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Physicochemical and Rheological Characterization and Antioxidant Activity of the Juice of "Puro Puro" (*Passiflora pinnatistipula*) †

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Abstract: The genus *Passiflora* spp. stands out as an important source of bioactive compounds and health promoters; however, not all its species have been studied in depth, as in the case of *Passiflora pinntistipula*, or "puro puro". In this sense, the main objective of this research is to study the antioxidant capacity, physicochemical properties and rheological behavior of "puro puro" juice from the Pamuri Community, Tayacaja Province, Huancavelica. The physicochemical characterization was carried out according to the official methods of international analysis (AOAC). The parameters evaluated were the percentage of humidity (66%), ashes (3%), fat (1.5%), proteins (4.5%), carbohydrates (25%), total energy (100%), pH (4.5), total acidity (1.3%), and soluble solids (18.5%). The color was determined on the CieLab scale and was L* (34.5), a* (1.2), b (4.4), C* (4.5) and h* (74.3). The antioxidant capacity was evaluated with the 2,2′-azino-bis (3-ethylbenziozoline-6)-sulfonic acid (ABTS) method, and the value obtained was 5.2 µmol-Equi. Trolox/g. The rheological parameters of the pure cigar juice were evaluated using a rotary viscometer at temperatures of 15, 20, 25, and 30 °C; and in concentrations of 18.5, 20, 25, 30, and 35 °Brix. The power law model fitted the experimental results and showed non-Newtonian flow behavior. The value of the flow behavior index (n) was less than unity at all temperatures and concentrations, indicating the pseudoplastic nature of the juice. The Arrhenius model was able to relate apparent viscosity to temperature. The consistency index (k) varied in the range between 0.42 and 1.38 Pa s^n, and flow behavior index (n) was in the range between 0.26 and 0.55.

Keywords: *Passiflora pinnatistipula*; antioxidant capacity; rheological behavior; pseudoplastic fluid

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1. Introduction

The demand for high-quality health foods is growing today. Consumers are looking for organic foods, such as fruits, that are a source of bioactive phytochemical compounds that contribute to disease prevention. There is research demonstrating the beneficial effects of fruit-rich diets as healthy food [1]. The consumption of tropical fruits is gaining public interest, due to the supply of minerals, vitamins, and carbohydrates required to minimize human health complications [2].

Passiflora is a genus comprising about 520 species distributed in the tropical regions of Africa, America, and Asia, 96% of which are found in South and Central America [3]. Although there is a great diversity of *Passiflora* species, only some are used in agriculture, mainly to produce fruits, which are directly consumed or processed, such as juices, jams, ice cream, and sweets [4]. Wild and cultivated species of this genus have been shown to be sources of potential bioactive molecules for developing new anti-diabetic, antimicrobial, anti-inflammatory, and antiproliferative drugs, in addition to natural antioxidants for food applications [5].

Passiflora pinnatistipula (family Passifloraceae), is commonly known as "gulupa" (Colombia), "tacso", and "purupuru" in Ecuador, and, "puro puro" and "tin tin tin" in Peru [6]. *P. pinnatistipula* is a woody climbing plant that originates from the tropical regions of Peru and Bolivia, but is cultivated from Colombia to central Chile, with a greater presence in northern Bolivia and southern Peru [7,8]. Fruits generally grow between 2500 and 3800 m; however, they can be cultivated up to 4000 m, allowing for production in cold climates [9].

The fruit of *P. pinnatistipula* is spherical, 4 to 6 cm in diameter, with a thin grayish-green or yellow pericarp and a leathery, but brittle exocarp [6,10]. The pulp is whitish to yellowish, with a sweet or slightly sour taste, and the seeds are relatively large, round and hard [6,9]. It can be consumed as a fruit or used to prepare juices [6].

The fruits of *P. pinnatistipula* are harvested at various times of the year in small quantities and traded at fairs and local markets due to low productivity [11,12]. *P. pinnatistipula* fruits are of great interest to the villagers, as they depend on these resources for subsistence; however, the fruit has a low monetary value (PEN 0.50 (USD 0.15) per unit), despite its high nutritional value and scarcity in the market [13]. Therefore, the processing of these fruits is of great relevance to develop new products with high added value, such as juices and beverages, capable of providing a wide range of nutrients to human health, which stand out as a new source of economic income for producers. The present study aims to increase the scarce knowledge on the physicochemical composition, antioxidant capacity, and rheological behavior of "puro puro" (*P. pinnatistipula*) juice to promote the revaluation and production of the fruit, developing new proposals for its processing.

2. Materials and Methods

2.1. Materials

The puro puro fruits were collected from the Community of Pamuri, District of Acraquia, Tayacaja–Huancavelica.

2.2. Preparation of the Puro Puro Juice

The puro puro ripe fruits were selected (free of mechanical damage), washed, and disinfected with sodium hypochlorite solution (0.75 mL/L of water). Then, they were cut longitudinally in halves, peeled, destemmed, and filtered manually. The yield of the puro puro juice obtained was 71%. The samples (puro puro juice) were refrigerated (4 °C) until the day of rheological analysis.

2.3. Determination of Physicochemical Properties

Acidity was determined with titration, with the addition of phenolphthalein as an indicator; the results were expressed as a percentage of ascorbic acid. The pH was measured by means of a digital potentiometer (SCHOTT, PH11). Total soluble solids were measured with a digital refractometer (SCHMIDT-HAENSCH, DHR-60) and expressed as Brix. Proximate chemical composition was determined using the A.O.A.C. method [14]: moisture, ash, fat by using the Soxhlet system; hexane and protein using the Kjeldahl method, and carbohydrate by using difference. Color was measured using the CIE lab scale (L*, a*, b*) with a Konica colorimeter (Minolta, CR-10).

Antioxidant activity was determined using ABTS+ radicals, following the methodology of [15]. The results were expressed as µmol Equi. Trolox/g sample.

2.4. Rheological Behavior

The puro puro juice (18.5 °Brix) was concentrated to 20, 25, 30, and 35 °Brix using vacuum evaporation. Rheological measurements of the samples were carried out in a

Brookfield viscometer (model DV III Plus) with a CV N°2 spindle, at seven rotation speeds (0.5, 1, 1, 4, 10, 20, 20, 50, and 100 rpm) with a torque of 10%. Measurements of the different levels of concentrations were carried out at temperatures of 15, 20, 25, and 30 °C, in a shear rate range of 0.1–100 s^{-1}.

Rheological parameters were determined according to the conversion method of Mitschka (1982). The rheograms (shear stress versus shear rate plots) were fitted to the Ostwald de Waele model to predict the rheological behavior of the juice. They were calculated with:

$$\tau = k(\gamma)^n \tag{1}$$

where τ is the shear stress or shear strain (Pa), k is the flow consistency index (Pa s^n), γ is the shear rate or strain rate (s^{-1}), and n is the flow behavior index (dimensionless).

The apparent viscosity was determined using the following equation:

$$\eta_{ap} = k\left(\frac{\gamma^n}{\gamma}\right) \tag{2}$$

where η_{ap} is the apparent viscosity (Pa s).

To determine the effect of juice temperature on rheological properties, the Arrhenius model was used:

$$k = k_0 \cdot \exp(Ea/RT) \tag{3}$$

where η_{ap} is the apparent viscosity (Pa s), k_0 is the so-called infinite deformation viscosity constant, Ea is the activation energy at flow (J mol^{-1}), R is the universal gas constant (8.3143 J K^{-1} mol^{-1}), and T is the absolute temperature (K).

3. Results and Discussions

3.1. Physicochemical Characterization, Color and Antioxidant Activity of Cigars

The puro puro is a fruit that has not yet been investigated, but passion fruit and granadilla are part of the *Passiflora* genus and have similar physical characteristics. The soluble solids have a sweet taste, due to their total sugar content and low acidity, both percentages being higher than those of passion fruit and granadilla, and the pH is in the range of the genus *Passiflora*. In addition, the results (Table 1) indicate that the pure juice has excellent chemical characteristics in terms of total ash, total fat, crude protein, and carbohydrates (including fiber), all above the values of passion fruit (*Passiflora ligularis* and *Passiflora edulis*).

Table 1. Physicochemical characterization and antioxidant capacity of puro puro juice.

Physicochemical Characterization	Value
pH	4.50
Acidity (% ascorbic acid)	4.48
Total Soluble Solids (Brix)	18.50
Humidity (%)	66.00
Total ash (% d.b.)	8.82
Total fat (% d.b.)	4.41
Crude protein (% d.b.)	13.24
Carbohydrates (by difference, % d.b.)	73.53
Color	
L*	
a*	1.2
b*	4.4
ΔE	20.8
Antioxidant Activity	
Antioxidant activity of the "puro puro" juice (μmol Equi. Trolox/g)	5.20

From Table 1, the puro puro pulp presented colorimetric parameters of lightness (L*: 34.5, indicating a light yellow, low lightness for being close to 0; 4.4 for b*, and 1.2 for a*, expressing a dull yellow color). These data are very similar to those reported by [16], who reported that the color of fresh passion fruit (*Passiflora edulis*) pulp was mainly due to the yellow contribution (positive value of b*) and in smaller proportion to the red contribution (positive value of a*), and the combination of both resulted in an intense yellow color with orange hues, mainly due to the presence of carotenoid and flavonoid pigments that are generally yellow in color. The total difference ΔE was 20.8, which indicates that the color can be observed with the human eye, since $\Delta E > \pm 5$ is perceptible by the human eye.

The puro puro juice possesses 5.2 µmol of Equi. Trolox/g of antioxidant capacity (Table 1), lower in comparison with other species of the genus *Passiflora*. The differences may be due to different chemical components (such as flavonoids), degree of fruit ripening, and the influence of external environmental conditions (temperature, humidity, and exposure to sunlight) [4,17].

3.2. Rheological Characterization

The rheological parameters of the flow behavior index (n) and consistency coefficient (k) obtained at different concentrations and temperatures were satisfactorily fitted to the Power Law equation and resulted in correlation coefficients of $r^2 \geq 0.955$ (Table 2), indicating that this model adequately describes the flow behavior of "puro puro" juice. This model has been successfully used by several authors [13,18] in the rheological study of various foods; for example, [19] in guava (*Psidium guajava*) pulp, [20] in mango (*Mangifera indica*) pulp, [18] in guabirá (*Campomanesia xanthocarpa*) jams, and [21] in fruit drinks ("mango" *Mangifera indica*, "apple" *Pyrus malus*, "melon" *Citrullus lanatus*, "banana" *Musa acuminata*, "pear" *Pyrus communis*) [22].

Table 2. Variation in flow behavior index (n) and consistency coefficient (K) of puro puro juice at different temperatures and concentrations.

Temperature (°C)	Rheological Parameters	Concentration (°Brix)				
		18.5	20	25	30	35
15	K	0.99	1.10	1.16	1.28	1.38
	N	0.31	0.29	0.30	0.27	0.26
	r^2	0.985	0.993	0.990	0.978	0.973
20	K	0.84	0.89	1.09	1.16	1.30
	N	0.34	0.32	0.31	0.29	0.27
	r^2	0.982	0.997	0.984	0.984	0.977
25	K	0.68	0.82	0.99	1.05	1.22
	N	0.37	0.31	0.33	0.31	0.28
	r^2	0.986	0.992	0.989	0.973	0.974
30	K	0.42	0.62	0.79	0.94	1.09
	N	0.55	0.40	0.40	0.34	0.30
	r^2	0.961	0.976	0.995	0.968	0.995

Figure 1 shows the rheograms of puro puro juice at different concentrations and temperatures. The ascending curves show that the samples show non-Newtonian behavior of a pseudoplastic type, because the value of the flow behavior index in all cases is lower than unity ($n < 1$) [5,23]. According to the results, the consistency index decreases as the temperature rises and the flow behavior index increases, while, as the concentration increases, the consistency index shows an increasing trend and the flow behavior index decreases. This behavior is reported in other juices, such as in totapuri mango juice [1,17,24].

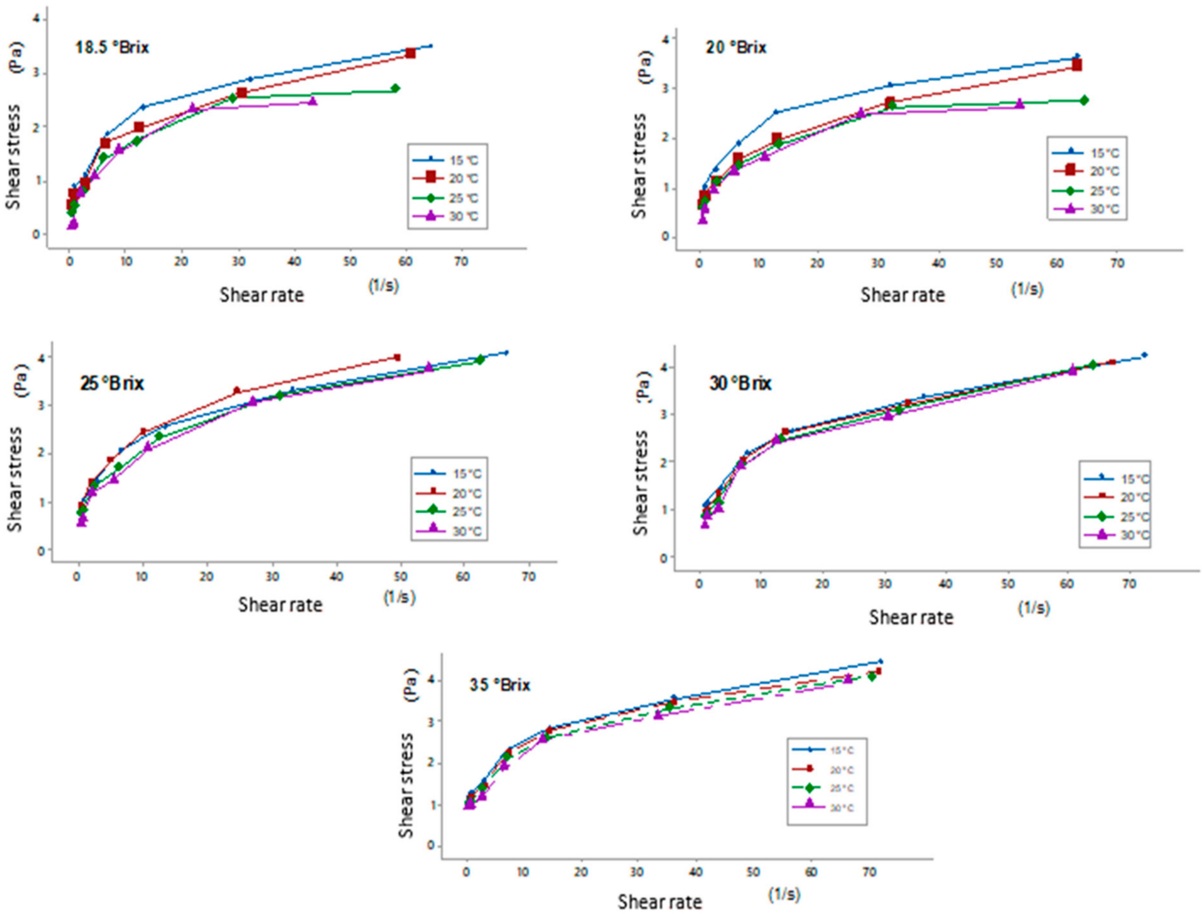

Figure 1. Rheogram for puro puro pulps at different concentrations (18.5, 20, 25, 30, and 35 °Brix) and temperatures (15, 20, 25, and 30 °C).

The influence of different temperatures and concentrations on the apparent viscosity is shown in Table 3, and the relationship between apparent viscosities and strain rate is shown in Figure 2. Under a fixed temperature and strain rate, the apparent viscosity increases as the concentration increases. This is explained by the fact that, at low concentrations, the puro puro juice molecules are separated from each other. In contrast, as the concentration increases, the molecules remain bound together, eventually forming an entangled network [6,25]. Viscosity tends to increase with concentration, especially during the entanglement stage and, at the same time, viscosity becomes more dependent on the shear rate [16,20,26].

Table 3. Variation in apparent viscosity of puro puro juice with rotational speed at different temperatures and concentrations.

Temperature (°C)	Rotation Speed (rpm)	Concentration (°Brix)				
		18.5	20	25	30	35
15	0.5	2.16	2.37	2.52	2.70	9.27
	1.0	1.34	1.45	1.55	1.62	5.40
	4.0	0.51	0.54	0.58	0.59	1.83
	10.0	0.27	0.28	0.31	0.30	0.89
	20.0	0.17	0.17	0.19	0.18	0.52
	50.0	0.09	0.09	0.10	0.09	0.25
	100.0	0.06	0.06	0.06	0.06	0.15

Table 3. *Cont.*

Temperature (°C)	Rotation Speed (rpm)	Concentration (°Brix)				
		18.5	20	25	30	35
20	0.5	1.83	1.96	2.37	2.51	7.87
	1.0	1.16	1.22	1.47	1.54	4.61
	4.0	0.46	0.47	0.56	0.58	1.58
	10.0	0.25	0.25	0.30	0.30	0.78
	20.0	0.16	0.16	0.18	0.19	0.46
	50.0	0.09	0.09	0.10	0.10	0.23
	100.0	0.06	0.05	0.06	0.06	0.13
25	0.5	1.50	1.80	2.18	2.30	6.00
	1.0	0.97	1.12	1.37	1.43	3.49
	4.0	0.40	0.43	0.54	0.55	1.18
	10.0	0.22	0.23	0.29	0.29	0.57
	20.0	0.14	0.14	0.18	0.18	0.33
	50.0	0.08	0.08	0.10	0.10	0.16
	100.0	0.05	0.05	0.06	0.06	0.09
30	0.5	0.84	1.36	1.74	2.06	6.14
	1.0	0.61	0.90	1.15	1.31	3.54
	4.0	0.33	0.39	0.50	0.53	1.18
	10.0	0.22	0.23	0.28	0.29	0.57
	20.0	0.16	0.15	0.19	0.18	0.33
	50.0	0.10	0.09	0.11	0.10	0.16
	100.0	0.08	0.06	0.07	0.06	0.09

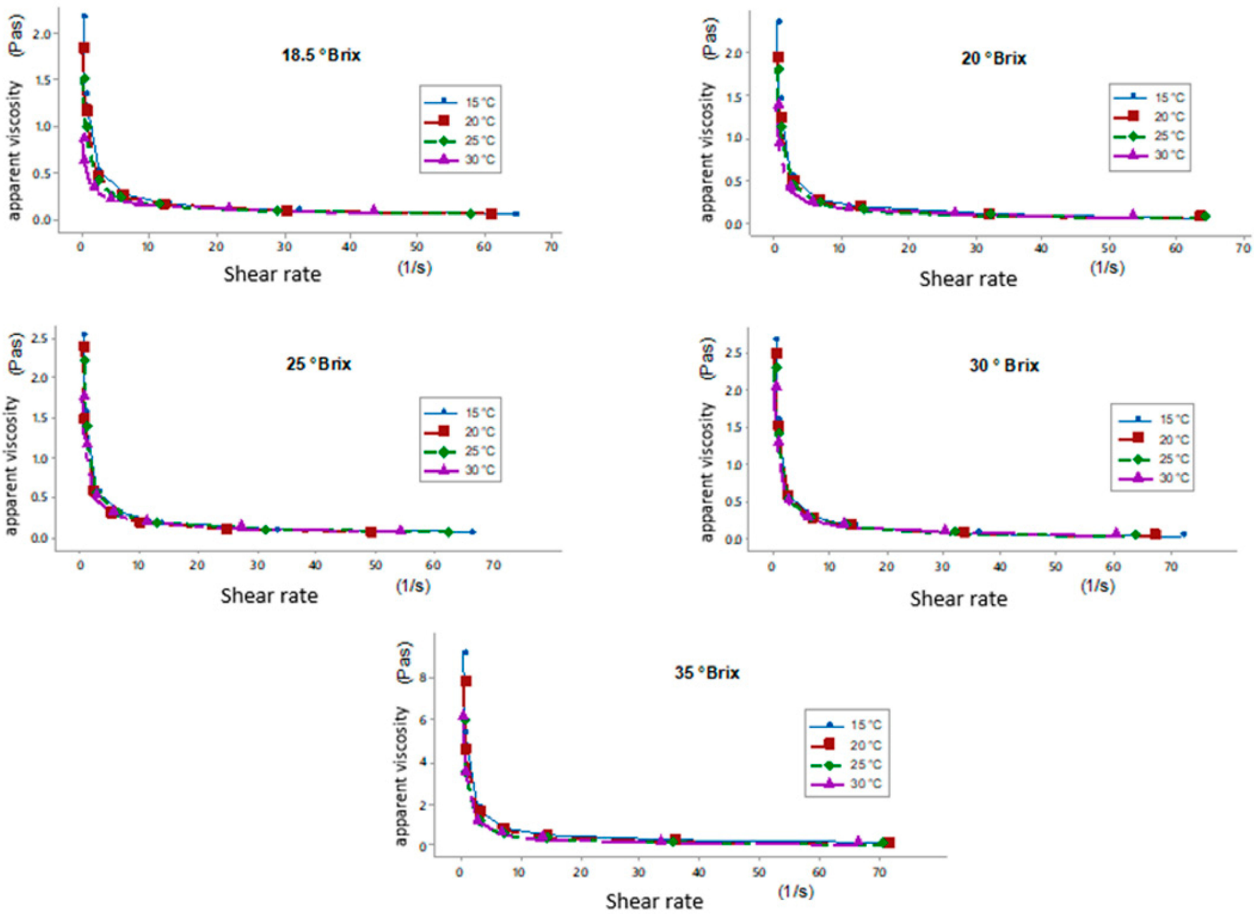

Figure 2. Apparent viscosity vs. shear rate of "puro puro" juice at different concentrations (18.5, 20, 25, 30, and 35 °Brix) and temperatures (15, 20, 25, and 30 °C).

The results indicate (Figure 2) that, at a given concentration, the apparent viscosity decreases with increasing temperature because, at elevated temperatures, liquids flow easily [21,27].

The puro puro juice has excellent physicochemical properties. The content of total ash, total fat, crude protein, and carbohydrates (including fiber) are higher than the values of other species of the genus *Passiflora*. However, its antioxidant activity is low, being its value lower than the antioxidant activity of other species of the same genus. The rheological characterization of the pure juice was developed in the range of temperatures (15, 20, 25, and 30 °C) and concentrations (18.5, 20, 25, 30, and 35 °Brix). The puro puro juice was considered as a pseudoplastic fluid, and is described according to the Power Law model. The results also reported that, as the temperature increases, the consistency index decreases and the flow behavior index increases, while, as the concentration increases, the consistency index increases and the flow behavior index decreases. Apparent viscosity is proportional to concentration and inversely proportional to temperature.

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