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Chemical composition, fatty acid profile and bioactive compounds of guava seeds (*Psidium guajava* L.)

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Abstract

This study aimed to characterize the chemical composition, determine the fatty acid profile, and quantify the bioactive compounds present in guava seed powder (*Psidium guajava* L.). The powder resulted from seeds obtained from guava pulp processing. The agro-industrial seeds from red guava cv. paluma were used, and they were donated by a frozen pulp fruit manufacturer. They contain varying amounts of macronutrients and micronutrients, with a high content of total dietary fiber (63.94 g/100g), protein (11.19 g/100g), iron (13.8 mg/100g), zinc (3.31 mg/100g), and reduced calorie content (182 kcal/100g). Their lipid profile showed a predominance of unsaturated fatty acids (87.06%), especially linoleic acid (n6) and oleic acid (n9). The powder obtained contained significant amounts of bioactive compounds such as ascorbic acid (87.44 mg/100g), total carotenoids (1.25 mg/100 g) and insoluble dietary fiber (63.55 g/100g). With regard to their microbiological quality, the samples were found suitable for consumption. Based on these results, it can be concluded that the powder produced has favorable attributes for industrial use, and that use of these seeds would be a viable alternative to prevent various diseases and malnutrition in our country and to reduce the environmental impact of agricultural waste.

Keywords: guava seeds (*Psidium guajava* L.); chemical composition; fatty acid; bioactive compounds; *Artemia salina* sp.; microbiological quality.

1 Introduction

Brazil is considered the third largest producer of fruits, after China and India (Food and Agriculture Organization of the United Nations, 2010; Brasil, 2012). According to the Brazilian Fruit Institute – Ibraf (Instituto Brasileiro de Frutas, 2013), in 2012 the production of fresh fruit in Brazil was 43,598 million tons. A total of 427,314 tons were imported and 693,020 tons were exported; 22,906,000 tons of fresh fruits were consumed and 20,692,000 tons of fruits were processed. In the same year, 9,162,000 tons of fruits went to waste and per capita consumption was 78.84 kg/habitant/year.

Worldwide, millions of tons of waste from agro-industrial activities are generated. Some of it is used as animal feed or in the fields; however, most of it is still discarded without treatment causing damage to the environment. In addition, the destination of this waste, as currently practiced, causes economic deficit in the supply chain since a substantial part is rich in bioactive compounds, but some of them are capable of preventing the oxidative damage caused by free radicals (Melo et al., 2011; Omena et al., 2012).

In this scenario, guava, a fruit widely grown in tropical and subtropical regions, is particularly important. The major producers of guava are South Africa, India, Hawaii, Colombia, Puerto Rico, Jamaica, and Brazil. This fruit has attracted attention of agro business due to some of its characteristics such as flavor, appearance, and functional nutrients that can promote health. Guava can be consumed processed as jellies, jams, and juices (McCook-Russell et al., 2012). It is particularly rich in minerals and functional compounds such as vitamin C, dietary fiber, carotenoids, and phenolic compounds (Corrêa et al., 2011; Osorio et al., 2011; Usman et al., 2013).

A residue composed mainly of pulp and seed accounting for 4% to 12% of the total mass of the fruit is obtained from the processing of guava fruit pulp. It is estimated that about 202 tons of guava are processed each year by the frozen fruit pulp manufacturers in Brazil, which corresponds to approximately 12 tons of waste that are disposed of affecting the environment. However, waste disposal has become an environmental problem for fruit processing industries (Fontanari et al., 2008). Since this kind of waste is characterized as potential pollutant source, alternatives for reducing its amount are of great importance. Consequently there have been efforts to develop new food products and the extraction of bioactive compounds from these products to increase the options for reuse and recovery of waste (Aghajanzadeh-Golshani et al., 2010). Several studies on the composition of fruits and waste in the Brazilian agro-industry have been conducted focusing on their proper use.

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To add value to these fruits, it is necessary to determine their chemical constituents through scientific and technological research (Vieira et al., 2009; Sousa et al., 2011).

Considering the increase in waste arising from the processing of tropical fruits and the attempt to minimize the environmental impact caused by the accumulation of agro-industrial waste, this study aimed to characterize the chemical composition, determine the fatty acid profile, and quantify the bioactive compounds present in guava seed powder (*Psidium guajava* L.).

2 Materials and methods

2.1 Waste production, processing, and preparation of powder

The agro-industrial waste (seeds) from the guava (*Psidium guajava*) cv. Paluma was donated by an industry that produces frozen pulp fruit (Nutri Vita), located in the city of Teresina, Piauí, Brazil. The samples were collected on different production dates (April 2011 and May 2012). The seeds were taken directly from the production line, after the frozen guava fruit pulp had been processed, and were immediately transported in an isothermal box to the Food Laboratory of the Federal Institute of Science and Technology of Piauí - IFPI. Upon arrival, they were stored in polyethylene bags in a freezer at a temperature of –18°C. The seeds were dried in an air circulation oven (Tecnal, model TE-394 /L) at 60°C for approximately 16 hours. After dehydration, the seeds were triturated using a domestic blender (Walita) and the powder obtained was sieved using a set of seven sieves (10, 30, 40, 60, 80, 100, and 200 mesh corresponding to the opening sizes of 2, 0.60, 0.42, 0.25, 0.18, 0.15, and 0.075 mm, respectively). The powder was packaged in lidded polyethylene containers until analysis. The powder was then subjected to chemical analysis for determination of chemical composition**,** bioactive compounds**,** minerals, fatty acid profile, and microbiological quality. Subsequently, the powder was subjected to extractions using diferent solvents, and the extracts were subjected to toxicological analysis.

2.2 Chemical analysis

Acidity, pH, moisture, and ash

Acidity was determined by titration with 0.1 N NaOH, and the results were expressed in grams of citric acid/100g. The pH was determined by direct reading on the potentiometer (MS Tecnopon, model mPA210) calibrated in buffer solutions of pH 4.0 and 7.0. Moisture determination was performed by drying the sample in an air circulation oven (Tecnal, model TE-394 /L) at 105°C to constant weight. Moisture was calculated by measuring the difference between the mass of the sample before and after drying, and the result was expressed as a percentage of moisture. Ash was determined by incineration in a furnace at 550°C until constant weight. These analyses were performed in triplicate according to the method described by the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008).

Lipids, protein, total dietary fiber, and total carbohydrate

Lipids were obtained by Soxhlet extraction using hexane under reflux as the solvent for six hours, according to the analytical standards of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008). Protein was determined using the micro-Kjeldahl method. The conversion factor of 6.25 was used to covert nitrogen into protein, as recommended by the Association of Official Analytical Chemistry (1995). Total dietary fiber was obtained by adding the soluble and insoluble fractions, according to the enzymatic-gravimetric method of Prosky et al., (1984). Total carbohydrate was determined by the difference method: 100 - (weight in grams [moisture + ash +protein + total fat + total dietary fiber in 100 g of food).

Pectin, fructose, and starch

Pectin was determined following the Pearson method (Pearson, 1976) and consisted of the neutralization of the overall charge of free uronic acid residues by calcium ions causing gelation and precipitation of pectin.The results were expressed in grams of calcium pectate per 100g of sample. The amount of fructose was determined according to the method of Feinberg & Burgner (1992) based on the extraction of sugars from an aqueous medium and determining their levels by high performance liquid chromatography. The amount of starch was determined according to the method of Diemair (1963), through which a known amount of starch was solubilized, and the optical rotation of starch and the sugars present was determined.

Total energy value

The total energy was calculated based on the energy nutrient results obtained using the conversion factors of Atwater, as described by Osborne & Voogt (1978), considering 4 kcal/g for carbohydrate, 4 kcal/g for protein, and 9 kcal/g for lipids.

2.3 Determination of minerals

The analyses of minerals were performed at the Laboratory of Water and Soil of the EMBRAPA (Brazilian Agricultural Research Corporation – Tropical Agroindustry), in Fortaleza, Ceará, Brazil. Initially, nitric perchloric acid digestion of the sample was performed. The minerals calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and manganese (Mn) were determined by atomic absorption spectrophotometry using a Perkin Elmer AAnalyst 300 spectrophotometer. The minerals Sodium (Na) and potassium (K) were determined by fame photometry using a fame photometer (Digimed, DM62). Phosphorus (P) and sulfur (S) were determined using a spectrophotometer at a wavelength of 660nm for phosphorus and 420nm for sulfur. All determinations of these minerals followed the method described by Silva (1999) and were performed in triplicate.

2.4 Fatty acid composition (GC/MS analysis)

The lipid fraction was initially subjected to esterification of fatty acids, which were converted to fatty acid methyl esters (FAMEs) using the method described by Hartman & Lago (1973). The analysis of FAMEs was performed using

a gas chromatograph-Flame Ionization Detector (GC-FID) (*Shimadzu* GC-2010), with a SP-2560 (bis-cyanopropyl polysiloxane) fused silica capillary column with a stationary phase (100 m × 0.25mm, df 0:20 m; Supelco Bellefonte, PA). Split injection mode (1:50) was used, and hydrogen was used as carrier gas with a constant flow of 1.5 mL min^{-1}. The temperatures of the injector and detector were both 220°C. The oven temperature was programmed as follows: initial column temperature 80°C, rising up with a ramp initial rate of 11°C min^{-1} to 180°C and once again at the rate of 5°C min^{-1} to 220°C, and held at this temperature for 19 minutes. The identification of the peaks in the chromatogram was performed by comparison of their retention indices with those of known compounds of a fatty acid standard solution previously injected following the same method. The contribution of each compound to the mixture is given by the relative area (%) of its respective peak in the chromatogram.

2.5 Bioactive compounds

Dietary fiber: soluble and insoluble fiber

The values of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were obtained by the enzymatic-gravimetric method, according to Prosky et al. (1992). The samples were subjected to the action of α-amylase (*Sigma* A-5426) and subsequently protease (*Sigma* P-3910) and amyloglucosidade (*Sigma* A-9913). Based on this hydrolysate, the insoluble fiber content was determined by washing in water and acetone, and the soluble fiber was obtained from the filtrate by precipitation with ethanol 98% and filtration with ethanol and acetone.

Vitamin C

The content of vitamin C was determined according to the method described by Pearson & Cox (1976), which is based on the reduction of 2,6-dichlorophenol indophenol sodium (ITD) by ascorbic acid. The result was expressed in milligrams of ascorbic acid/100g sample.

Total carotenoids

Total carotenoids were determined according to the method described by Higby (1962); the extraction was performed using a solution of isopropyl alcohol: hexane (3:1). The reading was performed on a spectrophotometer (Coleman 33 D) at 450 nm. The result was expressed as mg carotenoid/100g sample and calculated by the formula: Carotenoids = (A x 100)/ (250 x L x W), where: A = absorbance; L = width of the cuvette in cm; and W = the quotient between the original sample weight in grams and the final volume of dilution in ml.

2.6 Microbiological analysis

The microbiological quality of the samples was determined by counting standard Coliforms at 45°C, *Bacillus cereus*, and *Salmonella*, according to the method described by the American Public Health Association (Downes & Ito & American Public Health Association, 2002) and Silva et al. (2001). The results were compared with the standards of the Resolution No. 12, dated January 2, 2001, established by the National Agency for Sanitary Surveillance in Brazil, which sets standards for the microbiological quality of the flour group: pasta, bakery products and similar products; and the starch subgroup: flours and powdered or flaked starch and cornmeal (Brasil, 2001).

2.7 Toxic potential using Artemia salina sp.

Preparation of extracts

From the guava seed powder, extraction was performed using diferent solvents and the Soxhlet apparatus, as described by the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008). For the extraction, the following solvents were used, hexane (non-polar), ethanol, acetone, and methanol (polar) to obtain a water-soluble extract without interference. The extraction was controlled for 6h at 60°C. The material extracted was concentrated under vacuum using a rotary evaporator (Fisatom, model 801) at a temperature of 50°C. Afer the process, the extracts were subjected to a thermostatic bath at a temperature of 60°C until there was no trace of solvent. The extracts were stored with protection from light in glass containers until the analysis.

Toxic potential

The toxic potential of the extracts was determined using the larvae of *Artemia salina* sp., according to the method described by Meyer et al., (1982) and McLaughlin et al. (1991). The eggs of *Artemia salina* sp. were hydrated in an aquarium containing synthetic saline water adapted to 12 ppm in ambient temperature around 25°C. After a period of time of approximately 48 hours, the eggs hatched and produced larvae, which were collected for bioassays. The dilutions of ethanol, acetone, and methanol extracts and a blank test were conducted in synthetic saline, 0.5 mL of dimethyl sulfoxide concentration (DMSO), to which ten larvae were added in 50 mL plastic cups. For the negative control, larvae were kept only in synthetic saline. After 24 hours incubation, living and dead larvae were counted to calculate survival percentage, which was used to determine the LC_{50} (lethal concentration for 50% of larvae).

2.8 Statistical analysis

Analyses were carried out in triplicate. All results were expressed as means ± standard deviation (SD) using the software Origin® for Windows, version 7.0 (OriginLab Corporation, 2002).

3 Results and discussion

3.1 Characterization of guava seed powder

The pomace was passed through a sieve using five different mesh openings and standardized to the powder particle size between 0.42 mm and 0.60 mm in diameter. Taking the wet sample into account, it can be said that there was a yield of approximately 54%.

In fruits of other Myrtaceae, such as pear-guava (*Psidium acutangulum*) and guava-boi (*Eugenia stipitata*), some authors found the yield of 55.01% and 63%, respectively (Ferreira, 1992; Rebouças et al., 2008). The good yield of powder obtained from waste guava can be attributed to the large numbers of seeds in its composition.

3.2 Physicochemical characterization

The results of the physicochemical analyses are shown in Table 1. Acidity is an important parameter in assessing the conservation status of a food product. The acidity value found in the powder was 2.18 ± 0.08g of citric acid/100g. This value is higher than that found by Uchôa et al., (2008), who obtained a value of 1.21±0.16 g of citric acid/100g for guava seed powder. The variations in the results can be explained by factors such as the variety and ripeness of the fruit studied. The pH value found in this study was 4.30 ± 0.03, which is below or very close to 4.5 (value that limits the development of micro-organisms) and, thus it can be classified as an acid product and consequently realatively impervious to microbial attack. Marquina et al., (2008) studied the chemical composition of guava pulp and peel and found pH values of 4.1 and 3.9, respectively.

The moisture values found in this study are lower than those found by Fontanari et al., (2008), who obtained a moisture value of 8.3 ± 0.03 g/100g for guava seed powder. According to Resolution No. 263, dated 2005, 22 September by the National Agency for Sanitary Vigilance, the maximum value of moisture for flours should be 15% (Brasil, 2005a). The the ash value found was 1.18 ± 0.02 g/100g. This value is close to that found by other authors, such as Martínez et al., (2012), who obtained an ash content of 2.4 ± 0.10 g/100 g for guava. McCook-Russell et al. (2012) comparing the nutritional composition of fruits of Jamaica, found ash values of 3.12 ± 0.03 g/100g for the common guava and 3.05 ± 0.01 g/100g for the araçá, a fruit of the same genus.

Observing the results of lipid analysis shown in Table 1, the results presented in this study are close to those reported by by Silva et al., (2009), 11.71 g/100g for the guava seed on a dry basis. Fontanari et al., (2007) reported that the seed protein has functional properties similar to those of other seeds which have been used as food ingredients and may be an alternative source of protein for future use in processed foods. Comparing the protein analysis results, it was shown that the protein content of the powder obtained from guava seed was 11.19 ± 0.28 g/100g (Table 1). Fontanari et al., (2008) obtained the protein value of 9.2 ± 0.10 g/100g when studying protein isolated from guava seed (*Psidium guajava* L.).

Typically, fruits are considered rich in reducing sugars (glucose and fructose), and this measurement is important to evaluate the potential of the fermentation product. The fructose content of 0.29 ± 0.01 g/100g (Table 1) was found in the guava seed powder. Higher levels of total pectin are important for postharvest since pectins influence the texture of the fruit and its conservation. The total pectin values found in this study are lower than those found by Osorio et al. (2011), who obtained total pectin value of 1.52 ± 0.01 g/100g for mashed guava (wet basis).

According to the technical regulation from 13/01/1998 of ANVISA-MS concerning the disclosure of nutritional supplement (Ordinance No. 27), a food item can be considered a source of dietary fiber when it has 3g/100g (integral basis) in the finished product for solid foods and 1.5 g/100 mL (integral basis) for liquids; if it has twice as much as this the amount, it can be considered as high fiber food (Brasil, 1998). Fontanari et al., (2008) obtained the value of 67.00g/100g for total dietary fiber for guava seed powder. The results presented suggest that new products based on fibers obtained from residues of this fruit can be formulated to prevent diseases, especially those related to the gastrointestinal tract and the cardiovascular system. Regarding the caloric value, the powder obtained from guava seed had a lower value than that found by Abud & Narain (2009), who found values of 266.65 ± 1.73 Kcal/100g for flours obtained from guava residue. These data suggest that the flour produced from the seed obtained from processing guava fruit pulp waste, which has a low calorific value, can be used as an ingredient in the food industry in order to add value and reduce the number of calories.

3.3 Minerals

According to the results of the mineral analysis shown in Table 2, it can be seen that the minerals iron and zinc were present in higher concentrations, and low sodium and magnesium concentrations were observed. Gondim et al., (2005) reported that magnesium and sulfur are essential minerals in

Table 1. Physicochemical characterization of powder produced from the seed obtained from processing guava fruit pulp (*Psidium guajava* L.).

Parameters (% dry basis)	Results (mean ± SD)
Moisture (g/100g)	6.68 ± 0.00
Ash (g/100g)	1.18 ± 0.02
Total Lipids (g/100g)	13.93 ± 0.03
Protein (g/100g)	11.19 ± 0.28
Carbohydrate (g/100g)	3.08
Pectin (g/100g)	0.58 ± 0.01
Frutose (g/100g)	0.29 ± 0.01
Starch (g/100g)	0.17 ± 0.00
Total Dietary Fiber (g/100g)	63.94 ± 0.10
Total Calories (Kcal/100g)	182

Table 2. Composition of minerals in powder produced from the seed obtained from processing guava fruit pulp (*Psidium guajava* L.).

Minerals (mg/100g)	Results (mean ± SD)
Calcium	0.05 ± 0.14
Magnesium	0.13 ± 0.02
Sulfur	0.09 ± 0.27
Iron	13.8 ± 2.95
Manganese	0.44 ± 0.47
Zinc	3.31 ± 2.52
Sodium	0.05 ± 0.02
Potassium	0.20 ± 0.02
Phosphorus	0.30 ± 0.45

many biological reactions. This study found lower values than those obtained by Uchôa et al., (2008), who, when evaluating the composition of minerals in guava seed, obtained values of 0.19 mg/100g for magnesium. The concentration of the mineral iron found in the present study represents 92% of the dietary reference intake (DRI) for adults, which is 14 mg/day (Brasil, 2005b). However, it is important to remember that not all of the iron present in food is absorbed by the body; therefore, we cannot state that the flour studied is rich in this mineral because we need to check how much of this mineral our body can absorb. The value of 3.10 ± 2.52 mg/100g was obtained in the analysis of zinc concentration in the powder prepared from guava seed. This value represents 20.7% of the recommended intake daily intake for adults, which is 15 mg/day (Brasil, 2005b).

It is important to note that the intake of the mineral sodium by the Brazilian population is often high due to the consumption of salty and processed foods. The amount of sodium in this study was considered low since the World Health Organization (2013) recommends an intake of less than 1.7 g/day of this mineral.

3.4 Fatty acid profile

The fatty acid lipid profile of the guava seed powder showed a predominance of unsaturated fatty acids (87.06%), especially linoleic acid (n6) and oleic acid (n9), 77.35% and 9.42%, respectively. Göktürk Baydar et al. (2007), when assessing the marc derived from four grape varieties, found an average degree of unsaturation lower (86.42%) than that found in the present study. The high levels of unsaturation are highly relevant since they have an important role in reducing blood cholesterol levels and in the treatment of atherosclerosis (Rockenbach et al., 2010). The stearic (4.48%) and palmitic (8.00%) were the saturated fatty acids found in the largest quantities. Leite et al., (2009) in their lipid analysis, found palmitic acid (21.3%), palmitoleic acid (1.6%), stearic acid (2.2%), oleic acid (24.1%), and linoleic acid (27.6%) in the hexane extract of avocado seeds.

Table 3. Fatty acid profile of the powder produced from the seed obtained from processing guava fruit pulp (*Psidium guajava* L.).

Fatty acid	N° Carbon	Average ± SD
Lauric acid	(C12:0)	0.07 ± 0.00
Myristic acid	(C14:0)	0.10 ± 0.00
Palmitic Acid	(C16:0)	8.00 ± 0.04
Heptadecanoic acid	(C17:0)	0.07 ± 0.00
Stearic Acid	(C18:0)	4.48 ± 0.17
Oleic Acid (n-9)	(C18:1)	9.42 ± 0.26
Linoleic Acid (n-6)	(C18:2)	77.35 ± 0.35
Arachidic acid	(C20:0)	0.12 ± 0.00
Gondoic acid	(C20:1)	0.14 ± 0.00
Linolenic acid	(C18:3)	0.15 ± 0.00
Behenic acid	(C22:00)	0.10 ± 0.00
Σ SFA[a]		12.94
Σ MUFA[b]		9.56
Σ PUFA[c]		77.5
PUFA/MUFA		8.11
Σ USFA[d]		87.06

The results are expressed as mean ± standard deviation for analysis in three replicates. [a]SFA= saturated fatty acids; [b]MUFA= monounsaturated fatty acids; [c]PUFA= polyunsaturated fatty acids; [d]USFA= unsaturated fatty acids; n-6= omega 6 fatty acid, n-9=omega 9 fatty acid.

With regard to the general classification of the fatty acids (Table 3), it was found that the guava seed had the following sequence: PUFA (77.5%) >MUFA (9.56%) >SFA (12.94%). The PUFA/SFA ratio in the present study (8.11) was considered above the minimum recommended by United Kingdom (1994), which is equal to 0.45.

3.5 Bioactive compounds

From the results shown in Table 4, one can observe an ascorbic acid content of 87.44 ± 1.70 mg/100g. Comparing the value found in the sample with the dietary reference intake (DRI) (DRI) for adults (Brasil, 2005b), which is 45 mg/day, it can be stated that the sample can be considered a good source of this bioactive compound. Osorio et al. (2011) obtained a value of 118.7 ± 0.10 mg/100g for mashed guava on wet basis. The amount of carotenoids present in the powder sample obtained from the guava seed was 1.25 ± 0.14 mg/100g. Corrêa et al., (2011) studied the antioxidant content in guava and araçá grown in different regions of Brazil and obtained the value of total carotenoid concentration ranging from 0.13 to 2.54 mg/100 g in white and red guava pulp, respectively. The carotenoid concentration in araçá, a similar fruit, was 0.73 mg/100g.

The soluble dietary fiber fraction (SDF) consists of pectins, beta-glucans, gums, mucilages, and some hemicelluloses and may be found at higher concentration in the skin than in the seed of the fruit. According to Table 4, the value found was lower than that found by Martínez et al., (2012) of 1.1 ± 0.09 and 0.6 ± 0.03 g/100g, for pineapple and guava flour, respectively. The powder obtained from guava seed is a better source of insoluble fiber than the fractions of the seed and skin of the jabuticaba, a fruit belonging to the same family, which, according to Lima et al., (2008) has an insoluble fiber value of 26.93 and 26.43 g/100g, respectively. Ramírez & Delahaye (2009) determined values of insoluble fiber for guava and soursop of 54.65 ± 0.54 and 40.43 ± 0.00 g/100g, respectively.

3.6 Microbiological analysis

The results of microbiological analysis, shown in Table 5, obtained for the powder produced from the seed obtained from processing guava pulp, show that it is suitable for consumption since it is in accordance with the standards recommended by the Resolution No. 12 of January 2, 2001 (Brasil, 2001).

These results suggest that the powder obtained meets the sanitary conditions established by law, and therefore it is satisfactory for human consumption since both processes

Table 4. Bioactive Compounds of powder produced from the seed obtained from processing guava fruit pulp (*Psidium guajava* L.).

Bioactive Compounds	Results (mean ± SD)
Vitamin C (mg ascorbic acid/100g)	87.44 ± 1.70
Carotenoids Totals (mg/100g)	1.25 ± 0.14
Soluble Dietary Fiber (g/100g)	0.39 ± 0.02
Insoluble Dietary Fiber (g/100g)	63.55 ± 0.12

Table 5. Microbiological analysis of the powder produced from seeds obtained from processing guava fruit pulp (*Psidium guajava* L.).

Microorganism	Result	Tolerance*
Salmonella (25g)	Absent	Absent
Coliformes at 45° (NMP/g)[a]	3,6	10^2
Bacillos cereus (UFC/g)[b]	<100	3 x 10^3

*According to RDC No. 12 of 02 January 2001, the National Agency of Sanitary Vigilance, for flour, pasta, and bakery (processed and packaged) and similar products (Brazil, 2001). [a]MPN/g = Most Probable Number per gram. [b]CFU/g = Colony Forming Unit per gram.

used in the extraction of fruit pulp and in the preparation of guava waste flour were conducted in accordance with good manufacturing practices. This indicates that the flour obtained from guava residue may be suitable for the development of new food products.

3.7 Toxic potential using the larvae of Artemia salina sp.

In the toxicological assay against *Artemia salina* sp., it was found that all the extracts evaluated showed no toxicity. Meyer et al., (1982), established a relationship between the degree of toxicity and median lethal dose, LC_{50}, of plant extracts against larvae of *Artemia salina* sp. since it is known that when the values are higher than 1000µg/mL, they are considered nontoxic. Leite et al. (2009) found that hexane extract from avocado seeds showed toxicity to *Artemia salina* (LC_{50} of 2,37mg/mL^{-1}). The absence of toxicity may be an advantage when considering a possible use of this extract in the development of new herbal medicines and for human use.

4 Conclusion

Based on the results obtained, it can be concluded that the powder produced from the seed obtained from the processing of frozen guava pulp has favorable attributes for industrial use. Its composition exhibits a high content of total dietary fiber, protein, iron, zinc, and reduced calorie content. In addition, it can also be considered a potential source of bioactive compounds such as vitamin C, carotenoids, insoluble dietary fiber, and unsaturated fatty acids, especially linoleic acid (n6) and oleic acid (n9). Therefore it can be used as an ingredient in the development of new food products since it is suitable for human consumption. The utilization of these seeds would be a viable alternative to combat various diseases and malnutrition in our country and reduce environmental contamination. It is suggested, however, that further studies should be conducted on this residue to evaluate the presence of other bioactive compounds including the evaluation of antioxidant activity and phenolic compounds in the seeds of this fruit.

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