Proximate Composition, Nutrient Mineral and Fatty Acid of the *Bunchosia glandulifera* Fruit

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Abstract *Bunchosia glandulifera* is an exotic species found in southern of Brazil, of which the pulp and toasted seed are much used because of their stimulating properties and nutritional values. However, few scientific data are reported about the chemical composition of the *B. glandulifera* fruit and the benefits its consumption. The aim of this study was to determine the fatty acid, proximate and mineral composition of the fruit. The results revealed that soluble solids and sugars are present at high concentration in the pulp, whereas the seed predominantly contained protein and ash. According to the particle-induced X-ray emission (PIXE) measurement, the elemental composition revealed that K, P, Ca, and Mg are the most abundant minerals in the fruit. Interestingly, the ingestion of 200 g of the fruit provides 100% of the recommended daily ingestion (RDI) of some elements. Moreover, among the seventeen fatty acids found in the pulpy seed, palmitic acid (C16:0) was found to be the most concentrated.

Keywords: mineral analysis, proximate composition, fatty acid, *Bunchosia glandulifera*

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1. Introduction

Fruits are important constituents of diet, providing energy, antioxidants and minerals, which are essential to the maintenance of several physiological functions. According the World Health Organization, the regular consumption of fruits is a determining factor for body maintenance in a healthy diet. It is estimated that low consumption of fruits and vegetables causes about 2.7 million deaths every year, thus being one of the top-10 risk factors contributing to mortality [1].

Among the huge variety of fruits found in Brazil, *B. glandulifera* is an exotic species that is cultivated at private residences or farms, mainly in the south of the country such as in the Santo Antonio da Patrulha region (29° 50' 18" S, 50° 30' 58" W). Popularly known as “guaraná, caferana, or falso-guaraná”, the *B. glandulifera* fruit presents small size and red color when ripe [3]. The color is associated with the presence of carotenoids, which are bioactive compounds known to stimulate cancer and cardiovascular diseases. The main carotenoid present in the pulp of the *B. glandulifera* fruit is lycopene. In addition, the fruit is also rich in phenolic compounds totaling approximately 2.193 mg/100g, which is higher than the values found for most fruits [4].

According to the local population, the fruit possesses stimulating and nourishing properties, and its frequent consumption provides longevity, physical vigor, and mental clarity. These effects may be associated with the presence of caffeine in the pulp and seed of *B. glandulifera* at high concentration. The caffeine is a powerful stimulant of the central nervous system and, when consumed in adequate amounts, the consumer experiences reduced drowsiness and fatigue, and increased intellectual activity [4].

Knowledge of the elemental composition and nutritional factors of fruits is essential for the evaluation of consumption and development of new food products. A healthy diet should include minerals and lipids, which are important nutrients found in different types of fruits. A balanced intake of fatty acids is directly associated with cardiovascular disease prevention [2]. In addition, since minerals play important roles in several physiologic events, it is necessary to maintain a balanced mineral concentration to avoid diseases related to their excess or deficiency.

However, the literature contains only a few reports on *B. glandulifera*, which emphasizes the need for additional research on this species. Therefore, this study aimed to determine the elemental composition of *B. glandulifera* fruit using particle-induced X-ray emission (PIXE), and the fatty acid and proximate content using chemical approaches.
2. Material and Methods

2.1. Sample Collection

*B. glandulifera* fruits were collected on January 2015 at Santo Antonio da Patrulha, a city located near the northeast seacoast of the Rio Grande do Sul state, Brazil. A voucher specimen was deposited in the Herbarium of the Federal University of Rio Grande do Sul (UFRGS), under the number 167276ICN with designation *Bunchosia glandulifera* (Jacq.) Kunth (Malpighiaceae). After collection, fruits were washed with distilled water. Seed and pulp were separated and stored in polyethylene bags at −18°C until proximal composition and fatty acid analysis. For PIXE analysis, pulp and seed were dried for approximately 5 h at 100°C, macerated using mortar and pestle, and pressed into pellets.

2.2. Proximal Composition

The proximal composition analysis (pH, ash, soluble solids, total sugars, lipids, moisture, reducing sugars, proteins, and fiber) was performed in triplicate and in accordance with the Analytical Standards Institute Adolfo Lutz [5]. The total carbohydrate content, including sugars and fiber, was determined using the equation Total carbohydrate =100 – (moisture + ash + proteins + lipids). The total energy value was estimated using the water conversion values of 4 Kcal/g for protein and carbohydrates and 9 Kcal/g for lipids.

2.3. Fatty Acid Composition

The oil to fatty acid analysis was obtained using 20 g of pulp and seed samples, which subsequently suffered extraction with chloroform / methanol following method Bligh and Dyer [6].

Fatty acid methyl esters (FAMEs) were prepared by methylation, according to the methodology proposed by Joseph and Ackman [7]. In particular, 25 mg of oil was added to 1.5 mL of 0.50 M NaOH in methanol and the mixture was heated in water bath at 100°C for 5 min. Then, 2 mL of solution BF$_3$ (14% BF$_3$ in methanol) and heated in water bath at 100°C for 30 min. After cooling, 1 mL of isooctane was added and the solution was slowly stirred for 30s. Then, 5 mL of saturated NaCl aqueous solution was added. The supernatant was transferred into a clean flask, 1 mL of isooctane was added and the resulting solution was stirred. The solvent was removed under nitrogen flow until a final volume of 1 mL was reached for chromatographic analysis.

FAMEs were separated using a Shimadzu gas chromatographer (model CG-2010) equipped with flame ionization detector (FID) and Omegawax 250 capillary column (30 × 0.25 mm i.d. × 0.25 μm). The gas flow rates were 1.2 mL/min for H$_2$ (carrier gas), 30 mL/min for N$_2$ (makeup gas), and 30 and 300 mL/min for H$_2$ and synthetic air (flame gases), respectively. Each sample (1 μL) was automatically injected in triplicate, and the sample split rate was 1:50. The operational parameters were as follows: injection and detector temperatures were maintained at 250°C and 260°C, respectively; initial column temperature of 50°C was increased to 220°C at a rate of 4°C/min. For fatty acid (FA) identification, the retention times were compared to those of FAME standards.

2.4. PIXE Analysis

PIXE consists in a multi-elemental technique based on the characteristic X-ray emission induced by an energetic ion beam, with enough limit of detection for the most part of minerals (about 1 ppm) [8,9]. All measurements were performed using a 2-MeV proton beam generated in a 3-MeV electron accelerator. Samples were accommodated in a holder inside the PIXE reaction chamber, which was kept at a pressure of 10$^{-6}$ mbar. Each sample was irradiated for 400 s using an average current of approximately 3 nA. The X-rays emitted from the samples were detected by a Si(Li) detector with an energy resolution of approximately 160 eV at 5.9 keV. PIXE spectra were fitted using the GUPiXWIN software [10] and elemental concentrations were obtained. For PIXE standardization purposes, in this study, the apple leaves standard from the National Institute of Standards and Technology (NIST reference material 1515) was used.

2.5. Statistical Analysis

Statistical analysis of the elemental concentration consisted in performing the Student’s *t*-test (SPSS version) with 5% significance level (P < 0.05) to compare results obtained from seed and pulp.

3. Results and Discussion

Table 1 shows the proximate composition for the pulp and seed of the *B. glandulifera* fruit. According to the results, the pulp contains a higher concentration of total sugars and soluble solids than the seed does. Soluble solids are mainly constituted by sugars, which are important for fruit processing and make the fruit pulp the most appreciated part of the plant for consumption [11]. The seed contains a higher concentration of proteins and ash, which confer high nutritional value, than other parts of the plant. The pulp and seed of *B. glandulifera* exhibited total energetic values higher than 100 Kcal/100 g, which makes the fruit suitable for consumption in high-calorie diets.

|                | Pulp (%) | Seed (%) |
|----------------|----------|----------|
| Moisture (%)   | 72.00±0.06 | 55.00±0.55 |
| Ash (%)        | 1.30±0.09  | 2.90±0.04  |
| Fat (%)        | 0.13±0.04  | 0.30±0.07  |
| Proteins%      | 2.45±0.09  | 7.00±0.09  |
| pH             | 5.09±0.00  | 8.12±0.02  |
| Reducing sugars (%) | 3.57±0.08 | ----       |
| Total sugars (%) | 6.25±0.09 | 1.66±0.01  |
| Soluble solids (Brix) | 11.72±0.01 | 4.52±0.04 |
| Fiber (%)      | 0.43±0.01  | 1.94±0.09  |
| Total Carbohydrates (%) | 24.12±0.02 | 34.80±0.24 |
| Total energetic value (Kcal/100g) | 107.43±0.05 | 193.3±0.13 |

Values are for std deviations of carefully conducted triplicate experiments. Furthermore, mean carry different superscripted alphabets vary *(p<0.05)* with 95% confidence.
PIXE results showed that K is the most abundant mineral in the pulp and seed of *B. glandulifera* fruit, since the seed has a higher concentration of K than the pulp. The consumption of 200 g of *B. glandulifera* fruit pulp provides 65.5% of the recommended daily ingestion (RDI) value (4700 mg/d), while the same amount of seed provides 86% of the RDI value.

Usually, fruits are rich in K because this element presents great mobility in plants. In addition, its low affinity for organic chelates explains why this metal is present at high concentration in vegetal tissues [12]. When associated with Na in humans, K plays a role in maintaining water balance and neural impulse transmission. Interestingly, the K level in the *B. glandulifera* fruit pulp is similar to that found in the pulp of *Myrciaria cauliflora* (*jaboticaba*) and *M. cauliflora* seed [12], whereas it is higher than that found in *Citrus reticulata* (tangerine) (599 mg/100 g) [13].

The fruit pulp and seed of *B. glandulifera* also contain S, P, Ca, and Mg at high concentration, and the seed has a higher concentration of these minerals than the pulp. P concentration in the seed may be associated with the presence of phytin, a compound considered a reservoir of phosphorus during the germination period. P plays an important role in cell structure and many biochemical reactions such as energy metabolism [14]. Mg is associated with the green pigmentation of plants and is more concentrated in the seed than in other parts of the fruit [15]. Since the consumption of 200 g of the fruit pulp provides 57% of the RDI (260 mg/d) and the consumption of the same amount of seed provides 100% of the RDI value, the *B. glandulifera* fruit is an excellent source of this element.

Moreover, the results showed that the pulp showed higher concentration the Fe and Al than seed. The consumption of 200 g of the *B. glandulifera* pulp provides more than 100% of the Al and Fe RDI values. Despite its importance in several physiological processes such as oxygen transportation in the muscles [16], Fe excess (average intake is 14 mg/d) may be associated with oxidative stress, as well as Al excess (intake ranges from 0.3 to 3.6 mg/d) is involved in bone phosphate mobilization, thus influencing ionic balance [17,18].

The concentration of Si in the pulp of *B. glandulifera* was higher than in the seed and the estimated intake range of 10-25 mg / d. Although Si is an important mineral in the production of collagen, its accumulation in animal tissues may be related to increased oxidative stress [19].

Others important macronutrient and micronutrients (e.g., Zn, Ca, and Mn) were also detected in the *B. glandulifera* fruit whit higher concentration in the seed. This element participates in reactions involving the synthesis and degradation of secondary metabolites and nucleic acids. Ca, Zn, and Mg are required as cofactors in enzymatic processes and participate to the structure of the DNA self-repair system [20]. Interestingly, 200 g *B. glandulifera* fruit provide more than 100% of the Mn RDI value (2.3 mg/day). Mn concentration in *B. glandulifera* fruit is similar to that found in other exotic fruit such as *bacuri* and *ingá-açu* [21].

Table 3 shows the fatty acid composition of the pulp and seed of the *B. glandulifera* fruit (Table 3). Thirteen fatty acids were found, including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). The pulp showed higher fatty acids content than the seed did. In particular, palmitic acid (C16:0) was predominant. Among saturated fatty acids, palmitic acid is the most abundant in diets. Moreover, it is the main fatty acid (40%) found in the palm oil (*Elaeis guineensis*) [22]. A previous study reported that this acid elevates plasma concentration of cholesterol and low-density lipoprotein (LDL) [2].

Another saturated fatty acid found at high concentration is stearic acid. The seed showed higher concentration this acid than the pulp. The stearic acid is rapidly metabolized to oleic acid, a monounsaturated fatty acid beneficial to humans [23].

### Table 2. Elemental composition of *Banchosia glandulifera*

| Mineral | Pulp | Seed |
|---------|------|------|
| Na      | 5.37 ± 1.00 | 8.69 ± 2.10 |
| Mg      | 74.4 ± 6.90  | 152.41 ± 31.00|
| Al      | 16.66 ± 5.90 | 12.30 ± 3.60 |
| Si      | 31.62 ± 13.20 | 9.71 ± 2.30 |
| P       | 161.37 ± 14.80 | 364.81 ± 73.20 |
| S       | 86.94 ± 9.80  | 181.96 ± 35.00 |
| Cl      | 32.79 ± 2.10  | 37.19 ± 7.80 |
| K       | 1538.89 ± 117.50 | 2022.63 ± 378.90 |
| Ca      | 132.70 ± 11.50 | 207.96 ± 40.00 |
| Ti      | 0.41 ± 0.01   | 0.37 ± 0.02 |
| Cr      | 0.32 ± 0.02   | 0.25 ± 0.08 |
| Mn      | 1.03 ± 0.20   | 3.93 ± 0.80 |
| Fe      | 7.96 ± 4.50   | 5.72 ± 2.60 |
| Ni      | 0.55 ± 0.47   | 0.19 ± 0.07 |
| Cu      | 0.58 ± 0.18   | 0.55 ± 0.15 |
| Zn      | 2.29 ± 0.20   | 4.01 ± 0.80 |
| Rb      | 2.82 ± 0.90   | 3.56 ± 0.80 |
| Sr      | 1.70 ± 0.70   | 1.39 ± 0.20 |

Values are for std deviations of carefully conducted triplicate experiments (mg/100 g). Furthermore, mean carrying different superscripted alphabets vary (p<0.05) with 95% confidence.

| Mineral | Pulp | Seed |
|---------|------|------|
| Lauric (C12:0) | 0.25 | - |
| Myristic (C14:0) | 1.23 | 0.67 |
| Myristoleic (C14:1) | 7.89 | 9.22 |
| Palmitic (C16:0) | 67.26 | 38.57 |
| Palmitoleic (C16:1) | 0.75 | 3.35 |
| Stearic (C18:0) | 7.40 | 13.28 |
| Oleic (C18:1, n-9) | 4.55 | 6.20 |
| Linoleic (C18:2, n-6) | 1.79 | 23.23 |
| Linolenic (C18:3, n-3) | 1.14 | - |
| Arachidonic (C20:0) | 0.72 | 1.80 |
| Behenic (C22:0) | 0.61 | 0.77 |
| Docosadienoic (C22:2) | 0.51 | - |
| Cerotic - C24:0 | 1.14 | - |

### Table 3. Fatty acid composition (%) of the fruit pulp and seed of *Banchosia glandulifera*

| Fatty acids | Pulp | Seed |
|-------------|------|------|
| SAF | 78.61 | 55.09 |
| MUFAS | 13.19 | 18.77 |
| PUFAS | 3.44 | 23.23 |
| Total | 95.24 | 97.09 |
The amount of unsaturated fatty acids is higher in the seed than in the fruit pulp. Linoleic acid is the PUFA found at the highest concentration (69.69 mg/100 g fruit) in the seed of B. glandulifera. Ingestion of this acid is crucial to human because it cannot be produced by our metabolism. PUFAs reduce total cholesterol and LDL levels in plasma, induce lipid oxidation, and elevate HDL levels when consumed in large quantities, which contributes to the prevention of cardiovascular diseases [24].

4. Conclusion

According the authors’ knowledge, this study reports for the first time on the fatty acids, proximal, and elemental composition of the B. glandulifera fruit. The fruit exhibited high value energy and rich in minerals. The seed had a higher concentration of minerals and PUFAs. This study revealed that the B. glandulifera fruit is an excellent source of essential macronutrients and micronutrients for human consumption.

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