

María N. Osorio¹ / Diego F. Moyano² / Walter Murillo² / Elizabeth Murillo² / Albert Ibarz³ / José F. Solanilla¹

Functional and Rheological Properties of Piñuela (*Bromelia karatas*) in Two Ripening Stages.

¹ Grupo de investigación CEDAGRITOL, Faculty of Agronomic Engineering, University of Tolima, Ibagué, Colombia, 730001, Ibagué, Colombia, E-mail: jfsolanilla@ut.edu.co

² Grupo GIPRONUT, Faculty of Science, University of Tolima, 730001, Ibagué, Colombia

³ Department of Food Technology, School of Agricultural and Forestry Engineering, University of Lleida, 25198, Lleida, Spain

Abstract:

The physicochemical characteristics and the activity of the polyphenol oxidase from piñuela fruit juices were determined at two ripening stages. The antioxidant capacity was evaluated by the superoxide anion yield. The ripening stage showed greater ability to inhibit (O_2^- , 35.3%). The inhibition of superoxide dismutase was higher for both ripe (88.29%) and unripe (95.94%) states. The rheological behaviour of the juice was satisfactorily described using Herschel-Bulkley model ($R^2 > 0.99$). The concentration effect on the rheological parameters was described by the potential law model, and the temperature effect on the viscosity was described based on the Arrhenius equation, finding activation energy values from 11.94 and 17.80 kJ/mol. These results make *Bromelia karatas* L. a promissory fruit due to their content of secondary metabolites and its antioxidant activity, which could be associated to the presence of phenolic compounds, specifically flavonoids. Variations in these metabolites could also account for structural changes, physicochemical properties, the integrity protection of the fruit against adverse and an alternative to food products.

Keywords: *Bromelia karatas*, piñuela, antioxidant activity, superoxide dismutase, polyphenol oxidase, rheological behavior

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1 Introduction

Nowadays, there is a growing demand of tropical fruits and their derivate products for international markets. A good knowledge of their composition (presence of metabolites with biological activity) and nutritional value becomes the main gateway to these markets [1]. That interest is promoted by the ability of some phytochemicals to fight against certain tropical diseases (antibacterial activity), induce apoptosis in neoplastic cells (cytotoxic activity), diminish the organic imbalance caused by free radicals (antioxidant activity), reduce cholesterol levels, decrease hypertension, and control the appearance of chronic cardiovascular diseases as well as other associated disorders [2]. A clear example of such, are the phenolic compounds. The antioxidant capacity could be due to the protective action revealed by some of its secondary metabolites (phenolic compounds, carotenoids, anthocyanins, ascorbic acid, among others) against oxidative stress [3]. Those compounds are found mainly in fruits and vegetables, food that should be included into the diet in order to find health benefits [4].

Colombian Andean tropical dry forest is the habitat of many fruits that remain anonymous or underutilized. *Bromelia Karatas* (Bromeliaceae), popularly known as piñuela, is a clear example [5]. The fruits of this species are ellipsoid-shaped berries, with pineapple flavour. Despite its limited scientific and commercial knowledge, it is used in traditional Latin American medicine and consumed by local people who can find it almost everywhere given that it grows wildly [6]. However, in Mexico, the varieties of this species have received attention by some researchers. Other research [5], for example, studied the antioxidant activity of the leaves, while in other studies [7], they isolated and characterized a protease enzyme (karatasin) contained in the fruit. In Colombia, the only interest has been on its exotic aroma, where researches focused on studying their organoleptic properties [8, 9]. These few findings are not an impediment in the recognition of the promising prospects of the fruit and instead, they are an alternative for the development and innovation of products.

Taking into account above references and recognizing that the possible industrialization of these fruits would require basic information for setting parameters for the design, operation and control processes, this

José F. Solanilla is the corresponding author.

 <http://orcid.org/0000-0003-0673-5174> (Albert Ibarz)

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work was focused in the physicochemical characterization of the piñuela juice in two stages of maturity (unripe and ripe). The evaluation of the antioxidant potential and the study of its rheological behaviour in order to determine the best statistical model that fits better in experimental data.

2 Methodology

2.1 Sample preparation

The fruit of Piñuela in Ambalema-Tolima-Colombia municipality (4°49'11" N, 74°48'30" W) in the central-western Colombian zone was collected. The juice, after washing, peeling and shredding the fruit, was obtained. Then, it was filtered and centrifuged at 1,118 g for 15 min (Hettich centrifuge EBA20). The supernatant was recover and used immediately. To determine the rheological behaviour of the samples, juices filtrates were concentrated by evaporation (70 °C, until 5.3 to 65.0 °Brix). Then, they were lyophilized and stored (4 °C). The samples with distilled water for analysis were reconstituted.

2.2 Physicochemical properties of juice

Titrate acidity [10], pH (Schott Instruments, Handylab pH 11, Germany) and soluble solids, e. g. °Brix (Atago PAL- α model, USA) were determined in the juice. Quantification of reduction and total carbohydrates were carried out according to the methodology proposed by Loewus [11] and Norris and Ribbons [12] using a spectrophotometer (Thermo Fisher Scientific Inc., Helios gamma, Waltham, USA) with quartz cell one centimeter wide. Finally, the pectin content was determined by the gravimetric method [13].

2.3 Total phenolic content and total flavonoids

The total phenolic content was measured using the Folin Ciocalteu (FC) method, proposed by Dastmalchi et al. [14]. The total flavonoid content was assessed using two complementary colorimetric methods: quantification of flavones and flavonols with aluminium chloride [15], and evaluation of flavanones and flavanonols by 2,4-dinitrophenylhydrazine reagent [16].

2.4 Antioxidant activity

2.4.1 Superoxide anion inhibition ($O_2^{\cdot-}$)

To assess the inhibitory capacity of superoxide anion, a detailed methodology proposed by Bermúdez et al. [17] was followed. In a final volume (3 mL), the reaction mixture contained Tris-HCl buffer (50 μ L, 50 mM, pH 8.2), EDTA (50 μ L, 1 mM), juice (100 μ L) and pyrogallol solution (50 μ L, 0.124 mM). The optical density was read (420 nm) in the first minute, and after 20 min of reaction against the butylated hydroxytoluene (BHT, 500 mg/mL) and ascorbic acid (500 g/mL) used as control; the results were expressed as percentage of inhibition of $O_2^{\cdot-}$.

2.4.2 Superoxide dismutase activity (SOD)

The enzyme SOD activity was determined by using a commercial kit (SIGMA 19160 SOD) according to the protocol provided by Sigma-Aldrich. WST solutions (water-soluble tetrazolium salt) and xanthine oxidase enzyme were prepared according to the specifications of the kit. In a 96-well microplate, a sample aliquot (20 μ L), the WST solution (200 μ L) and the enzyme solution (20 μ L) were mixed against 3 blank batteries to ensure the accuracy of the response samples. Blank 1 (B_1) containing distilled deionized water (20 μ L), WST solution (200 μ L) and enzyme solution, blank 2 (B_2) with sample (20 μ L), WST solution (200 μ L) and enzyme solution (20 μ L) and blank 3 (B_3) with distilled water (20 μ L), WST (200 μ L) and solution enzyme (20 μ L). After incubating the plate for 20 min (37 °C), the absorbance was measured at 450 nm in a microplate reader (Biotek ELX800). The results were expressed as inhibition percentage of SOD activity and calculated by the following eq. (1).

$$\text{Inhibin} : \frac{[(Abs_{B1} - Abs_{B3}) - (Abs_M - Abs_{B2})]}{(Abs_{B1} - Abs_{B3})} \quad (1)$$

Where, Abs is the absorbance at 450 nm, M is the sample and B₁₋₃ (Blanks, see methodology).

2.5 Polyphenol oxidase activity (PPO)

The PPO activity in juices was measured using the method described by Falguera [18] with some modifications. The increase of the absorbance was measured at 420 nm for 60 min with 10 min intervals using catechol as substrate (prepared in citrate buffer at 50 mM, pH 4). 1 mL of juice was mixed with the catechol solution (2.5 mL). One PPO unit was defined as the amount of enzyme responsible for the increase of one unit of absorbance at 420 nm in 10 min.

To find the best activity conditions of the PPO enzyme, pH scan was performed using catechol in citrate buffer (pH = 4.0, 5.0, 6.0) and phosphate buffer (pH = 7.0 and 8.0); then, with the pH of highest activity, temperature scan (10–60 °C) was performed with 10 °C intervals.

2.6 Rheological behaviour

Viscosity was assessed at 20 °C using a Haake RS-80 RheoStress Rheometer (Gebrüder Haake GmbH, Karlsruhe, Germany) with a Z40-DIN concentric-cylinder sensor system (radii ratio 1.0847) [19]. For temperature control, a Thermo Haake C25P bath (Gebrüder Haake GmbH) was used, using a glycol–water solution (50 % w/w) as coolant fluid, which allows an interval variation of ± 0.2 °C. The samples were sheared at a constant shear rate of 400 s⁻¹ for 3 min, after which a downward ramp to 0 s⁻¹ and another upward ramp until 400 s⁻¹ were accomplished. The average shear stress values of these two ramps were used to calculate the juice viscosity and to build the corresponding rheogram, which gives information about the flow-behavior of the different samples [20]; eqs (2) to (7).

$$\text{Herschel – Bukley} := \sigma_0 + K(\dot{\gamma})^n \quad (2)$$

$$\text{Newton equation} : \sigma = n \cdot \dot{\gamma} \quad (3)$$

$$\text{Bingham equation} : \sigma_0 + \eta\dot{\gamma} \quad (4)$$

$$\text{Powerlaw (Ostwald de Waele equation)} : \sigma = K\dot{\gamma}^n \quad (5)$$

Where, σ shear stress (Pa), σ_0 yield stress (Pa), $\dot{\gamma}$ shear rate (s⁻¹), η viscosity (Pa•s), K consistency index (Pa•sⁿ), n flow behavior index (dimensionless).

2.7 Statistical analysis

The tests were performed with three replicates, reported as the average of three determinations, standard deviation (SD). On data, a check for homogeneity of variances was carried out, whereas one-way analysis of variance (ANOVA) and multiple comparisons Fisher's LSD post hoc tests were then applied to identify noted differences among ripening stages. A *P* value of less than 0.05 was considered statistically significant. In order to determine the level of association between the assessed parameters, a principal components analysis (PCA) using the statistical program InfoStat/Professional® version 1.2 was used.

3 Results and discussion

3.1 Physicochemical parameters and activity of polyphenol oxidase

The results of the physicochemical characterization of piñuela fruit juices are shown in Table 1. These values reveal significant changes in the piñuela that may be associated with fruit ripening process. These changes are:

(a) decrease of acidic organic substances in cells reflected in changes of pH from unripe to ripe state; (b) the greater buffering capacity of intracellular components, evidenced by the higher free acidity (titratable) of the ripe fruit; (c) loss of texture and increased permeability of the fruit due to the fact that the content of soluble solids is three times higher in the ripe state than in unripe state. Similarly, it is possible to notice that the change from unripe to ripe causes the oxidative degradation of reserve materials such as starch, resulting in the formation of reducing and non-reducing simple sugars, with the consequent increase of sweetness and astringency in the plant product.

Table 1: Physicochemical properties and polyphenol oxidase activity of *B. karatas*. juices.

Parameter	Unripe fruit juice	Ripe fruit juice
pH	4.02 ± 0.01 ^a	3.83 ± 0.02 ^b
Soluble solids (°Brix)	5.3 ± 0.0 ^a	17.9 ± 0.0 ^b
Titratable acidity (g AM/L)*	0.2814 ± 0.00 ^a	0.3484 ± 0.00 ^b
Total sugars (g G/L)**	7.18 ± 0.01 ^a	11.64 ± 0.05 ^b
Reducing sugars (g G/L)**	N.D.	4.69 ± 0.09
Total content of pectin (%)	0.12 ± 0.01 ^a	0.54 ± 0.04 ^a
PPO activity (U/ml)	0.0071 ± 0.00 ^a	0.0192 ± 0.00 ^a

n = 3 ± standard deviation, values with the same superscript are not statistically significant at P ≤ 0.05 level, * AM: malic acid, ** G: glucose, ND: not detected.

The piñuela maturation process could also be accompanied with an increase of the soluble pectin fraction because of the rupture of the interpolymer cross-links [21–23] and with an increase in the activity of the Polyphenol Oxidase (PPO). However, the statistical analysis did not show significant difference (p = 0.4084) between unripe and ripe state (20 °C and natural fruit pH).

It is important to recall that the fruit ripening requires significant amounts of energy to perform inherent physiological processes in that state [23], resulting in the increase of respiratory mechanisms. Energy generation through respiration in turn produces free radicals, but those are counteracted by the active cell antioxidant defence mechanisms that include phenolic compounds production. Therefore, the relationship between enzyme activity and phenolic compounds is clearly understood, as these specific compounds are the specific substrates of the enzyme (PPO) [24]. Table 1 reveals that the enzyme activity is ten times higher in ripe than in unripe fruit.

A principal component analysis applied to the above referenced variables, revealed that PPO activity in piñuela has a close correlation with pH (r = 0.94), titratable acidity (r = 0.97), flavones/flavonols system (r = 0.97) and total carbohydrates (r = 0.94) but in a lesser extent with soluble solids (r = 0.86) and total phenols (r = 0.81). Studies on apple [25], banana [26] and avocado [27] have shown the influence of environmental conditions in the enzymatic browning process, which is closely linked to the PPO functionality.

A PPO activity scanning in piñuela juices over time (Figure 1), clearly shows that the maximal enzyme activity is reached after 60 min in the ripe fruit, undergoing dramatic changes in enzyme activity before and after that time (more in ripe than in unripe fruit). Later, the activity decreases in both states, perhaps due to exhaustion of the substrate as other authors have found [28, 29]. Meanwhile, it can be seen in Figure 2 that changes between pH 5 and 8 on ripe fruit are not significant; however, for unripe state there is a steady increase as the pH rises to a maximum activity peak at pH 8.

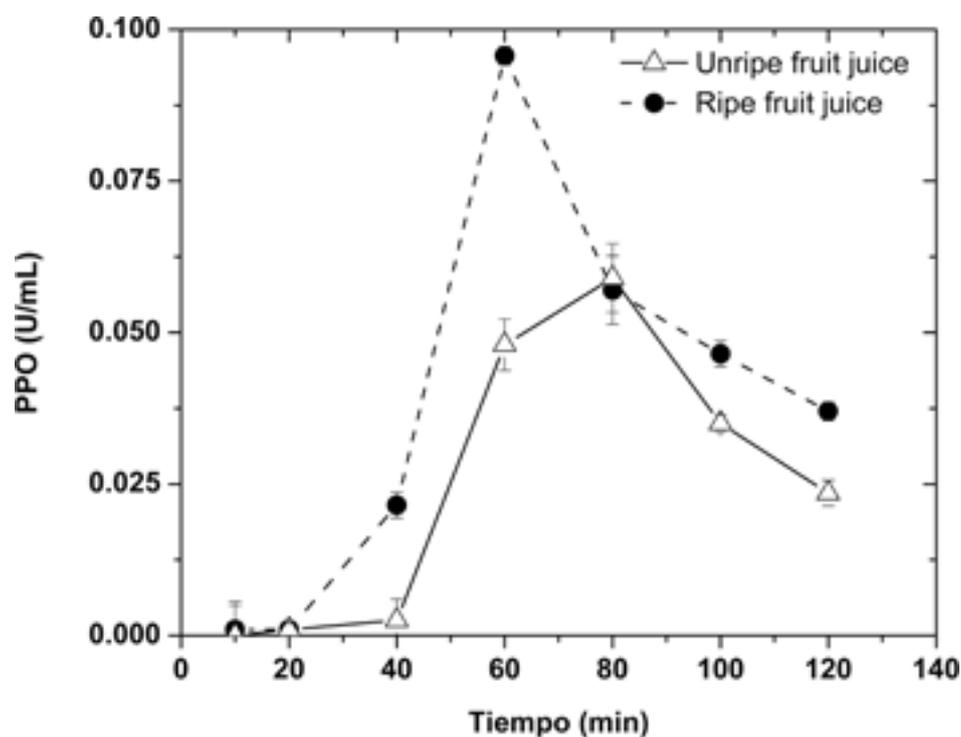


Figure 1: Polyphenol oxidase activity of piñuela juices in natural conditions at 20°C.

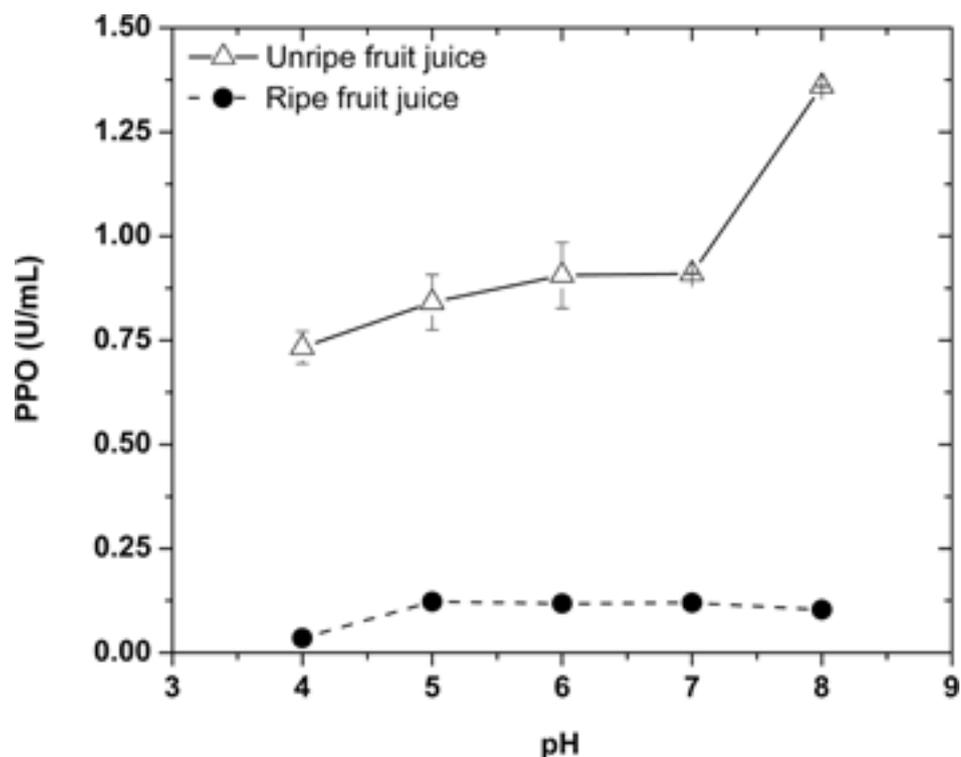


Figure 2: Polyphenol oxidase activity of piñuela juices in natural conditions at 20 °C and pH between 4 and 8.

The results obtained in this study demonstrate that the enzyme, either in unripe or ripe fruit, works at its full capacity at 20 °C; being more critical in unripe, rather than in ripe state (Figure 3). Assuming that at 20 °C the maximum enzyme activity (100 %) is achieved, it appears that at a temperature of 30 °C, an inhibition of more than 89 % of the enzymatic activity in the ripe state can be reached, while in the unripe state inhibition is around 93 % [30].

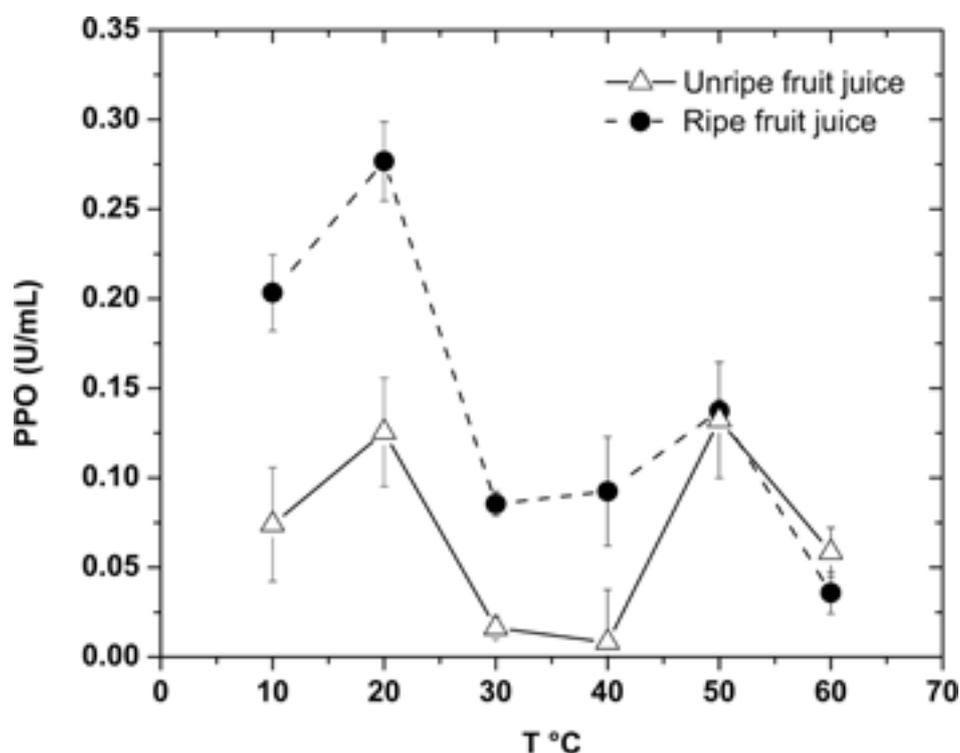


Figure 3: Polyphenol oxidase activity in piñuela juices at a temperature range from 10 to 60 °C.

Temperature is a parameter of careful observation in all processing operations and food preservation. In the control of heat treatments, it is essential in reducing and inactivating the enzyme activity [25, 31–33].

Results showed that the PPO of *B. karatas* appears to be activated between 20 and 80 min as maximum, it is relatively tolerant to acid pH and temperatures oscillating amongst 10 and 20 °C. From the results also, pH and temperature greatly influence when the fruits are immature, and decline as they hit peak ripeness.

3.2 Antioxidant capacity

In a previous study in GIPRONUT (Research Group of Natural Products), phenolic compounds such as coumarins, flavonoids and phenylpropanoids were identified [34]. Thus, in this work it was found that total phenolic compounds increased 40 % during fruit development, which could be interpreted as an indicator parameter of the physiological processes. That occurs during fruit ripening or as evidence that these compounds are required for protection of the nutrients against the attack of pathogens and predators [35–37], perhaps to protect the cells against oxidative stress caused by the ripening process or because of both processes.

Table 2 shows that flavonoids are a representative group within phenolic compounds in piñuela, especially those of flavone and flavonol type (0.7 mg/100 g in the unripe tissue and 20 mg/100 g in ripe tissue). It can also be noted that during the transition from unripe to ripe, the percentage of flavanones decreases (relative to the total flavonoids) by 9 %, while flavones increase by 9.4 %, which could be explained by the fact that flavanones are considered precursors in flavones biosynthesis [38]. Based on the above, there were significant differences ($P < 0.0001$) in flavones/flavonols content between the two stages of maturity, occurring the opposite with flavanone/flavanonol type ($P = 0.9373$).

Table 2: Antioxidant activity of *B. karatas* fruit.

	Unripe	Ripe	Control Ascorbic acid*	SOD**
Superoxide anion (% inhibition)	4.0 ± 0.00 ^a	35.3 ± 0.00 ^b	95.8 ± 0.00 ^c	-
SOD Activity (% inhibition)	88.29 ± 0.02 ^a	95.94 ± 0.04 ^a	-	86.86 ± 0.00 ^a
Total phenolics (mg EGA/100 g)	280.28 ± 6.83 ^a	394.54 ± 2.99 ^b		

Flavanone and flavanonol (mg EN/100 g)	187.29 ± 1.01 ^a	187.86 ± 0.95 ^a
Flavone and flavonol (mg EQ/100 g)	0.71 ± 0.01 ^a	19.99 ± 2.21 ^b

$n = 3 \pm$ standard deviation; values with the same superscript are not statistically significant at $P \leq 0.05$ level. mg E: equivalent milligrams; GA: gallic acid, N: naringenin, Q: quercetin, *100 µg/ml; ** 400 µg/ml

Likewise, Table 2 reveals the results from *in vitro* antioxidant activity exhibited by fruit juices. The antioxidant activity of ripe fruit expressed as superoxide inhibition percentage was significantly higher ($P < 0.0001$) than the juice from unripe fruits; however, it did not exceed the control activity (ascorbic acid) at any conditions, which is a renowned exogenous antioxidant used in industry [39]. The activity of the Superoxide Dismutase (SOD) enzyme (first line cellular defence against oxidative stress), showed a high capacity to stabilize the superoxide anion generated by xanthine oxidase during the reaction [40] in both maturation states without statistical difference ($P = 0.2651$).

In order to find the changes in antioxidant activity through maturation, a separate principal component analysis for each state was performed. It was demonstrated that in ripe stage the superoxide anion inhibition appears to be greatly associated to flavanones/flavanonols system ($r = 0.99$); two types of flavonoids that also act as vascular pigments contributing with the whitish colour of piñuela fruits [41]. For unripe fruit, the same superoxide anion inhibitory activity but with total phenols ($r = 0.96$) was observed.

In turn, independently whether the fruits are ripe ($r = 0.78$) or unripe ($r = 0.80$), the SOD activity displayed moderate association with flavanones/flavanonols system for ripe ($r = 0.90$) and unripe fruit ($r = 0.81$) and somewhat less with the flavones/flavonols system. These results indicate that the phenolic compounds (including flavonoids) contribute significantly to the antioxidant activity of piñuela fruits. Besides, the SOD enzyme activity maintains a direct relation with phenolic compounds, meaning that the enzyme manifests its protective action only when soluble antioxidants have been reduced to low levels, and vice-versa. The change in the behaviour of the antioxidant activity is associated with fruit development. The flavonoids, in addition to their antioxidant functions, act as messengers inhibiting protective enzymes [42] as well as in the possible exerted regulatory properties on the development and communication pathways between plant and adequate environment for their colour and aroma; considering the increase in flavonoids content as a clear indicator of fruit maturity [43]. These metabolites can be produced as a result of environmental stresses and can act as plant protection product in different ways; variations in these metabolites could also account for structural changes such as aroma, colour, taste and other properties that are evident while the ripening process takes place, process that also includes the integrity protection of the fruit against adverse conditions.

3.3 Rheological behaviour

Among the different rheological models, Henschel-Bulkley was found to be the best model that fits the piñuela juices flow behaviour always with high coefficients of determination ($R^2 > 0.99$). For juices that contain less soluble solids, the model that best fits the experimental data was Newton equation. For flow, the rheograms were accomplished with a downward ramp to 0.12 s^{-1} and another upward ramp until 400 s^{-1} . To verify whether the samples show thixotropy, they were sheared at a constant $\dot{\gamma}$ of 400 s^{-1} for 3 min. It was not observed any variation in σ . Therefore, the samples do not show thixotropy, accordingly, the ramps are matching. On the other hand, the equipment used does not allow to work with $\dot{\gamma}$ lower than 0.12 s^{-1} .

The results (Table 3 and Table 4) show that juices in both ripening stages exhibited values less than one for shear σ_0 and n in all temperatures tested, except in, as already stated, for the juices with less soluble solids content ($n = 1$). These are typical characteristics of flow behaviour for pulps and fruit juices that are not clarified and have moderate amounts of pectin, showing a time-dependent behaviour and following in most cases the behaviour of pseudoplastic materials with yield stress [44–46].

Table 3: Flow rheological behavior of piñuela juices in ripe state, depending of the temperature and soluble solids.

SS (°Brix)	$T(^{\circ}\text{C})$	$\sigma_0(\text{Pa})$	$K(\text{Pa}\cdot\text{s}^n)$	n	$\eta_{ap}(\text{mPa}\cdot\text{s})$	R^2
65.0	10	13.602	8.912	0.554	1278.8	0.9995
	20	11.196	7.083	0.527	914.0	0.9994
	30	9.562	6.157	0.498	705.7	0.9993
	40	8.461	5.745	0.470	585.0	0.9991
	50	7.542	5.701	0.444	515.9	0.9989

44.3	10	4.561	3.598	0.482	376.8	0.9996
	20	3.603	3.275	0.454	301.0	0.9996
	30	3.094	3.155	0.430	259.5	0.9995
	40	2.610	3.197	0.405	232.5	0.9993
	50	2.281	3.506	0.380	224.6	0.9992
20.9	10	0.240	0.086	0.732	27.4	0.9997
	20	0.213	0.061	0.749	21.3	0.9995
	30	0.187	0.045	0.767	17.3	0.9995
	40	0.109	0.046	0.754	15.9	0.9976
	50	0.167	0.026	0.808	12.4	0.9986
17.9	10	0.0080	1	8.0	0.9997
	20	0.0068	1	6.8	0.9956
	30	0.0057	1	5.7	0.9927
	40	0.0049	1	4.9	0.9935
	50	0.0043	1	4.3	0.9930

T is temperature, σ_0 is yield stress, η is viscosity, η_{ap} is apparent viscosity, K is consistency index, n is flow behavior index, R^2 is coefficient of determination

Table 4: Flow rheological behavior of piñuela juices in green state, depending of the temperature and soluble solids (SS).

SS (°Brix)	T (°C)	σ_0 (Pa)	K (Pa·s ⁿ)	n	η_{ap} (mPa·s)	R^2
12.5	10	2.685	1.771	0.486	192.9	0.9987
	20	2.113	1.484	0.484	159.0	0.9978
	30	1.673	1.303	0.475	132.9	0.9975
	40	1.435	1.258	0.461	119.5	0.9969
	50	1.321	1.872	0.411	137.5	0.9979
8.3	10	1.376	0.727	0.582	119.8	0.9988
	20	1.193	0.615	0.580	100.8	0.9985
	30	1.003	0.514	0.577	83.3	0.9975
	40	0.867	0.444	0.574	71.1	0.9984
	50	0.727	0.358	0.577	58.3	0.9975
5.3	10	0.0057	1	5.7	0.9930
	20	0.0044	1	4.7	0.9911
	30	0.0035	1	3.8	0.9911
	40	0.0026	1	3.0	0.9981
	50	0.0022	1	2.2	0.9970

T is temperature, σ_0 is yield stress, η is viscosity, η_{ap} is apparent viscosity, K is consistency index, n is flow behavior index, R^2 is coefficient of determination

It is important to note that the consistency index is strongly influenced by the content of soluble solids, which is directly reflected in the sharp increase of the consistency of K and σ_0 when the content of total soluble solids and the apparent viscosity increase, as shown by the results (Figure 1). Some other authors have described that behaviour [44–48].

However, the flow n , which in most cases tends to increase with T [44, 45, 49], did not follow an increasing trend in all tested concentrations. Data reported by other authors does not follow a clear trend, either [50].

In Figure 4, it is important to warn about the influence of temperature on the reduction of shear stress, which is taken by industry as an advantage to reduce energy consumption in transport processes since the reduction of the apparent viscosity also reduces the power required in the process of pumping and agitation. A fluid is able to flow due to the cohesive forces in their molecules and generally it continuously deforms when it is subjected to shear stress [47, 48].

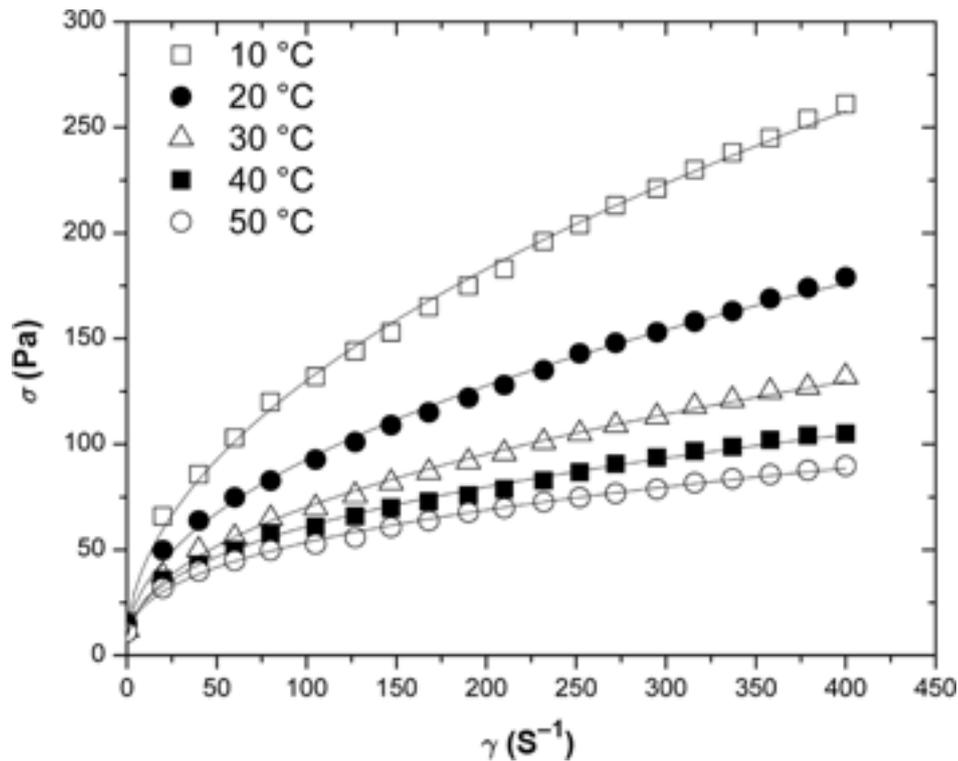


Figure 4: Rheograms for ripe piñuela juices of 65 °Brix at different temperatures.

3.3.1 Effect of temperature and soluble solids content

The Arrhenius equation is widely used to describe the behaviour of the apparent viscosity at different temperatures [19, 45].

$$\eta = K_0 \exp\left(\frac{E_a}{RT}\right) \quad (6)$$

Where η is the viscosity, K_0 is a constant, E_a is the activation energy of flow, R is the gas constant and T is the absolute temperature in Kelvin.

Table 5 shows the parameters of the Arrhenius equation for a 100 s^{-1} strain rate. The E_a values are within the normal range as in other pulps and fruit juices; finding activation energy values between 9.92 and 17.80 kJ/mol [19, 45, 48, 50]. However, an increase in soluble solids content did not correspond to higher activation energy for unripe fruit samples, as expected [51]. This could be explained, perhaps because of the molecular interactions or irreversible breakdown in the matrix structure of pulps that could also influence the activation energy [48].

Table 5: Parameters for Arrhenius equation for piñuela juices to different concentrations and maturation states.

	SS (°Brix)	K_0 (mPa·s)	E_a (kJ/mol)	R^2
Ripe	65	0.777	17.30	0.9798
	44.3	5.279	9.92	0.9487
	20.9	0.061	14.31	0.9820
	17.9	0.050	11.94	0.9993
Unripe	12.5	1.19	11.94	0.9906
	8.3	0.377	13.59	0.9969
	5.3	0.003	17.80	0.9823

K_0 is a constant, E_a is the activation energy of flow, SS is soluble solids and R^2 is coefficient of determination

The effect of the solids content was described by a potential model (5), which fits very well to the experimental

data ($R^2 > 0.98$). In the case of unripe piñuela juice, the pulp amount and its yield were very low ($33.34 \pm 1.32\%$), for this reason the sample was insufficient and the data corresponding to the three tested solid concentrations were not sufficient to fit the data for this model. Table 6 shows the parameters of the power law equation for concentrated juices in ripe state, in which a decrease in the frequency factor with the apparent viscosity of the samples is evident. In the Figure 5 and Figure 6 can be seen the influence of temperature and the effect of soluble solids on the viscosity of ripe piñuela juice, respectively. Being evident the influence of temperature in the solubility of solids in the juice from ripe fruit. Most of the solids have positive dissolution heats, and therefore an increase in temperature that favours the solubility, dissolution rate and influences the fluid viscosity. According to Le Chatelier's law, an endothermic process is favoured by temperature increase but no from those exothermic processes that exhibit negative dissolution heats.

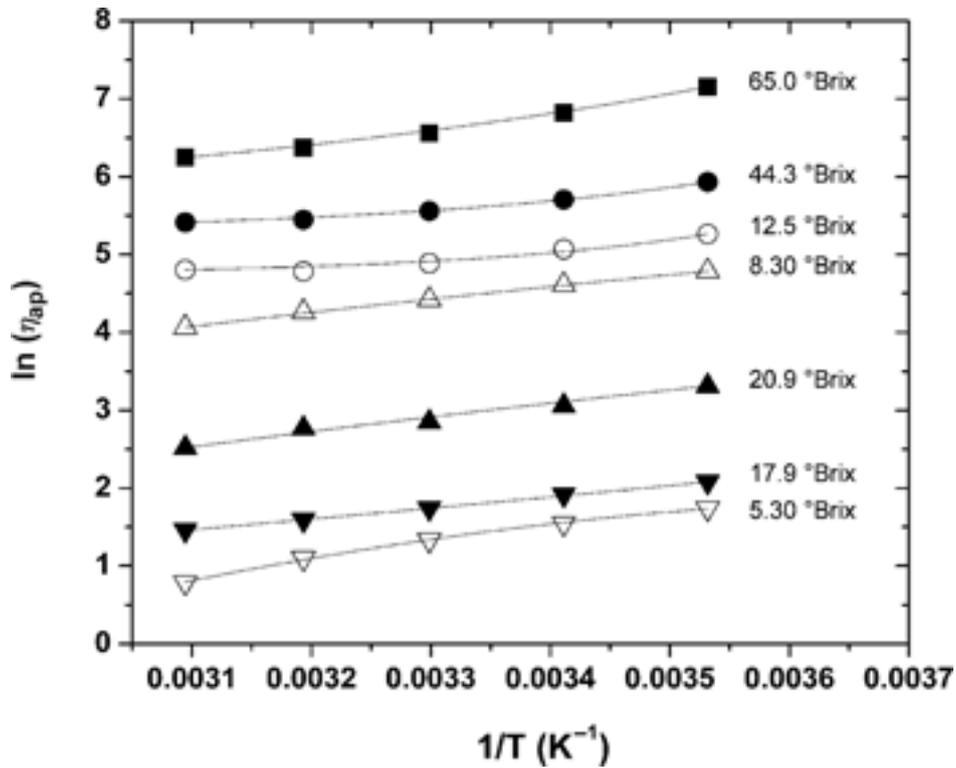


Figure 5: Effect of temperature on the viscosity of ripe piñuela juices at different soluble solids content.

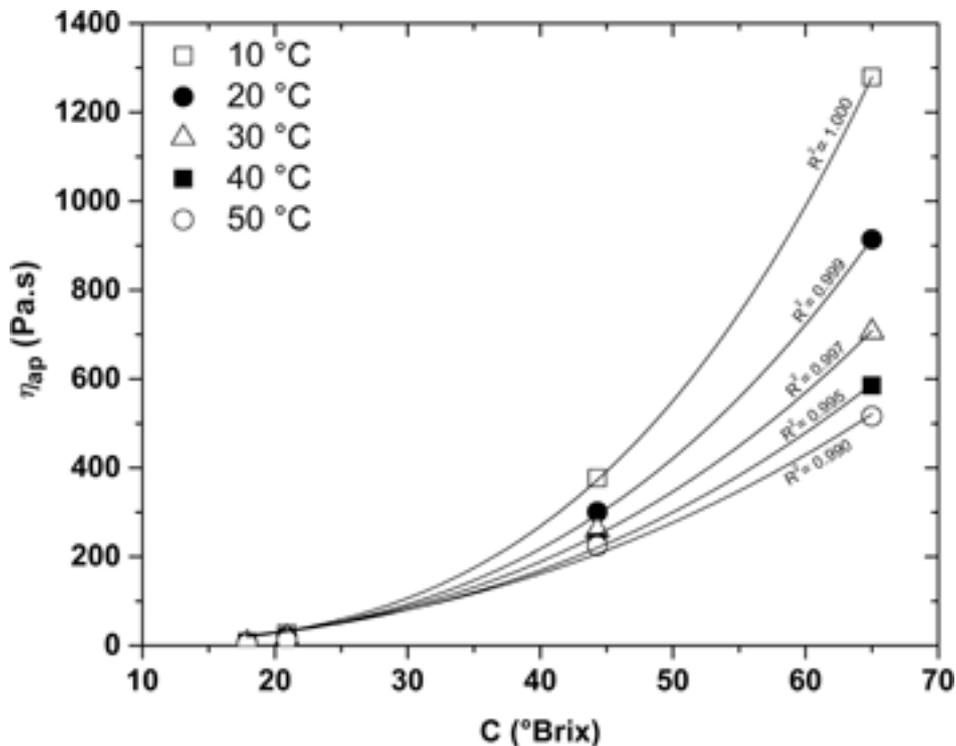


Figure 6: Effect of soluble solids content on the viscosity of piñuela ripe juice as function of temperature.**Table 6:** Exponential equation parameters of soluble solids effect on ripe bromelia karatas of juice ripe piñuela viscosity at different temperatures.

T (°C)	η_0 (mPa·s)	b (°Brix ⁻¹)	R ²
10	2.040×10^{-4}	3.7771	0.9856
20	2.195×10^{-4}	3.6859	0.9865
30	2.007×10^{-4}	3.6548	0.9850
40	1.994×10^{-4}	3.6182	0.9801
50	1.341×10^{-4}	3.6937	0.9812

$$\eta = \eta_0(C)^b \quad (7)$$

Where η is the viscosity, η_0 is the viscosity when the soluble solids content is 0°Brix, b is a constant and C is the concentration expressed in °Brix.

Studying the effect of factors such as temperature and soluble solids on the viscosity of a product is needed in order to determine the rates of heat transfer, energy consumption (with increasing concentration) and to control the temperature and flow rates so it is possible to ensure continuous product flow [52]. In general, the temperature and solids content affect some of the physical properties of the juices such as density, viscosity, refractive index, boiling point and specific heat. This is explicable because the concentration of dissolved solids in the primary juice (glucose and fructose) directly affects those properties mentioned above [53].

Then, studying the variations experienced by the viscosity and density with concentration and temperature provides guidelines for designing and optimizing resources in processes of food products derived from fruits (pumping, evaporation, membrane filtration, etc.).

4 Conclusions

The results obtained from the tests applied to *Bromelia karatas* fruits support the conclusion that ripe piñuela is a promising fruit due to their content of secondary metabolites and its antioxidant activity, which could be associated to the presence of phenolic compounds (specifically flavonoids) and to the activity of enzymes such as SOD. Variations in these metabolites could also account for structural changes, physicochemical properties and integrity protection of the fruit against adverse.

The piñuela industrialization would demand refrigeration temperatures or the use of mild heat treatments, as well as acidic pH to inactivate the polyphenol oxidase enzyme in order to prevent browning of the products. These variables influence in unripe fruit at greater extent.

The rheological behaviour of piñuela juices with the highest soluble solids concentration showed that Henschel-Bulkley model is the one that best fits the experimental data. Besides pseudoplastic behaviour, the juices presented yield stress that increased the soluble solids content. The apparent viscosity, increased when the soluble solids content was higher than expected. A rise in temperature had a reduction in shear stress. For the ripe fruit, a higher content of soluble solids corresponded to higher activation energy, which was not clear for the unripe stage. Meanwhile, a potential model satisfactorily described the effect of the soluble solids concentration on the consistency index.

This appears to be the first work to study the antioxidant and rheological properties of piñuela juice and the information provided here could be helpful to the successful development of new food functional products.

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