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Scientific Research

Phytochemical Composition and Antioxidant Capacity of *Psidium guajava* Fresh Fruits and Flour

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Abstract

***Psidium guajava* fruits are consumed fresh or processed (beverages, syrup, ice cream, jams and jellies). Guayaba is a fruit highly perishable and susceptible to damage during the postharvest. One strategy to overcome this problem is its processing by using techniques that preserve its organoleptic, nutritive and functional properties and allow getting food with added value. The purpose of this study was to obtain flour from fresh fruits cultivated in Argentina Northwestern by lyophilization and to determine the antioxidant activity and the main phytochemicals present in fresh fruits and flour. Nutritional composition (sugar, protein and fat) and the bioactive phytochemicals (total phenolic compounds, flavonoid phenolic, condensed and hydrolizable tannin, ascorbic acid, pigments such as anthocyanin and carotenoids) as well as fiber content, were evaluated. The flour preserved flavor, aroma and color of pulp from fresh fruits. The flour contained around 30% of sugar, 20% of total protein, 0.5% of fat and high level of crude fiber. Carotenoids and ascorbic acid were the dominant phytochemicals in flour as well as in fresh fruits. The guayaba flour showed antioxidant activity with SC_{50} values similar to fresh fruits. The flour showed nutraceutical characteristics that are demanded by functional food and could be used as a dietary supplement.**

Keywords

Fresh Fruit; Flour; *Psidium guajava*; Nutritional Properties; Functional Properties

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1. Introduction

The *Psidium* genus is a member of Myrtaceae family and is represented by approximately 120 - 150 species [1]. *Psidium guajava* L., (popular name in Argentina "guayaba") native to tropical America, is a tree which is now widely distributed throughout the tropic and subtropical areas, and its fruits are consumed fresh or processed. The major producers of "guayaba" are South Africa, India, Hawaii, Colombia, Puerto Rico, Jamaica, Brazil, and Israel. In Argentina, the production area is limited but is currently in active expansion. Guayaba fruits can be round, oval, or pear-shaped, 3 - 10 cm in diameter. The color of the peel of the ripe fruit is yellow-orange, and the color of the flesh can be white, pink, yellow, salmon, or carmine, depending on the variety. Guayaba fruits are good sources of vitamin C and carotenoids [2]-[4]. Guayaba passes through different stages of harvesting and post-harvest conservation. Later, the ripe fruits are processed by the consumer. The products obtained from them (juices, fruit salads, beverages, syrup, ice cream, jams and jellies) have a limited useful life time principally due to oxidation [5]. The slicing of fruits promotes contact between enzymes such as oxidases and peroxidases and its substrate (in presence of light and oxygen), which may cause degradation of phytochemical components. Cutting also promotes the synthesis of ethylene, accelerating the senescence processes with increases in oxidase activity, including lipoxygenase enzyme activity, causing the oxidation of fatty acids and carotenoids [6]. One strategy to overcome this problem is its processing using techniques that preserve its organoleptic, nutritive and functional properties and allow getting food with added value. The aim of this study was to obtain flour from fresh fruits cultivated in Argentina Northwestern by lyophilization, to determine the antioxidant activity and the main phytochemicals and to compare with fresh fruits.

2. Materials and Methods

2.1. Abbreviations

Glucose equivalent (GE); bovine serum albumin equivalent (BSA-E); fresh weight (FW); gallic acid equivalents (GAE); 4-dimethylamino-cinnamaldehyde (DMAC); procyanidin B2 equivalents (PB2E); cyanidin-3-glucoside equivalents (C3-G E); β-carotene equivalents (β-CE); L-ascorbic acid (L-AA); 2,2'-azinobis-(3-ethylbenzthiazoline-6- sulphonic acid (ABTS); butyl hydroxi toluene (BHT).

2.2. Plant Material

The ripe fruits of *P. guajava* L. (**Figure 1**) were harvested in Horco Molle, Tucumán, Argentina.

2.3. Preparation of Forms Extractive from Fresh Fruits

Fruits were cut and homogenized with a Waring blender (20% w/v) and maintained at −20˚C.

2.4. Flour Preparation from "Guayava" Fruits

Ripe fruits were lyophilized and then were grounded to obtain flour (**Figure 1**).

2.5. Nutritional Components Measurement

2.5.1. Sugar

Sugar determination: The phenol-sulphuric acid method [7] was used to determine total neutral sugars. Reducing sugars were measured using the Somogyi-Nelson method [8] [9]. Results were expressed as g of GE/100g flour or fresh weight.

2.5.2. Protein

Soluble protein concentration was determined by the method of Bradford [10] using BSA as standard. Results were expressed as mg BSA-E/100g flour or FW. The total Nitrogen (N) content of fresh fruits or flour was determined by Kjeldahl method [11] (AOAC, 1998). Crude protein content was calculated as % N × 6.25.

2.5.3. Fat

An aliquot of 20 g of sample was used to determine crude fat by extracting with petroleum ether (40˚C - 60˚C) in a Soxhlet apparatus during 4 h [12] (AOCS, 1989).

(a) (b)

Figure 1. Guayaba fruits (a), flour from fruits (b).

2.6. Functional Phytochemical

2.6.1. Total Polyphenols Extraction and Measurement

Ripe fruits and flour were macerated in ethanol 96˚ (1 g of tissue per 5 mL of ethanol) for 1 day with stirring (40 cycles/min) at room temperature in glass vessels in dark conditions. Then, the preparation was maintained in an ultrasonic bath for 30 min at room temperature and then centrifuged at 12,000 *x g* during 10 min. The remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined and evaporated in vacuo (40˚C).

Total phenolic content of the samples was determined using the Folin-Ciocalteu reagent [13]. Results were expressed in mg GAE/100g flour or FW. Non-flavonoid phenols were measured by determination of total phenol content remaining after precipitation of the flavonoids with acidic formaldehyde [14]. Results were expressed in mg GAE/100g flour or FW.

2.6.2. Tannins Extraction and Measurement

Flour or fresh fruits (1 g) was extracted with 12.5 mL acetone: water (70:30, v:v) in an ultrasonic bath for 30 min at room temperature and then centrifuged at 9000 *x g* during 10 min. The pellet was extracted exhaustively with the same solvent system. All organic extracts were combined and the acetone was evaporated in vacuo (40˚C), then the final volume was adjusted to 5 mL.

Condensed tannins: The total condensed tannins content was determined with DMAC according to Prior *et al*. [15]. 450 µL of DMAC solution (0.1% in acidified ethanol) was added to 150 µL of tannin extraction. The absorbance was measured at 640 nm after 20 min at 25˚C. Data were expressed as mg PB2E/100g flour or FW.

Hydrolized tannins: The aqueous fraction was subjected to acid hydrolysis by adding sulphuric acid (2N) and heating at 100˚C during 26 h and the gallic acid released was determined with the rhodanine method [16]. The hydrolyzed fraction and non hidrolyzed fraction were freeze-dried and resuspended in 200 µL of 0.2 N H_2SO_4. Two hundred µL of 0.2 N H_2SO_4 and 300 µL rhodanine (0.67% in methanol) were added to the diluted extracts. After 5 min, 200 µL of 0.5 N potassium hydroxide and 4 mL distilled water were added and the absorbance at 520 nm was determined. Gallotannin concentrations were expressed as mg GAE/100g flour or FW of fruits obtained by difference between the amount of gallic acid present in hydrolized sample and without hydrolysis.

2.6.3. Pigments Extraction and Measurement

1) Total monomeric anthocyanins

Flour or fresh fruit (1 g) was extracted with 5 mL 1% HCl in methanol overnight at 5˚C and then centrifuged at 12,000 *x g* during 10 minutes. The pellet was extracted three times with the same solvent and combined, vacuum-concentrated at 40˚C and resuspended with 5 mL MILLIQ water. Total anthocyanins were evaluated by the pH differential method [17]. The content of total anthocyanins was expressed as mg C3-G E/100g flour or FW.

2) Total carotenoids

Samples (1 g) were extracted with 5 mL of hexane:acetone:ethanol (50:25:25, v/v). After centrifugation at 13,000 *x g* for 10 min at 4˚C, the top hexane layer was recovered and the absorbance was measured at 450 nm [18]. Total carotenoid content was calculated as mg β-CE/ 100g flour or FW.

2.6.4. Ascorbic Acid Extraction and Measurement

Samples (0.2 g) were extracted with 500 µL of H_3PO_4 2% and centrifuged at 12,000 *x g* during 10 min. The su-

pernatant was reserved to determine the AA content using 2,6 dichloroindophenol sodium salt hydrate according to Barros *et al*. [19]. AA content was expressed as mg L-AA/100g fresh fruit or flour.

2.6.5. Crude Fiber

Fiber content was determined according to Jaafar *et al*. (2009) [20]. Two grams of samples were put into a 250 mL conical flask and 1.25% sulphuric acid solution was added. The sample was heated for about 30 min, filtered using a vacuum filter and washed until traces of acid were undetected. A Whatman 5B paper was placed in the Buchner flask. After the acid digestion, the sample was transferred to a 250 mL conical flask and 3.52% NaOH solution was added. The sample was heated again for 30 min. It was filtered using a vacuum filter and washed with water until base was undetected. The whole material was placed in a crucible and dried for 12 h at 120˚C. The crucible was heated in a muffle oven at 550˚C for 12 h and weight of the crucible was recorded.

2.7. Measurement of Antioxidant Capacity

The antioxidant capacity assay was carried out by the improved $ABTS^{\bullet+}$ method [21]. $ABTS^{\bullet+}$ solution (1 mL) was added to extractions or commercial antioxidant (BHT, AA and quercetin) and mixed thoroughly. Absorbance was recorded at 734 nm during 6 min. The concentration of guayaba extract required to scavenge 50% of $ABTS^{\bullet+}$ (SC_{50} values) was calculated as µg GAE/mL.

3. Results

3.1. Nutritional Composition of Fresh and Lyophilized (Flour) Fruits

Fresh fruits of guayaba were cut and homogenized to obtain a “juice” or lyophilized and grounded to obtain “flour”. The weight of fruit pulp before and after lyophilization was measured. After freeze drying, weights of fruits pulp were 15% of initial wet weight.

Fresh fruits from Horco Molle (Argentina) showed lower level of sugar (2.6%) than the reported to fruits from Brasil (13%) [22] and similar to those from Ecuador (4.5%) [23], **Table 1**. The content of sugar of fresh fruit was lower than kiwi, apple, orange and grape [24]. The total protein content (4.2%) was similar to that reported to *P. guajava* from Jamaica [25] and higher than variety from Venezuela (1.0%) [26]. The results showed that the chemical composition of a fresh fruit depends on its stage of maturity, the moisture, the collection season and the zone where it has grown.

When the fruits were lyophilized and grounded, the flour preserved flavor, aroma, color (**Figure 1**) and macronutrients content of pulp from fresh fruit (**Table 1**). The flour contained around 30% of total sugar; 20 % of total protein and 0.5% of fat. The crude fiber content in fresh fruit and flour was 11.9% and 12.7%, respectively. According to international normative the fruits could be included as fruits with high fiber level. This content of fiber is an indicator of the considerable amounts of celluloses and hemicelluloses present. A high proportion of fiber could be considered an advantage. The flour can be used by the food industry as an ingredient to increase the content of indigestible insoluble compounds. In addition, high fiber content could have beneficial health effects related to increases in satiety and in the volume and weight of faecal mass, thus promoting improved functioning of the digestive system [27] [28].

3.2. Phytochemical Composition of Fresh Fruits and Flour

3.2.1. Ascorbic Acid

The content of AA was approximately 20 mg/100g of fresh fruit (**Table 2**), about half of the content of AA in the kiwi (55 mg/100g), which is an excellent source of vitamin C, and similar to tree tomato (20 mg/100g) and lima (21 mg/100g).

According to National Sanitary Surveillance Agency (ANVISA, 1998), fruits of “guayaba” that grow in Argentina could be considered as a good source of vitamin C because it contributes with around 30% of the index of dietary uptake. The AA content of guayaba from Argentina was lesser than *Psidium guajava* of Brazil and higher than guayaba from Peru (9.8%) [29]. The differences may be due to several factors, such as cultivars, seasons, country of origin (soil, climate and cultivation techniques), harvest, postharvest, analytical methods for extraction and quantification of vitamin C, moisture, etc.

The AA was an abundant component in flour (143 mg/100g flour) as well as fresh fruit. Hence, the consump-

Table 1. Macronutrients in fresh fruits and flour from guayaba fruits.

	Fresh fruit	Flour
Total sugar g G-E/100g FW or flour	2.56 ± 0.1	29.87 ± 0.1
Reducing sugar g G-E/100g FW or flour	0.18 ± 0.1	1.48 ± 0.1
Soluble Protein mg BSA-E/100g FW or flour	50 ± 0.2	369.24 ± 3
Total protein (%)	4.2	20
Fat (%)	0.50 ± 0.05	0.05 ± 0.02
Crude fiber (%)	11.9 ± 0.05	12.7 ± 0.05

Grams of glucose equivalent/100g of fresh weigth or flour (gGE/100 gFW or flour); milligrams of bovine serum albumin equivalent/100g of fresh weigth or flour (mg BSAE/100g of FW or flour).

Table 2. Phytochemicals in fresh fruits and flour from guayaba fruits.

	Flour	Fresh fruits
Phytochemical contents	expressed in 100 g flour	expressed in 100 g Fresh Weight
TP	970.34 ± 7	50.35 ± 2
nF-P	13.22 ± 0.2	0.56 ± 0.05
FP	957.12 ± 9	49.79 ± 2
CT	208.02 ± 0.8	8.51 ± 0.8
AN	16.0 ± 0.01	2.3 ± 0.02
AA	143.49 ± 2	19.73 ± 2
C	2300 ± 20	821.49 ± 30

Data expressed as mg GAE/100g [total phenolic (TP), non-flavonoids phenolics (nF-P), flavonoid phenolic (FP)]; mg procyanidin B2/100g [condensed tannins (CT)]; mg C3GE/100g [anthocyanin (AN)]; mg AA/100g [ascorbic acid (AA)] and mg E β-C/100g [carotenoids (C)].

tion of 50 g of flour or 300 g of fresh fruits may be necessary to cover these requirements in ascorbic acid.

3.2.2. Polyphenolic Compounds

According to the classification of Vasco *et al*. (2008) [30], guayaba fresh fruits would belong to Category 1 with a low level of total polyphenols (<100 mg GAE/100g). The flavonoids were the major polyphenolic with recognized functional potential. The flavonoid content was 49.79 mg/100g of guayaba.

The total polyphenol content of flour was around 970 mg GAE/100g flour (**Table 2**). Flour from guayaba would be considered to be in the category 3 with high phenolic content (>500 mg GAE/100g). Flavonoid phenolics were also the dominant phenolics in the flour (957 mg/100g flour).

Since the average value of uptake of flavonoids is 23 mg per day, 100 g guayaba fresh fruits per day (2 ripe fruits) or 3 g of flour are sufficient to meet the requirements of flavonoids.

3.2.3. Pigments

Differential extractions were performed from ripe fruits and the anthocyanin content was determined (2.3 mg EC-3G/100g FW). The content was similar to the brazilian guayaba fruits (2.7 mg EC-3G/100 FW). In addition to the health effects have a direct impact on the quality of the products that contain them, contributing to their sensory properties. Anthocyanins are one of the pigments responsible for the colors pink, red and violet to dark blue fruits.

Other pigments are carotenoids, which act as precursors of vitamin A, anti-inflammatory and antioxidant [28], [31] [32]. Guayaba is rich in carotenoids (0.82 g E β-C/100g FW). Lower levels were reported for guayaba fruits of Colombia [33], which may result from differences in the methods of extraction and quantification techniques, storage conditions, maturity, varieties/cultivars, including others [34].

The anthocyanin content in flour was similar to fresh fruit while the carotenoid content of fresh fruit was higher than flour (**Table 2**) taking into account the moisture of fresh fruit (85%).

3.3. Antioxidant Activity of Fresh Fruits and Flour

The antioxidant activity of juice and extracts obtained from fresh fruits and flour was analyzed. All preparations

exhibited ABTS cation radical reducing capacity (**Figures 2** and **3**). Ethanolic and acetonic extracts were more active than juice and aqueous extract obtained from fresh fruit and flour, respectively (**Table 3**). All extracts obtained from flour were more active than fresh fruits (SC_{50} values of 4.1 to 10.2 µg GAE/mL for extracts of flour and 5.2 to 14.2 for extracts from fresh fruits).

In all cases, a dose-response relationship between the scavenging activity percentage and phenolic compound content was observed (**Figures 2** and **3**). The scavenging activity in general was higher than commercial natural and synthetic antioxidants used in food industry such as quercetin (SC_{50}= 18 µg/mL), ascorbic acid (SC_{50} = 54 µg/mL) and BHT (SC_{50} = 55 µg/mL).

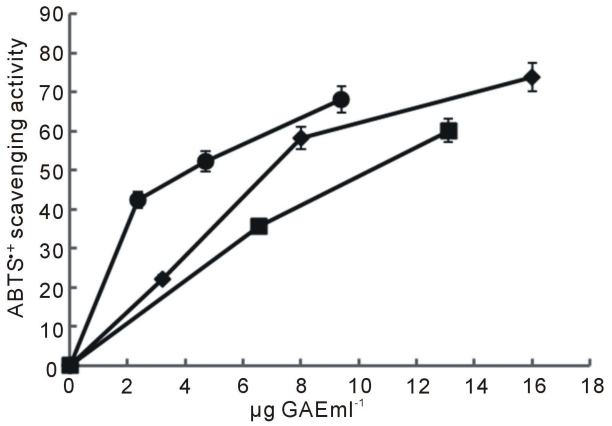

Figure 2. ABTS$^{\bullet+}$ scavenging activity in aqueous (■), ethanolic (♦) and acetone-water (●) extracts from "guayava" flour.

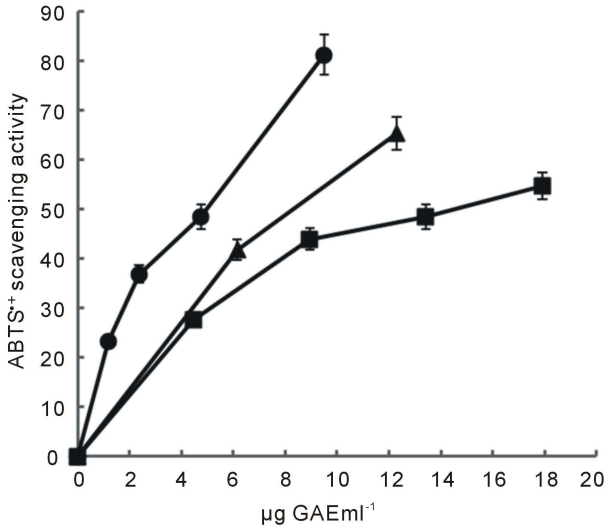

Figure 3. ABTS$^{\bullet+}$ scavenging activity in juice (■), ethanolic (▲) and acetone-water (●) extracts from fresh fruits.

Table 3. Phytochemicals in fresh fruits and flour from guayaba fruits.

	SC_{50} (µg GAE/mL)	
	Fresh fruits	Fruit Flour
Juice/aqueous extract	14.2 ± 1.2	10.2 ± 1.2
Ethanolic extract	8.2 ± 0.8	7.0 ± 0.7
Acetone-water extract	5.2 ± 0.05	4.1 ± 0.3

4. Conclusions

In conclusion, not only fresh fruits have potential as an antioxidant but also the flour obtained from "guayava" fruits maintained this potential. The antioxidant effect can be explained by their content of flavonoids phenolic compounds, vitamin C and carotenoids.

The flavonoids usually act by neutralizing free radicals and by chelating transition metals. The antioxidant capacity of them could be attributed to the reducing power [35]. The mechanism by which the carotenoids protect biological systems depends on the energy transfer from the excited oxygen to carotenoid [36]. Vitamin C can also act by oxygen radicals scavenging [37].

Scientific evidence on the health benefits of "guayaba" flour in addition to their nutritional quality provides added value to the "guayaba" fruits. The flour could be used as a dietary supplement or nutraceutical.

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