteu estimates, fermentation produced only slight redistributions within the phenolic profile of cherimoya juice rather than major compositional changes. The most noticeable differences among strains were related to slight variations in the relative distribution of procyanidin oligomers based on semi-quantitative estimates within an otherwise largely preserved phenolic matrix, mainly involving changes in dimer, trimer and tetramer isomers in fermented juices compared with unfermented cherimoya juice (Table 3). Among strains, *L. rhamnosus* CRL2049 showed compositional changes consistent with possible strain-dependent metabolism of some procyanidin isomers during fermentation, increasing both dimer and tetramer isomers contents compared to NFChJ. By contrast, *Lpb. plantarum* CRL2051 showed lower relative levels of hydroxycinnamic acids after fermentation, reducing caffeolquinic acid and coumaric acid-*O*-glucosides, as well as epicatechin and procyanidin trimer isomers in FChJ. Therefore, FChJ-CRL2051 showed a slight, non-significant tendency toward lower total phenolic content. The three studied strains, *Lpb. plantarum* CRL2051 and CRL2030, and *L. rhamnosus* CRL2049, showed lower relative gallic acid levels and higher relative levels of procyanidin dimer type A isomers among flavanols, and secoisolariciresinol I among lignans.

It is well-known that LAB can metabolize (poly)phenolic compounds within fermented matrices through diverse biochemical pathways. These transformations are strictly dependent on the specific enzymatic repertoire of each strain, as well as the matrix pH, temperature, and time of fermentation, among others. Bacterial polyphenol metabolism involves enzymes such as decarboxylases, reductases, and glycosidases, which may contribute to modest strain-dependent redistributions of specific phenolic subclasses during fermentation. Significantly increased concentrations of procyanidin, catechin, and epicatechin content after 12 h of lactic grape fermentation have been reported (Wu et al., 2021). Also, fermentation of elderberry and cherry juices increased the phenolic content by lactic fermentation (Ricci, Cirlini, Calani et al., 2019; Ricci, Cirlini, Maoloni et al., 2019). Decarboxylation of hydroxycinnamic acids was previously reported by *L. plantarum* and *L. rhamnosus* during food fermentations, predominantly reducing caffeic acid and p-coumaric acid to their respective dihydro-derivatives, in agreement with strain-dependent compositional trends observed here (Filannino et al., 2015; Ricci, Cirlini, Maoloni et al., 2019).

Flavonoids are among the most abundant classes of dietary polyphenols found in plant-derived foods, and flavan-3-ols, including catechins and proanthocyanidins, constitute a major subclass within this group, particularly in many fruits and vegetables (Thompson et al., 2024).The abundance of procyanidin oligomers in cherimoya is remarkable. Procyanidins intake has been associated with reported health-related effects in experimental and epidemiological studies, especially to alleviate pathologies associated with metabolic syndrome, such as type 2 diabetes and obesity (Li, X. et al., 2016; Masumoto et al., 2018). Although gut microbiota can biotransform procyanidin oligomers into simpler phenolic forms (Pierre et al., 2013), it appears that LAB are generally tolerant to their presence, while only a limited

Table 3
Content of individual phenolic compounds of *Anonna cherimola* juices.

Identified Compound	NFChJ	FChJ-CRL2051	FChJ-CRL2030	FChJ-CRL2049
HIDROXICINNAMIC ACIDS AND HIDROXICINNAMATES				
Caffeoylquinic acid	1.94	0.51	1.94	1.94
Caffeic acid	ND	ND	ND	0.31
Caffeic acid-*O*-glucoside	0.68	0.58	0.58	0.62
Caffeic acid-*O*-glucoside	0.02	ND	0.02	0.06
Coumaric acid-*O*-glucoside	0.70	0.39	0.33	0.33
Caffeoylquinic acid	2.06	1.61	1.71	2.01
Total	**5.41** (0.47 %)	**3.09** (0.29 %)	**4.58** (0.43 %)	**5.27** (0.44 %)
OTHERS SIMPLE PHENOLIC ACIDS				
Gallic acid	0.15	0.05	0.05	0.08
Protocatechuic acid	0.16	0.22	0.20	0.18
Total	**0.28** (0.02 %)	**0.27** (0.02 %)	**0.25** (0.02 %)	**0.26** (0.02 %)
FLAVANOLS				
Catechin	2.93	3.06	2.93	3.35
Epicatechin	77.93	63.51	66.85	71.37
Gallocatechin	1.82	1.98	1.28	1.54
Epicatechin-*O*-glucoside	0.72	0.91	1.04	0.97
Procyanidin dimer type B	77.75	79.01	71.75	78.39
Procyanidin dimer type B	25.33	29.29	28.95	28.03
Procyanidin dimer type B	375.70	351.27	337.22	347.62
Procyanidin dimer type B	13.54	11.86	12.25	12.33
Procyanidin dimer type B	22.41	22.66	23.17	23.48
Procyanidin trimer type B	35.16	36.53	35.58	38.53
Procyanidin trimer type B	30.71	32.70	33.83	33.49
Procyanidin trimer type B	232.49	191.16	217.4	226.15
Procyanidin trimer type B	15.40	16.85	16.00	18.78
Procyanidin trimer type B	47.92	42.33	53.07	50.96
Procyanidin trimer type B	9.73	7.34	8.18	9.18
Procyanidin tetramer type B	10.67	11.70	11.16	14.29
Procyanidin tetramer type B	19.63	22.71	20.02	24.91
Procyanidin tetramer type B	95.69	96.24	99.54	125.49
Procyanidin tetramer type B	11.04	14.29	12.89	23.41
Procyanidin tetramer type B	26.41	27.66	28.79	31.72
Procyanidin tetramer type B	13.17	14.65	14.58	22.18
Procyanidin dimer type A	3.42	4.33	4.97	5.05
Procyanidin dimer type A	2.40	3.08	3.53	5.19
Total	**1152.00** (99.4 %)	**1085.16** (99.6 %)	**1068.42** (96.2 %)	**1196.43** (99.4 %)
FLAVONOLS				
Calabricoside A	0.16	0.12	0.12	0.16
Quercetin-rutinoside	0.33	0.28	0.28	0.37
Rutin	0.11	0.13	0.14	0.14
Kaempferol	0.03	0.04	0.02	0.02
Total	**0.63** (0.05 %)	**0.57** (0.05 %)	**0.56** (0.05 %)	**0.65** (0.05 %)
LIGNANS				
Lariciresinol-glucopyranoside	0.28	0.29	0.31	0.26

(*continued on next page*)

Table 3 (*continued*)

Identified Compound	NFChJ	FChJ-CRL2051	FChJ-CRL2030	FChJ-CRL2049
Secoisolariciresinol I	0.10	0.19	0.20	0.21
Secoisolariciresinol II	0.21	0.20	0.21	0.24
Total	**0.59** (0.05 %)	**0.68** (0.06 %)	**0.72** (0.06 %)	**0.71** (0.06 %)
TOTAL POLYPHENOLS (mg/L)	**1158.91**	**1089.77**	**1110.63**	**1203.32**

number of strains are able to metabolize them during fermentation (Tabasco et al., 2011). Consistent with this and after lactic fermentation of ChJ, mainly simpler phenolics and a limited number of oligomeric forms showed strain-dependent variations within an otherwise conserved phenolic profile, without evidence of major restructuring of the overall phenolic composition. Importantly, the chemical structure of polyphenols has been related to enzyme inhibitory effects in experimental systems; however, the present correlations do not allow predictive structure–function conclusions due to the limited number of observations. In this context, it has been reported that a higher degree of polymerization of proanthocyanidins may enhance the inhibitory activity against α-glucosidase and α-amilase (Ćorković et al., 2022).

To evaluate the relation between changes on main polyphenols compounds and inhibition of carbohydrate-digesting enzymes, correlation and linear regression analyses revealed statistically significant associations between specific flavan-3-ols and enzyme inhibitory responses within the limited dataset analyzed between specific flavan-3-ols and the inhibitory responses of α-amylase and α-glucosidase. The statistically significant associations are presented in Table 4. Epicatechin content was positively correlated with IC_{50} values for α-glucosidase ($r = 0.99$, $p < 0.01$, $R^2 = 0.98$) and α-amylase ($r = 0.96$, $p < 0.05$, $R^2 = 0.93$), suggesting a possible inverse association between epicatechin levels and inhibitory potency within the experimental conditions evaluated. Procyanidin dimer B (4) also exhibited positive correlations with IC_{50} values for α-amylase ($r = 0.99$, $p < 0.01$, $R^2 = 0.98$) and α-glucosidase ($r = 0.98$, $p < 0.05$, $R^2 = 0.97$), consistent with a similar association pattern within the experimental dataset. In contrast, procyanidin dimer B (2) showed strong negative correlations with IC_{50} values (α-amylase: $r = -0.96$, $R^2 = 0.93$, $p < 0.05$; α-glucosidase: $r = -0.99$, $R^2 = 0.98$, $p < 0.01$), consistent with a stronger association with lower IC_{50} values within the experimental conditions evaluated. Other phenolic constituents displayed higher correlations without significant associations and, therefore, cannot be considered reliable predictors under the present experimental conditions, due to the limited number of observations included in the analysis. These exploratory associations suggest that specific flavan-3-ols may contribute to differences in the inhibition of α-amylase and α-glucosidase activities under the experimental conditions evaluated, suggesting that changes in individual flavan-3-ols during fermentation may be associated with differences in inhibitory activity of ChJ. These correlations should be interpreted cautiously, as they were calculated from only four juice conditions, and they should be interpreted as hypothesis-generating trends rather than as evidence supporting predictive structure–function relationships.

Table 4
Correlation of phenolic compounds with α-amylase and α-glucosidase IC_{50} values*. Correlation coefficients were obtained from four experimental conditions and therefore provide exploratory association patterns rather than robust predictive relationships.

Compounds	α-amylase IC_{50}		α-glucosidase IC_{50}	
	r Pearson	*p*	r Pearson	*p*
Hidroxicinnamic Acids and Hidroxicinnamates	0.814	0.186	0.804	0.196
Simple Phenolic Acids	0.493	0.507	0.595	0.405
Total Flavannols	0.457	0.543	0.590	0.410
Epicatechin	0.965*	0.035	0.991**	0.009
Procyanidin dimer type B (2)	−0.964*	0.036	−0.991**	0.009
Procyanidin dimer type B (4)	0.990**	0.010	0.982*	0.018
Procyanidin trimer type B (3)	0.857	0.143	0.837	0.163
Procyanidin trimer type B (6)	0.901	0.099	0.930	0.070

* Two-tailed test; $p < 0.05$ (*significant). $p < 0.01$ (**highly significant).

3.5. Sensory analysis

A hedonic sensory analysis was conducted by 105 untrained panelists, who were asked to compare NFChJ and FChJ-CRL2051, the latter selected based on its higher α-glucosidase inhibitory activity in comparison to the other fermented juices, in order to determine whether fermentation with strain CRL2051 had affected sensory perception, and to evaluate its potential market success. NFChJ and FChJ-CRL2051 juices were scored similarly for the seven evaluated attributes: colour, brightness, odour, acidity, astringency, texture, and taste, as well as overall acceptability (Fig. 3A). There were no significant differences in mean scores for any attribute between both juices, under the evaluated refrigerated post-fermentation storage conditions. In all cases, the scores ranged from 5.1 to 6.6, corresponding to “like moderately”.

Preference analysis (Fig. 3B) showed that 43% of participants favoured NFChJ, 31% preferred FChJ-CRL2051, and 27% expressed no clear preference. However, no statistically significant differences were observed in overall acceptability scores between the two evaluated juices. This indicates that fermentation did not significantly modify overall sensory acceptance under the conditions tested and suggests that both formulations were comparably well-received by the panellists. However, the preference distribution hints a modest tilt toward the unfermented formulation that should be considered when interpreting the sensory results. In addition, in a real market context, consumers who choose a fermented beverage are often motivated not only by sensory attributes but also by the expectation of added functional or health-related benefits associated with fermentation. Taken together, these aspects support the potential acceptability of FChJ-CRL2051 as a fermented cherimoya juice formulation under the conditions evaluated, while acknowledging the observed preference tendency.

Pearson's correlation coefficients were calculated to evaluate linear relationships among sensory attributes in NFChJ and FChJ-CRL2051 (Fig. 4). Most sensory attributes showed significant positive correlations in both juices, except for brightness vs. acidity, and brightness vs. astringency in NFChJ, which exhibited the weakest and non-significant correlations. Interestingly, no significant differences were detected when correlation patterns were compared between NFChJ and FChJ-CRL2051.

Colour and brightness were strongly correlated within each juice and showed a non-significant tendency toward higher correlation values in the fermented juice ($r = 0.723$) compared to the unfermented juice ($r = 0.663$) (Fig. 4). In line with this trend, increased lightness and colour intensity have been reported in LAB-fermented fruit beverages, often associated with changes in polyphenol content (Kwaw et al., 2018). Notably, correlations between these visual attributes (colour and brightness) and the other sensory variables (odour, acidity, astringency, texture, taste and overall acceptability) were consistently below 0.5 in both juices. In addition, odour displayed low correlation coefficients (below 0.5) with most sensory attributes in both juices. The only exceptions were its correlations with texture in NFChJ ($r = 0.534$) and with colour in FChJ-CRL2051 ($r = 0.523$). Although no significant differences in odour perception were found between NFChJ and FChJ-CRL2051, these results suggest a limited contribution of odour variability to differences in perceived sensory quality between the two juices under the evaluated conditions.

The correlations for acidity, astringency, texture, taste, and overall acceptability were all higher than 0.5 in both juices (Fig. 4A and B). Interestingly, overall acceptability exhibited the strongest correlation with taste ($r = 0.873$ and $r = 0.815$ for NFChJ and FChJ-CRL2051,

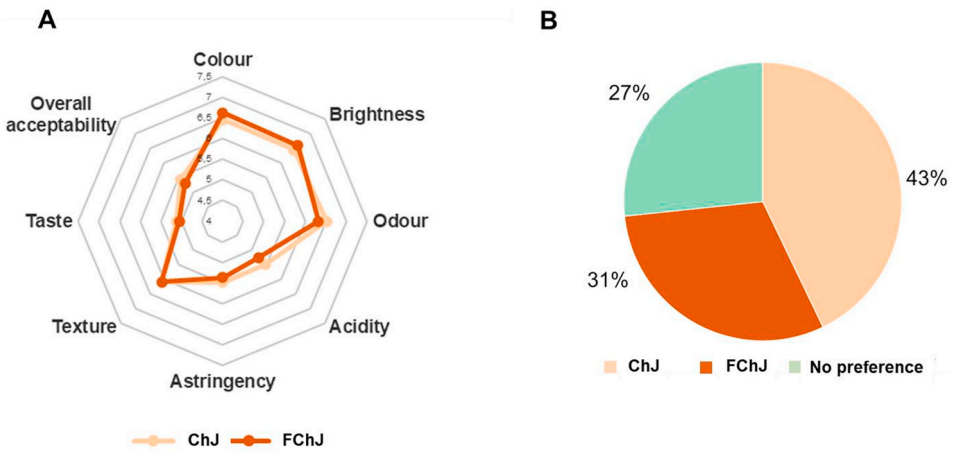


Fig. 3. (A) Overall sensory evaluation of unfermented (ChJ) and fermented (FChJ-CRL2051) cherimoya juices. Colour, brightness, odour, acidity, astringency, texture, taste, and overall acceptability correspond to 9-point hedonic evaluation (1, "dislike extremely"; 9 "like extremely"). (B) Preference test for NFChJ and FChJ-CRL2051. Percentage of participants who gave a higher score to one juice than the other for the overall acceptability.

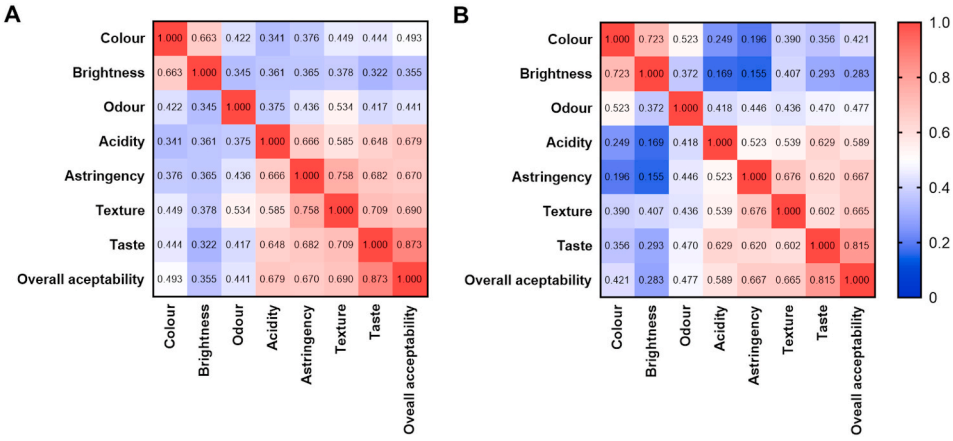


Fig. 4. Pearson's correlations amongst the scores for the attributes evaluated in the sensory analysis of (A) unfermented cherimoya juice (NFChJ) and (B) fermented cherimoya juice by *Lpb. plantarum* CRL2051 (FChJ-CRL2051).

respectively), in agreement with previous studies on fermented juices (Espirito-Santo et al., 2015; Rizzi et al., 2024). More importantly, fermentation with CRL2051 did not significantly modify overall sensory acceptance relative to the unfermented juice.

3.6. Principal component analysis (PCA)

PCA explained 72.17% of the total variance, with PC1 and PC2 accounting for 56.32% and 15.85%, respectively (Fig. 5), and was used as an exploratory visualization tool to examine relationships among sensory attributes. PC1 was responsible for the maximum variability, with an eigenvalue greater than 4, and all sensory attributes loading positively on this component.

Several sensory attributes displayed strong correlations, as indicated by small angles between vectors. In particular, there was a close relationship between colour and brightness, as well as between astringency and acidity, and between taste and overall acceptability. These patterns suggest possible covariation among sensory attributes within the exploratory PCA framework. In addition, colour, brightness, and odour were placed on the positive side of PC1, while all the other attributes were mainly on the negative side of PC2 (Fig. 5). Thus, variations in astringency, acidity, texture, and taste appeared to contribute to variability in the spatial distribution of samples within the exploratory PCA representation. Overall acceptability showed the strongest association with taste within the PCA representation, in agreement with the results shown in Fig. 5. It is worth noting that the samples did not form any clusters, indicating high variability in individual perceptions of the two beverages, as previously shown for other fruit-based juices (Bhalerao et al., 2020). Because samples did not form clearly separated clusters and individual variability was high, PCA results should be interpreted as descriptive visualization rather than inferential evidence of sensory differentiation between samples.

4. Conclusions

As the global consumption of fruits and vegetables increases, there is a real demand for novel foods, especially those based on highly perishable fruits. This work aimed to evaluate the effect of lactic fermentation by three LAB isolated from fruits on the phenolic profile, antioxidant capacity and *in vitro* inhibitory against carbohydrate-digesting enzymes, and the sensory attributes of cherimoya juice. The results suggest that lactic fermentation is a feasible strategy for developing novel fermented cherimoya-based beverages, maintaining a largely preserved procyanidin-dominated phenolic profile and antioxidant activity while enhancing the *in vitro* inhibitory activity against α-amylase and α-glucosidase relative to the unfermented juice, which may contribute to delaying starch digestion under *in vitro* conditions. The results suggest that *Lpb. plantarum* CRL2051 may represent a suitable candidate strain for fermented cherimoya juice under the experimental conditions evaluated. Finally, it is worth noting that

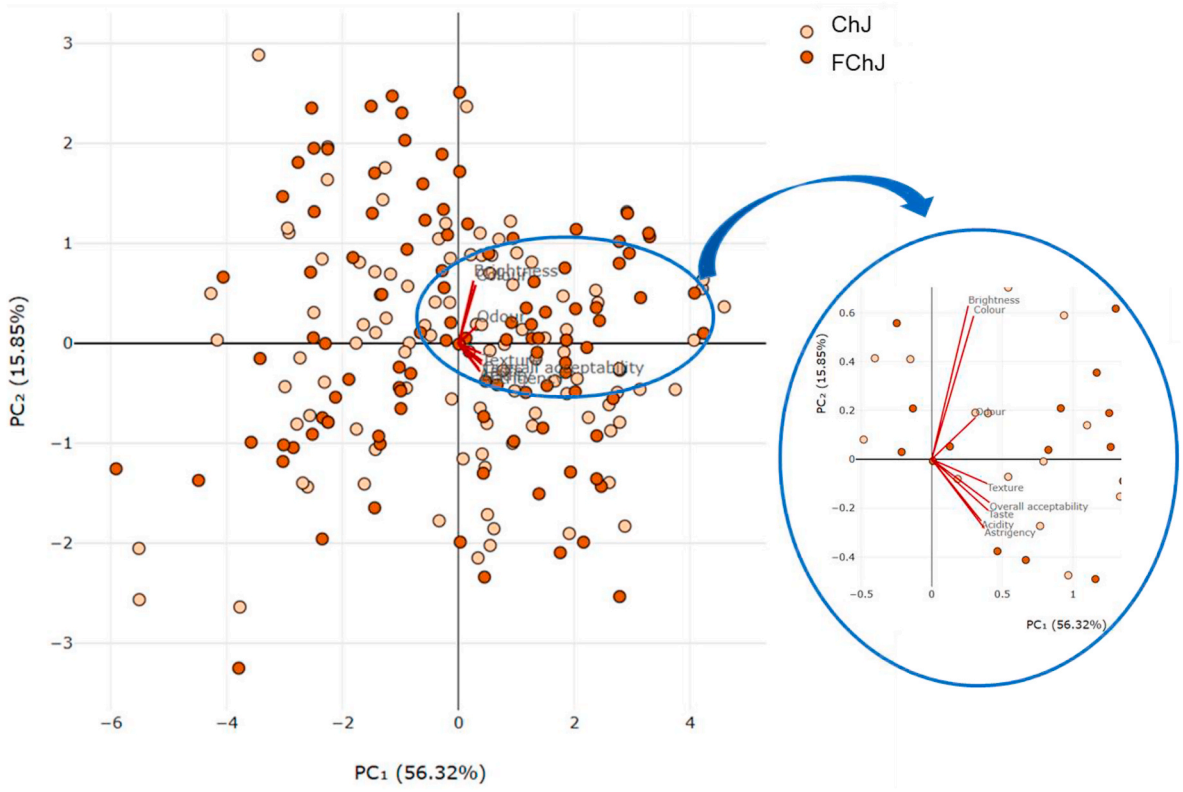


Fig. 5. PCA biplot showing the distribution of sensory attribute scores along the first two principal components (PC1 and PC2) for ChJ and FChJ-CRL2051. The representation is intended as exploratory visualization of attribute relationships rather than inferential separation between samples.

fermentation with CRL2051 maintained sensory acceptability at levels statistically comparable to the unfermented juices, and both fermented and unfermented cherimoya juices were well-accepted by the panelists. Further studies, particularly *in vivo* experiments, are required to confirm the physiological relevance of the enzyme inhibitory activity observed *in vitro*.

CRediT authorship contribution statement

Carina Van Nieuwenhove: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sonia Ramos:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Natalia García-Acosta:** Visualization, Supervision, Software, Methodology, Data curation. **Guido Van Nieuwenhove:** Writing – review & editing, Visualization, Supervision, Software, Data curation. **Ana Sofía Isas:** Validation, Supervision, Software, Methodology, Formal analysis. **Fernanda Mozzi:** Writing – review & editing, Visualization, Supervision, Investigation, Conceptualization. **Raquel Mateos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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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Data availability

Data will be made available on request.

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