



# Impact of juice processing of Costa Rican guava (*Psidium friedrichsthalianum*) on the physicochemical properties, total phenols and antioxidant capacity

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## ABSTRACT

*Psidium* plants are commonly consumed in processed forms, leading to a need to evaluate the impact of processing on bioactive compounds. The purpose of this study is to assess the effect of Costa Rican guava juice processing on physicochemical and antioxidant characteristics. Five samples corresponding to distinct phases in the juice processing were evaluated. Total polyphenols were measured using the Folin-Ciocalteu method, while antioxidant activity was assessed using various methodologies, including chemical methods (DPPH and ORAC) and cell assays with kidney cell cultures, liver homogenates, and erythrocytes. Results revealed variations in the impact of the juice processing stages on total polyphenols and for each antioxidant assay, evidencing that different polyphenols counteract in each technique. Significant decreases (ranging from 27 % to 58 %,  $p < 0.05$ ) were primarily observed during the pressing stage, while thermal treatments resulted in non-significant reductions. This study demonstrated a decline in antioxidant activity during the processing of Costa Rican guava juice underscoring the need for continued efforts to optimize processing conditions and enhance the retention of health-promoting properties in the juice.

## 1. Introduction

The *Psidium* genus comprises a diverse range of 150 species of shrubs and fruit-yielding trees that are cultivated in tropical and subtropical regions worldwide. However, only a few of the *Psidium* species are commercially exploited. The most widespread species used in the food industry is *P. guajava*, commonly known as guava (Menezes et al., 2020). Other *Psidium* species, such as *P. friedrichsthalianum* (Costa Rican guava), *P. cattleianum* (strawberry guava) and *P. guineense* (Brazilian guava), are also produced and consumed but in smaller quantities (Mani et al., 2011).

*Psidium* fruits are a rich source of several vitamins, minerals, carotenoids, and polyphenols, which are micronutrients and bioactive substances that increase their dietary value. Some of the biological properties reported for these compounds include antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, anti-tumoral, hepatoprotective and cardioprotective (Hall et al., 2023; Trujillo-Correa et al., 2019; Upadhyay et al., 2018). However, most of these studies had been mainly performed on *P. guajava* and not on other *Psidium* species.

In recent years, *Psidium friedrichsthalianum* has begun to be studied. This species is commonly called Costa Rican guava or sour guava. The fruit has a particularly good/appealing flavor due to its intense and pleasant aroma joined with its highly acidic taste. A detailed phenolic characterization of Costa Rican guava had identified more than 100 phytochemicals, highlighting as main compounds B-type proanthocyanidins derived from (*epi*) catechin units, corresponding to a 31 % of total polyphenols. Other important compounds reported are ellagitannins, such as vescalagin and geraniin, flavonols, mainly quercetin (Rojas-Garbanzo et al., 2019) and benzoic acid derivatives (Cuadrado-Silva et al., 2017b).

Limited information is currently available regarding the potential health effects of Costa Rican guava. However, few studies have reported its antioxidant activity, anti-inflammatory properties, and anti-platelet activity, primarily attributed to the presence of polyphenols and vitamin C (Cuadrado-Silva et al., 2017b; Flores et al., 2013; Rojas-Garbanzo et al., 2021). Furthermore, the essential oils extracted from the fruits and leaves of sour guava have shown anti-microbial activity (Granados-Chinchilla et al., 2016), and there are reports of leaf infusions used to treat cough (González-Ball et al., 2022). Recently, the bioaccessibility of phenolic compounds of Costa Rican guava has been

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studied using an *in vitro* digestion model. Results demonstrated that half of the total polyphenols are non-extractable and only in the colon would be potentially accessible. (Durán-Castañeda et al., 2023). Finally, an *in vivo* model with rats evaluated the urinary metabolites excreted after Costa Rican guava juice consumption. This research evidenced a decrease in the excretion of lactate and Krebs Cycle intermediates, suggesting a potential to modulate redox balance by Costa Rican guava bioactive compounds (Montoya-Arroyo et al., 2020).

Costa Rican guava fruit is rarely consumed fresh, as it is primarily utilized for preparing homemade and commercially produced beverages and sorbets (Cuadrado-Silva et al., 2017). Because of this reason, it becomes important to conduct studies on this fruit to evaluate the impact of processing on its potential health-promoting bioactive compounds and their activities. The industrial processing of fruits to produce juice involves procedures such as pressing, enzymatic maceration, or thermal treatment, all of which may affect the bioactive components and sensorial quality (Saikia et al., 2015). Consequently, this study aims to evaluate the impact of different stages of juice processing on the physicochemical properties, the total phenols, and the antioxidant capacity. Particularly the antioxidant activity has not been described previously. Studies on Costa Rican guava have focused attention on changes in the polyphenol profile and concentration due to juice processing (Rojas-Garbanzo et al., 2019). Conversely, this study is focused on the impact of juice processing on the antioxidant capacity, using not only chemical traditional assays, but even more, models that resemble the biological context.

## 2. Materials and methods

### 2.1. Chemicals

Solvents utilized for polyphenol extraction were acquired from JT Baker (Griesheim, Germany). *Tert*-butyl hydroperoxide (TBHP), quercetin, thiobarbituric acid (TBA), 2,2-azobis-2-methyl-propion-amidine-dihydrochloride (AAPH), 2,2 diphenyl-1-picrylhydrazyl (DPPH), 2,7-dichlorodihydro fluorescein diacetate (DCFDA) and fluorescein were purchased from Sigma Aldrich (St. Louis, MO, USA). The reagents utilized for cell culture: fetal bovine serum, L-glutamine, trypsin, streptomycin, MEM medium, and penicillin were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

### 2.2. Experimental design

Costa Rican guava juice was prepared and five samples representing different stages were collected: Pulp (P), Pressed Juice (PJ), Microfiltrated Juice (MJ), Pasteurized Pressed Juice (PPJ) and Pasteurized Microfiltrated Juice (PMJ). Each sample was characterized by physicochemical properties (moisture, total soluble solids, and pH), total polyphenols and their antioxidant capacity was determined using chemical methods and a cellular culture model. Additionally, the inhibition of lipid peroxidation capacity was assessed using two assays with biological models in liver homogenates and erythrocytes.

Comparisons were performed for the results of each assay, to determine the effect of juice processing. The pulp sample served as the control (unprocessed sample) for comparing pressed and microfiltrated juices, while for pasteurized juices, comparison was made with their respective non-pasteurized counterparts. Finally, correlations between total polyphenols and each antioxidant assay were also examined.

### 2.3. Plant material

Three batches of 10 Kg each of Costa Rican guava (*Psidium friedrichsthalianum*) were purchased fresh from local farmers in the regions of Valle de Orosi, Cartago, Costa Rica (altitude 1077 m, latitude 9°48'0" N, 83°51'0" W). According to habitual agronomic practices, Costa Rican

guava fruits were collected when the fruit color is from light green to yellow and naturally detach from the tree. The fruits have its typical globose shape of 3 to 6 cm in diameter. Fruits were transported in buckets for one hour without refrigeration to the laboratory where overripe fruits were eliminated manually, and the remaining fruits were washed with a sodium hypochlorite solution for food industry applications (200 ppm/3 min) and were frozen at  $-20^{\circ}\text{C}$ . Peeling operations were not necessary because Costa Rican guava has a soft peel that is regularly consume with the fruit. The frozen fruits were used for the preparation of pulp and two types of juices using a pilot scale according to the process flow shown in Fig. 1.

### 2.4. Sample preparation

Costa Rican guava was ground by a food grinder, mixed to ensure a uniform lot, and used to prepare pulp and juices following a previously established protocol (Soto et al., 2016). To obtain the pulp, ground fruit was finished using a pilot scale finisher with 1.5 mm and 0.8 mm sieves consecutively, and the first sample was collected in this step (**Pulp - P**). For juice production, the ground fruit was pressed and macerated with 75 ppm of a commercial enzymatic preparation, Pectinex® (Novozymes, Bagsvaerd, Denmark) for 60 min at  $35^{\circ}\text{C}$ . This enzymatic maceration was carried out in a double-jacketed steam kettle, (Groen™, United States) with a capacity of 40 kg, with manual agitation every 5 min. Then, for a discontinuous pressing, it was used a water press (Enotecnica Pillan, Italy) with a volume capacity of 40 L and operated under water pressure. The fruit was enclosed in a cheesecloth bag and pressed at 3 bar for 10 min to obtain the second sample (**Pressed Juice - PJ**). Later, the pressed juice was microfiltrated in a tubular ceramic membrane Membralox® 1 P19-40 (Pall Exekia, Bazel, France), with an average pore size of  $0.2\ \mu\text{m}$ , an average cross-flow velocity of  $7\ \text{m/s}$ , a transmembrane pressure fixed at  $2.3 \pm 0.3\ \text{bar}$  and temperature of  $35 \pm 1^{\circ}\text{C}$  (Sandri et al., 2011; Vaillant et al., 2001), the juice obtained in this step was considered the third sample (**Microfiltrated Juice - MJ**).

Finally, the pressed and microfiltrated juices were pasteurized at a laboratory scale using an electric heater with a 1.7 L capacity and manually agitation. The temperature was monitored with a thermometer, and the conditions applied were  $92^{\circ}\text{C}$  for 3 min. This process yielded two more samples (**Pasteurized Pressed Juice - PPJ** and **Pasteurized Microfiltrated Juice - PMJ**). The selected temperatures for pasteurization represent conditions commonly used for fruit juices in previous publications (Mena et al., 2013; Santhirasegaram et al., 2013).

As a result of the above-described protocol, five samples were obtained: pulp (P), pressed juice (PJ), microfiltered juices (MJ), pasteurized pressed juice (PPJ) and pasteurized microfiltrated juice (PMJ).

### 2.5. Polyphenol purification

The pulp (P) and juice samples (PJ and PPJ) containing solids were subjected to extraction using acetone and water (70:30) with agitation and ultrasound for 10 min. Next, the mixture was filtered to remove the solids, and the liquid fraction was concentrated using a rotary evaporator to remove acetone ( $37^{\circ}\text{C}$ ). Once the acetone was removed, the resulting aqueous fraction was filtered using a syringe filter with a pore size of  $0.45\ \mu\text{m}$ , and the filtrated was reconstituted in water to reach the original volume. The samples without solids (MJ and PMJ), were directly utilized without undergoing the acetone extraction (Gancel et al., 2011). All five aqueous solutions of Costa Rican guava samples were frozen at  $-80^{\circ}\text{C}$  and reserved for further analysis.

### 2.6. Total phenolic quantification by Folin -Ciocalteu

The total phenolic content of all samples was determined using the modified Folin-Ciocalteu assay (Berker et al., 2013). For this purpose,

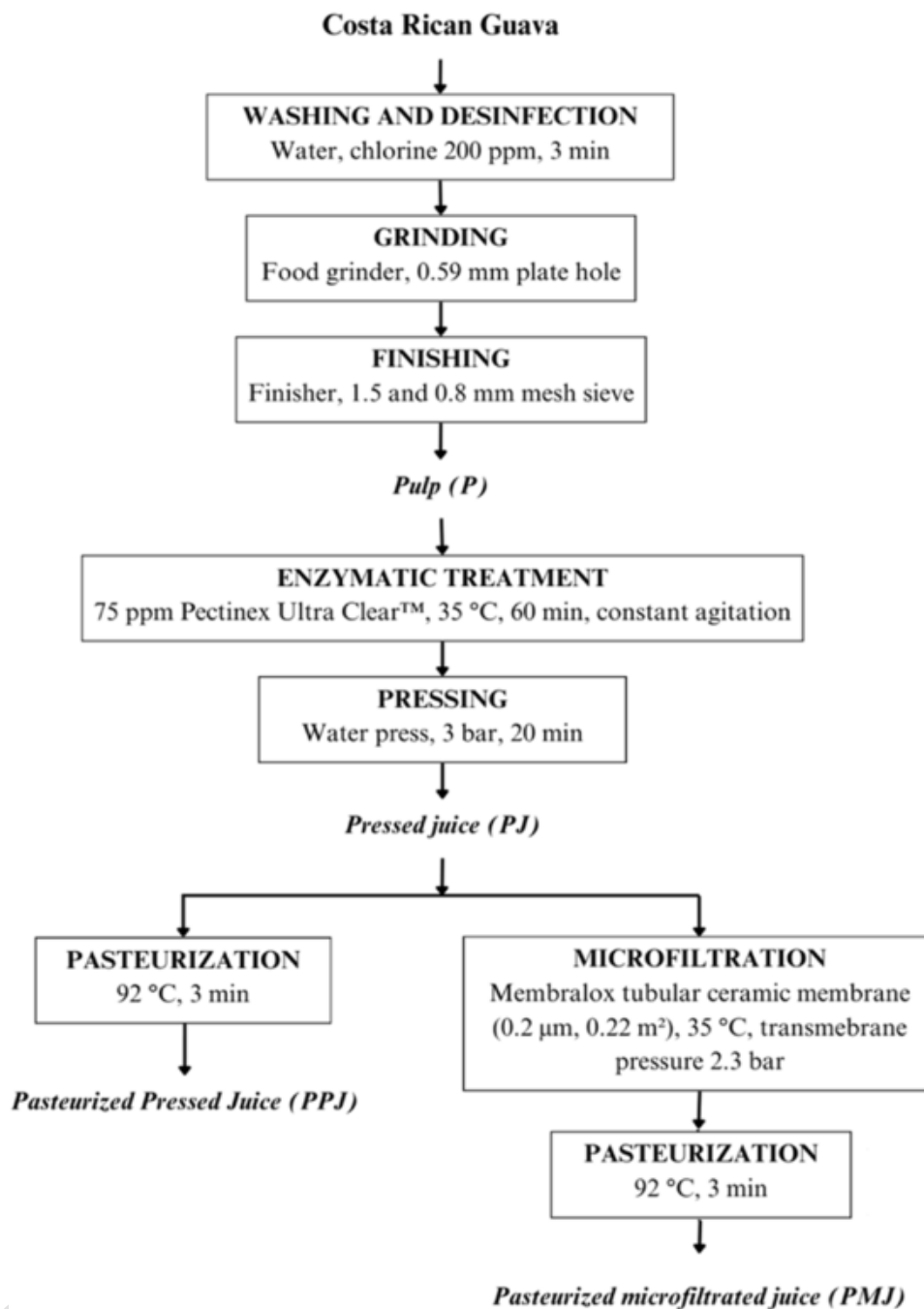


Fig. 1. Flow diagram for the preparation of Costa Rican guava juice samples.

0.25 mL of each sample was mixed with 1.25 mL of Folin Ciocalteu reagent and incubated at room temperature for 2 min. Later, 2 mL of sodium carbonate (75 g/L) was added to the mixture and further incubated at 50 °C for 15 min. After cooling, the absorbance was measured at 760 nm (Pharmaspec UV-Shimadzu, Kyoto, Japan). The quantification of phenolic compounds was performed using a calibration curve of gallic acid. Additionally, corrections were made to account for any interference caused by the juice components. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry matter. Each sample was analyzed in triplicate.

### 2.7. Physicochemical analysis

The values of moisture content of each sample were determined using standard AOAC methods (AOAC, 2005). In brief,  $20 \pm 5$  g of samples were weighed accurately and dried at 70 °C under a pressure between 50.8 - 84.6 kPa until consecutive weighing made at 2 h intervals varied by less than 3 mg. Total soluble solids were measured using an Abbe benchtop refractometer (Fisher Scientific Japan Ltd, Tokyo, Japan) with temperature control. Samples pH was determined using a Metrohm 827 Lab Meter (Metrohm AG, Herisau, Switzerland). Moisture values were used to express the results of phenolic content, antioxidant activity, and inhibition of lipid peroxidation assays in  $\mu\text{g}$  of dry matter (DM) per milliliter.

## 2.8. Antioxidant activity assays

### 2.8.1. DPPH radical scavenging activity (RSA)

The scavenging activity of Costa Rican guava samples against DPPH free radicals was measured in a reaction containing 0.5 mL of 0.25 mM DPPH solution dissolved in methanol and 1 mL of a diluted sample (ranging from 20 to 150 µg DM/mL). The reaction was agitated and allowed to incubate in darkness at room temperature for 30 min. A control reaction (without sample) and a sample blank (without DPPH) were prepared for every concentration to eliminate color interference. Following the incubation period, the absorbance of the reaction mixture was measured at 517 nm. The percentage of radical scavenging activity (% RSA) was estimated by the following equation: % RSA =  $[1 - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) / \text{Abs}_{\text{control}}] * 100$  (Azofeifa et al., 2015).

The % RSA was plotted against the sample concentration to calculate the IC<sub>50</sub>, defined as the concentration (expressed as µg DM/mL) that produces 50 % of radical scavenging activity. A low IC<sub>50</sub> indicates elevated radical scavenging activity. The % RSA was carried out in three independent experiments. For each experiment, the concentrations of the sample were analyzed in triplicate.

### 2.8.2. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed using fluorescein as a fluorescent probe, and oxidation was induced with AAPH. The Costa Rican guava samples were diluted in water and a calibration curve of trolox was used with concentrations range from 4 - 31 µmol/L. Assays were performed using spectrofluorimeter equipment (Biotek Instruments, Winooski, VT, USA). ORAC values were expressed as the mmol of trolox equivalents per gram of polyphenol extract (mmol TE/g of extract). Samples were analyzed in triplicate. (Azofeifa et al., 2015)

### 2.8.3. Inhibition of intracellular radical oxygen species (ROS)

The measurement of intracellular ROS inhibition was evaluated using the fluorescent probe 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) (Azofeifa et al., 2015). DCFH-DA can penetrate the cells, and once in the cytosol, it is cleaved by intracellular esterases into a non-fluorescent molecule (DCFH). Later, when exposed to the reactive oxygen species induced by tert-butyl hydroperoxide (TBHP), the probe is oxidized and becomes a highly fluorescent product (DCF). The fluorescence of DCF can be assessed using flow cytometry.

Monkey normal epithelial kidney cells (Vero) were cultivated in minimum essential Eagle's medium (MEM) supplemented with 10 % fetal bovine serum (FBS). The grown medium also contained 2 mmol/L glutamine, 100 IU/mL penicillin, 100 µg/L-streptomycin, and 0.25 µg/mL amphotericin B.

A 48-well plate was utilized to seed 1 mL of Vero cell suspension with a density of  $4 \times 10^5$  cells/mL. The cells were allowed to adhere for 4 h, after which they were treated with various concentrations of the samples (ranging from 50 to 1800 µg DM/mL) for 20 h. Following the incubation, the cells were washed twice with PBS and 4.2 µL TBHP (0.7 mM) was added for 2 h to induce oxidative stress. Thirty min before the end of the oxidative induction, 300 µL of DCFDA probe (5 µM) was added to each well. Then, cells were washed twice with PBS, trypsinized and % DCF positive cells were determined using a flow cytometer (FACSCalibur, Becton-Dickinson, Franklin Lakes, New Jersey, USA). Cell Quest program was used to count and analyze 10,000 events for each dosage treatment.

The % DCF positive cells were plotted against sample concentration to calculate the IC<sub>50</sub>, defined as the concentration (expressed as µg DM/mL) that decreased 50 % of DCF positive cells. A low IC<sub>50</sub> indicates elevated inhibition of intracellular ROS. Control wells were prepared without a sample (100 % DCF<sup>+</sup> positive cells). The measurement was carried out in three independent experiments, where each experiment involved duplicate analysis of all concentrations of Costa Rican guava.

## 2.9. Inhibition of lipid peroxidation assays

### 2.9.1. Inhibition of lipid peroxidation in liver homogenates

This procedure was approved by the Institutional Committee for Care and Handling of Experimental Animals at the University of Costa Rica (CICUA N° 036-15). The measurement of Inhibition of lipid peroxidation in liver homogenates was evaluated as described previously (Azofeifa et al., 2015). First, five male Sprague-Dawley rats (220 ± 20 g) were anesthetized with CO<sub>2</sub> and decapitated. Next, the liver was extracted, homogenized in PBS (20 %) using an ultraturax T-25 (Ika-Labortechnik, Staufen, Germany), and centrifuged at 9000 x g for 15 min at 4 °C. Then, 750 µL of liver homogenate was mixed with 75 µL of various concentrations of each sample (ranging from 1 to 28 µg DM/mL) and incubated at 37 °C for 30 min. Finally, to induce oxidative stress, 0.5 mL of TBHP in a final concentration of 1.7 mM was added, and the mixture was further incubated at 37 °C for 1 h.

The final products of the lipid peroxidation (TBARS) were measured by combining 0.25 mL liver mixtures with 0.25 mL of 35 % TCA (v/v) and 0.25 mL of Tris-HCl buffer (50 mM, pH 7.4). After an incubation of 10 min at room temperature, 0.5 mL of 0.75 % TBA was added, and the mixture was then heated in boiling water for 45 min. When the mixtures reached room temperature, 0.5 mL of 70 % TCA was added, followed by vortexing and centrifugation at 2500 x g for 15 min. The absorbance of the supernatant was measured at 532 nm, and the concentration of TBARS was determined using the molar absorption coefficient of MDA ( $1.5 \times 10^5 \text{ cm}^{-1} * \text{M}^{-1}$ ). A sample blank was prepared for each sample concentration to eliminate any potential interference of color.

Results were expressed in nmol MDA/g of liver tissue. The MDA concentration was plotted against sample concentration to calculate the IC<sub>50</sub>, expressed as µgDM/mL of the sample that reduces 50 % of the liver lipid peroxidation. A low IC<sub>50</sub> indicates an elevated inhibition of peroxidation. The measurement was carried out for each sample in three independent experiments.

### 2.9.2. Erythrocyte cellular antioxidant activity (ERYCA)

ERYCA assay was described previously (Quesada et al., 2020). It relies on differences in turbidity between intact and lysed human erythrocytes solution. AAPH, a peroxy radical generator, enhances the lipid peroxidation of the erythrocytes membrane and induces hemolysis, leading to a decrease in absorbance at 700 nm. The presence of antioxidant compounds scavenges peroxy radicals and inhibits hemolysis.

In a 96-well microplate, 500 µL of a human erythrocyte 2 % in PBS were mixed with 100 µL of samples at various concentrations (ranging from 9.6 to 360 µg DM/mL), and 100 µg/mL AAPH (100 µM). The absorbance at 700 nm was measured every 5 min for 6 h at 37 °C using a Cytation 3 reader (BioTek Instruments, Winooski, VT, USA). Prior to each measurement, a gentle shaking of 15 s was performed. A standard calibration curve was constructed using quercetin. The area under the absorbance decay curve (AUC) was calculated for each sample using GraphPad Prism 5 software. The results of ERYCA assay were expressed as millimoles of quercetin equivalents per gram of sample. The assessment was conducted in three independent experiments, with each experiment including triplicate analyses of the different sample doses.

## 2.10. Statistical analysis

The results from each experiment represent the means ± standard error of at least three independent assays. To compare the differences between values of each sample, an analysis of variance (ANOVA) followed by a Tukey post hoc test was performed using GraphPad Prism 5 software. Differences were identified between the pulp sample and both pressed and microfiltrated juices based on the Tukey *post hoc* data. Additionally, comparisons were made between pasteurized juices and their respective non-pasteurized counterparts using the same Tukey



post hoc data. A statistical significance level of  $p < 0.05$  was used to determine if the results were significantly different.

Correlations between total polyphenols and biological activities were also calculated using the same software.

### 3. Results and discussion

#### 3.1. Physicochemical analysis and total phenol content

This study quantified the physicochemical characteristics and total polyphenols content at different stages of Costa Rican guava juice processing. The physicochemical properties of pulp and juice samples do not exhibit significant differences. Moisture content ranges from 86 to 92 g /100 g, pH level varies from 2.66 to 2.69, and total soluble solids fluctuate from 9.01 to 10.35 °Brix (Table 1). In a previous study, physicochemical values were reported for Costa Rican guava fruit, showing a moisture content of 82 g /100 g and a pH level of 2.87. The observed variance could be attributed to differences in the maturity stage of the fruit and agroclimatic conditions of the crops. In terms of total soluble solids, previous reports for Costa Rican guava presented values from 9.6 to 12.7 °Brix (Rojas-Garbanzo et al., 2019, 2021; Montoya-Arroyo et al., 2020). This variance in total soluble solids of the fruits and juices could be explained by the deposition of insoluble compounds at the membrane surface (fouling layer) during the juice processing (Soto et al., 2016).

Total polyphenol concentration from Costa Rican guava pulp and juice samples ranged from 36 to 69 mg GAE /g DM (Table 1). The processed samples exhibited a significant reduction in total polyphenol content compared to the pulp, PJ decreased by 47 % and MJ 39 %. As observed, this decrease is caused mainly during the pressing stage and is likely attributed to the presence of non-extractable compounds associated with the cell wall, which are retained only in the pulp (Reynoso-Camacho et al., 2018; Soto et al., 2016).

The decrease in phenolic contents due to juice processing has been documented for other fruits. For example, microfiltration was found to cause a significant reduction of 26 % in total polyphenols in pomegranate (Fischer et al., 2011) and a 43 % decrease in açai juices (Donato Machado et al., 2012). The variation in these findings can be attributed to factors such as the concentration and type of polyphenolic compounds present in the plant material, as well as the concentration and type of insoluble solids content and the maturity stage of the fruit (Horvitz et al., 2017). Additionally, the decrease in polyphenol content is influenced by the operating conditions of microfiltration, including crossflow velocity, transmembrane pressure, volumetric reduction ratio, and temperature (Soto et al., 2016).

Table 1 showed that thermal treatments applied to processed juices (PPJ and MPJ) did not result in a significant additional decrease in polyphenol concentration. The thermal stability of polyphenols during juice processing has been previously described for fruits like pomegran-

ate (Benjamin & Gamrasni, 2020; Fischer et al., 2013) and blackberry (Azofeifa et al., 2015). Additionally, some authors have suggested that thermal treatments can enhance the extractability of bioactive compounds from small pulp particles (Benjamin & Gamrasni, 2020).

Although Table 1 showed that total polyphenols were not affected by pasteurization, a previous study involving Costa Rican guava pressed juice reported a reduction in specific polar secondary metabolites due to temperature. The decrease in these metabolites exhibited variable patterns. For instance, in the case of ellagitannins, the castalagin isomers experienced a loss of over 40 %, while pedunculagin showed a significant increase of 36 %. Other ellagitannins such as geraniin and vescalagin isomers did not exhibit significant changes (Rojas-Garbanzo et al., 2019).

#### 3.2. Antioxidant activity

Commonly employed techniques for assessing antioxidant activity often involve measuring scavenging activity against free radicals. As shown in Table 2, the antioxidant activity of Costa Rican guava, assessed using chemical assays such as DPPH and ORAC, exhibited losses due to processing operations. Specifically, during the pressing and microfiltration stages, significant reductions of 37 % and 34 % respectively, were observed in the DPPH assay, and 27 % and 33 % respectively, in the ORAC assay, compared to the pulp sample. Consistent with the trend observed in total polyphenol content, the primary decrease in antioxidant activity occurred during the pressing stage. This inference is supported by the fact that MJ was prepared from PJ, yet it does not exhibit any further significant reduction compared to PJ. Similar decreases in antioxidant activity due to pressing and microfiltration have been documented in other studies involving different juices. For instance, orange prickly pear juice exhibited a 45 % reduction in antioxidant activity (Mejía & Yáñez-Fernandez, 2021), pomegranate juice showed a 36 % reduction (Severcan et al., 2020), and pineapple juice displayed a 12 % reduction (Arikrishnan & Keshav, 2023).

The observed reductions in the DPPH and ORAC scavenging activity in PJ and MJ juices suggest the presence of non-extractable polyphenols that are retained in the fruit or pulp and not transferred to the juices. These polyphenols could be linked and consequently retain in the cell wall materials (pectin, cellulose, lignin) during the pressing of fresh pulp (Soto et al., 2016). Additionally, enzymatic treatment during juice production could release polyphenol oxidase from vacuoles, leading to the oxidation of certain polyphenols (Queiroz et al., 2008). Furthermore, exposure to light, heat, and oxygen may contribute to the oxidation of compounds (Mejía & Yáñez-Fernandez, 2021).

The ORAC values presented in Table 2 demonstrate a non-significant reduction in antioxidant capacity due to the thermal treatments applied. Contrarily, the DPPH scavenging activity values show a significant additional reduction because of the thermal treatments applied (92 °C, 3 min). PPJ juice exhibited a 14 % decrease in antioxidant

**Table 1**  
Physicochemical characteristics and total polyphenol content of the Costa Rican guava (*P. friedrichsthalianum*) samples.

Sample	Moisture (g /100 g FW)	pH	TSS (°Brix)	TP (mg GAE /g DM)	
<i>Effect of pressing and microfiltration*</i>					
Pulp (P)	86.0 ± 1.5 <sup>a</sup>	2.68 ± 0.01 <sup>a</sup>	9.51 ± 0.26 <sup>a</sup>	69.8 ± 1.7 <sup>a</sup>	
Pressed Juice (PJ)	91.5 ± 1.6 <sup>a</sup>	2.69 ± 0.01 <sup>a</sup>	9.58 ± 0.26 <sup>a</sup>	36.6 ± 0.5 <sup>b</sup>	(-47 %) <sup>#</sup>
Microfiltrated Juice (MJ)	92.0 ± 1.7 <sup>a</sup>	2.67 ± 0.01 <sup>a</sup>	9.08 ± 0.25 <sup>a</sup>	42.3 ± 0.4 <sup>c</sup>	(-39 %) <sup>#</sup>
<i>Effect of pasteurization**</i>					
Pasteurized Pressed Juice (PPJ)	89.5 ± 1.6 <sup>a</sup>	2.67 ± 0.01 <sup>a</sup>	10.35 ± 0.28 <sup>a</sup>	39.6 ± 0.5 <sup>b</sup>	
Pasteurized Microfiltered Juice (PMJ)	89.6 ± 1.6 <sup>a</sup>	2.66 ± 0.01 <sup>a</sup>	9.01 ± 0.25 <sup>a</sup>	43.2 ± 0.2 <sup>c</sup>	

Each value is the mean ± SE of three replicate experiments. TP: total polyphenols, TSS: total soluble solids, GAE: gallic acid, FW: fresh weight, DM: dry matter.

\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed sample (P).

\*\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed samples (PJ or MJ).

# Results in parenthesis are percentage of significant reductions compared to unprocessed sample (P).

**Table 2**  
Antioxidant activity of Costa Rican guava (*P. friedrichsthalianum*) samples.

Sample	DPPH	ORAC	Intracellular ROS
	IC <sub>50</sub> (μg DM/mL)	μmol Trolox/g FW	IC <sub>50</sub> (μg DM/mL)
<i>Effect of pressing and microfiltration*</i>			
Pulp (P)	59.1 ± 0.8 <sup>a</sup>	55.8 ± 2.2 <sup>a</sup>	684 ± 95 <sup>a</sup>
Pressed Juice (PJ)	80.7 ± 2.4 <sup>b</sup> (-37 %) <sup>#</sup>	40.6 ± 0.9 <sup>b</sup> (-27 %) <sup>#</sup>	771 ± 62 <sup>a</sup>
Microfiltrated Juice (MJ)	79.3 ± 1.4 <sup>b</sup> (-34 %) <sup>#</sup>	37.3 ± 2.0 <sup>b</sup> (-33 %) <sup>#</sup>	746 ± 79 <sup>a</sup>
<i>Effect of pasteurization**</i>			
Pasteurized Pressed Juice (PPJ)	89.7 ± 2.9 <sup>c</sup> (-14 %) <sup>##</sup>	45.3 ± 0.8 <sup>b</sup>	756 ± 91 <sup>a</sup>
Pasteurized Microfiltered Juice (MPJ)	92.5 ± 1.9 <sup>c</sup> (-22 %) <sup>##</sup>	44.2 ± 0.3 <sup>b</sup>	832 ± 80 <sup>a</sup>

Each value is the mean ± SE of three replicate experiments. DM: dry matter.

\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed sample (P).

\*\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed sample (PJ or MJ).

# Results in parenthesis are percentage of significant reductions compared to unprocessed sample (P).

## Results in parenthesis are percentage of significant reductions compared to unprocessed sample (PJ and MJ).

capacity compared to PJ, while MPJ showed a 22 % decrease compared to MJ. This lack of correlation between DPPH and ORAC has been reported and discussed previously (Niki, 2011; Azofeifa et al., 2015). However, reductions in the antioxidant activity of fruit juices caused by thermal treatments have been documented. Many studies have reported similar decreases in antioxidant capacity, consistent with the findings of this study. For instance, a 13 % reduction in DPPH scavenging capacity was observed after a treatment of 90 °C for 1 min in mixed fruit juice (Rodríguez-Roque et al., 2015). Similar reductions of 13 % were reported for blackcurrant juice under comparable conditions (Kruszewski et al., 2021), as well as a 16 % reduction for pineapple juice (Hoque et al., 2022). Furthermore, a higher reduction of 24 % in ABTS scavenging capacity was observed after a treatment of 85 °C for 30 s in pomegranate juice (Yuan et al., 2022).

Table 2 presents the measurement of the antioxidant activity using a cellular model with a ROS- sensitive fluorescent probe. This method provides a specific assessment of intracellular antioxidant activity, which closely resembles a biological context. The results obtained from this cellular method showed a non-significant reduction in the antioxidant activity of samples, indicating that neither the juice processing nor the thermal treatment had a significant impact on intracellular antioxidant activity. These findings suggest that the polyphenols derived from Costa Rican guava that reach the intracellular environment remain preserved under the unit operating conditions.

The antioxidant capacity demonstrated by Costa Rican guava could be attributed to the presence of ellagitannins and procyanidins. Other studies have characterized these compounds as remarkable antioxidants because of the presence of hydroxyl groups which favor the ability to donate hydrogen atoms and support the unpaired electrons (Burlando et al., 2023). A previous study with Costa Rican guava evaluated the antioxidant activity with ABTS assay and proposed the ellagic acid and the procyanidins as the polyphenols responsible for the antioxidant activity (Cuadrado-Silva et al., 2017).

### 3.3. Inhibition of lipid peroxidation

In addition to assessing the antioxidant capacity against oxidative species, this study also evaluated the capacity of Costa Rican guava polyphenols to inhibit lipid peroxidation, which is significant as damaged lipids are implicated in various pathological conditions (Ayala et al., 2014). Two models were employed to quantify the inhibitory capacity against lipid peroxidation. In rat liver homogenates, non-significant reductions were observed. However, in human erythrocytes, a significant decrease of 43 % and 58 % was evident for PJ and MJ, respectively. These results suggest that the loss of inhibitory activity is primarily attributed to the pressing process, given that MJ does not exhibit significantly lower activity compared to PJ (Table 3).

Thermal treatments in both rat liver homogenates model and on human erythrocytes do not show significant decrease in the inhibitory effect of lipid peroxidation (Table 3). Other studies reported similar results regarding the effect of pasteurization on the capacity to inhibit lipid peroxidation. For example, a pasteurized carrot juice incubated with linoleic acid (Ma et al., 2013), a pasteurized mandarin juice incubated with HepG2 cells exposed to H<sub>2</sub>O<sub>2</sub> (Di Nunzio et al., 2020), and a pasteurized blackberry juice incubated with erythrocytes exposed to AAPH (Azofeifa et al., 2015). None of these studies evidence a significant reduction in its lipid peroxidation inhibition capacity because of thermal treatments.

### 3.4. Correlation between total polyphenol content and biological activities

It is well established that juice processing often reduces polyphenol concentration, and it is assumed that this consequently affects antioxidant activity. However, the results of this study suggest that the variety of polyphenols present in each sample contributes to the antioxidant assays to a different extent. This implies that a decrease in total polyphenols does not necessarily correlate directly with significant reductions in antioxidant activity. Factors such as polyphenol structure, stability, and food matrix determine the antioxidant activity of polyphenols in each method (Lang et al., 2024).

A significant correlation between total polyphenols and antioxidant activity was only evident for the assay measuring the inhibition of lipid peroxidation in human erythrocytes, with a determination coefficient of  $R^2 = 0.778$  ( $p = 0.047$ ) (Fig. 2E). This significant correlation suggests that many of the polyphenols in the sample are involved in pro-

**Table 3**  
Inhibition of lipid peroxidation of the Costa Rican guava (*P. friedrichsthalianum*) samples in rat liver homogenates and human erythrocytes.

Sample	Rat Liver homogenates	Human Erythrocytes
	IC <sub>50</sub> (μg DM/mL)	(mmol Q/g DM)
<i>Effect of pressing and microfiltration*</i>		
Pulp (P)	9.4 ± 0.6 <sup>a</sup>	0.77 ± 0.08 <sup>a</sup>
Pressed Juice (PJ)	11.7 ± 0.7 <sup>a</sup>	0.44 ± 0.08 <sup>b</sup> (-43 %) <sup>#</sup>
Microfiltrated Juice (MJ)	11.0 ± 0.7 <sup>a</sup>	0.32 ± 0.04 <sup>b</sup> (-58 %) <sup>#</sup>
<i>Effect of pasteurization**</i>		
Pasteurized Pressed Juice (PPJ)	11.6 ± 1.2 <sup>a</sup>	0.37 ± 0.04 <sup>b</sup>
Pasteurized Microfiltered Juice (MPJ)	12.6 ± 0.9 <sup>a</sup>	0.29 ± 0.02 <sup>b</sup>

Each value is the mean ± SE of three replicate experiments. Q: quercetin, DM: dry matter.

\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed sample (P).

\*\* Columns followed by different letters differed significantly ( $p < 0.05$ ) compared to unprocessed sample (PJ or MJ).

# Results in parenthesis are percentage of significant reductions compared to unprocessed sample (P).

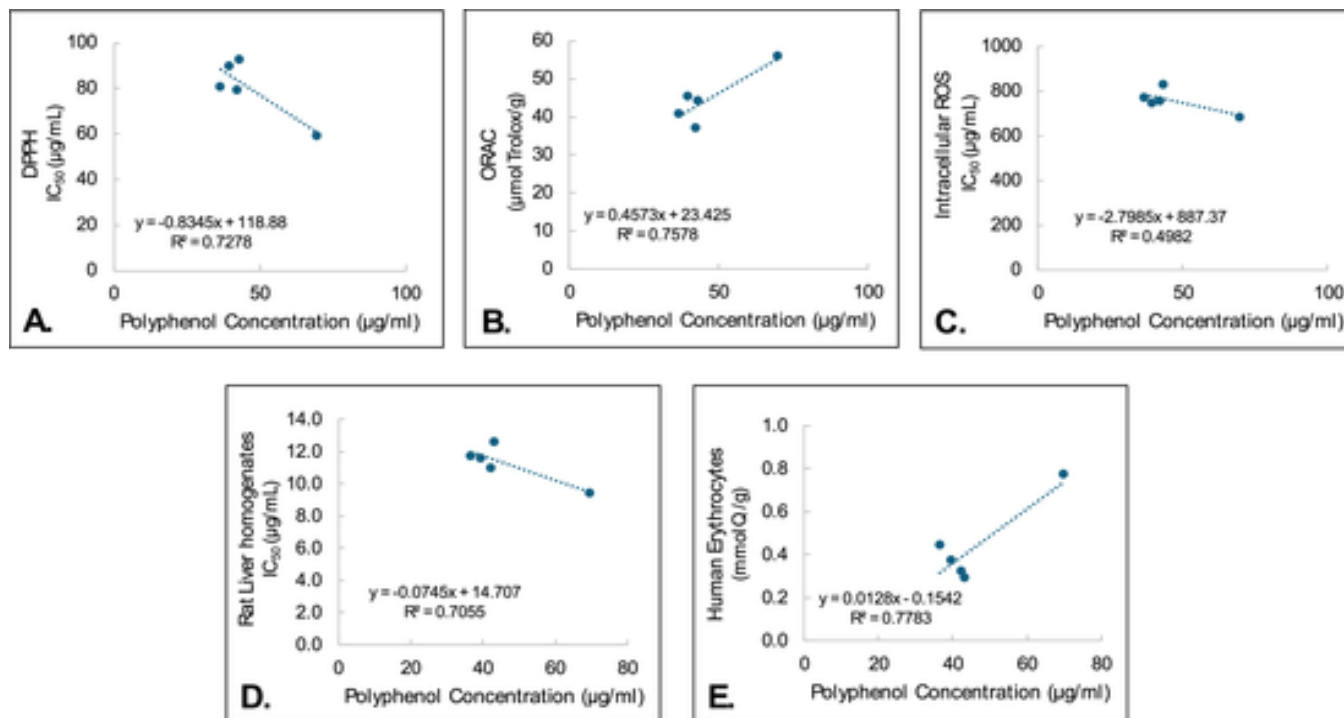


Fig. 2. Correlation scatterplots between total polyphenol concentration vs antioxidant activity (A, B, C) and total polyphenol concentration vs inhibition of lipid peroxidation activity (D, E). A, C and D methods showed negative correlations because the results of these assays are expressed as  $IC_{50}$ , where a lower value indicates a better antioxidant activity.

protecting the membrane lipids of erythrocytes, and their loss during juice processing impairs this antioxidant capacity.

Conversely, the antioxidant activity measured using other methods, showed a lower determination coefficient with total polyphenol concentration (Fig. 2A, 2B, 2C, 2D). DPPH exhibited an  $R^2=0.728$  ( $p > 0.05$ ), inhibition of lipid peroxidation in liver homogenates  $R^2=0.706$  ( $p > 0.05$ ), ORAC assay  $R^2=0.758$  ( $p > 0.05$ ) and the lowest correlation was observed for intracellular ROS inhibition, with  $R^2=0.498$  ( $p > 0.05$ ). The lower correlations for these methods suggest that the bioactive compounds involved in these assays are maintained despite juice processing, uncoupling the correlation between total polyphenol content and antioxidant activity. Specifically, the assay that evaluates the inhibition of intracellular ROS suggests that the specific fraction of polyphenols capable of entering the cell is the least affected by juice processing. The variability of the results and correlations shown in this study supports the importance of selecting the appropriate methodologies to evaluate the impact of processing (Sadeer et al., 2020; Zeng et al., 2020; Lang et al., 2024).

#### 4. Conclusions

The present study determined that the processing of Costa Rican guava juice did not lead to significant changes in its physicochemical properties. Nevertheless, a notable reduction in polyphenol concentration was observed, which has implications for antioxidant activity, as demonstrated by DPPH, ORAC, and lipid peroxidation in human erythrocyte assays. These methods indicate that the primary loss of antioxidant activity occurs during the pressing stage and showed that thermal treatment does not induce significant changes in antioxidant activity. Therefore, it is important to make ongoing efforts to develop better processing conditions during the pressing stage to minimize the reduction of bioactive compounds and ensure the retention of health-promoting activities in the juice.

One advantage of the present study is the evaluation of antioxidant activity through chemical assays that are fast and simple, combined with cell assays that have the advantage of achieving a better match with *in vivo* conditions. However, cell assays do not consider the digestion and absorption of bioactive compounds. Therefore, future *in vivo* studies are necessary to reflect the actual organism's antioxidant activity and evaluate the bioavailability of Costa Rican guava polyphenols.

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#### Ethical statement

Animal experiments used in this study were approved by the Institutional Committee for Care and Handling of Experimental Animals at the University of Costa Rica (CICUA N° 036-15) and comply with the ARRIVE guidelines.

#### Uncited references

Gordon et al., 2011; Pérez-Gutiérrez et al., 2008; Ribeiro et al., 2014.

#### CRediT authorship contribution statement

**Laura Navarro:** Investigation, Methodology, Formal analysis, Writing – original draft. **Silvia Quesada:** Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Ana M. Pérez:** Conceptualization, Formal analysis, Funding acquisition, Resources, Supervision, Writing – review & editing. **Gabriela Azofeifa:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Re-



sources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

### 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.

### Data availability

Data will be made available on request.

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