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Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil

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Abstract

Grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil was evaluated in relation to chemical composition, and content of minerals and functional properties. Its microbiological quality and toxic potential, using *Artemia salina* sp, were also investigated. The results showed that the flour obtained from these residues had below neutral pH (3.82), moisture (3.33g/100g), acidity of (0.64g of citric acid/100g), and ash (4.65 g/100g). The amount of total dietary fiber (46.17g/100g) stood out quantitatively compared to the content of carbohydrate (29.2g/100 g), protein (8.49g/100g), and lipids (8.16g/100g). The total energy was 224Kcal/100g. With regard to the compounds with functional properties, higher values of insoluble fiber 79% (36.4 g/100 g); vitamin C (26.25 mg of acid ascorbic/100g), and anthocyanins (131mg/100g) were found. The minerals iron, potassium, zinc, manganese, and calcium were present in higher concentrations. There were no significant copper values. The results showed that the grape residues are an important source of nutrients and compounds with functional properties suggesting that they can be incorporated as an ingredient in the diet and/or used as a dietary supplement aiming at health benefits. The residues did not show microbiological contamination and were considered nontoxic.

Keywords: grape pomace; chemical composition; minerals; bioactive compounds; microbiological quality; *Artemia salina sp*.

1 Introduction

Because of the beneficial effects on human health and its economic importance, grape is a fruit widely grown and eaten around the world. Historically, the production and export of grapes were controlled almost exclusively by traditional European countries; however, in recent years, South America has shown significant rate of growth in production and export of grapes with two crops a year (Ruiz, 2011). According to data from the Food and Agriculture Organization (2010), Brazil occupies the 20th position in terms of world production of grapes. In 2012, data show that the annual production of grapes in Brazil ranged from 1.3 and 1.4 million tons/year (Camargo et al., 2011; Instituto Brasileiro de Geografia e Estatística (2013).

Municipalities in the Northeast of Brazil, where the predominant climate is semi-arid and dry sub-humid, are characterized by low rainfall and relative and high air temperature and solar radiation (Andrade Júnior et al., 2005). Phytosanitary problems tend to be smaller and fruit quality tends to be better, which is essential for the expression of the productive potential of the European vine (Costacurta & Roselli, 1980; Coombe, 1987). According to the Brazilian Institute of Geography and Statistics, in 2012, the planting of grapes in northeastern Brazil was 9.437 hectares, with total production of 287.050 tons and average yield of 31.043 kg/ha. In February 2013, data indicated a 2% increase in production although a 0.4% decrease in the total area planted was observed (Instituto Brasileiro de Geografia e Estatística, 2013). Along with this intensive production, large amounts of agro-industrial residues are generated (Bustamante et al., 2008; Tangolar et al., 2009; Cetin et al., 2011; Deng et al., 2011; Lachman et al., 2013). According to a study conducted in 2011 by the Brazilian Ministry of Environment (Brasil, 2011), the production of grape waste in Brazil was 290.838.411 tons.

The agro-industrial residues of grape are mostly solid by-products such as stalks, pomace and the liquid filtrate. Depending on the conditions of the grapes when they are harvested, the residues may represent from 13.5 to 14.5% of the total volume of grapes, and may reach 20% (Ahmad & Ali Siahsar, 2011; Rockenbach et al., 2008). These residues are composed of water, proteins, lipids, carbohydrates, vitamins, minerals, and compounds with important biological properties such as fiber, vitamin C, and phenolic compounds (tannins, phenolic acids, anthocyanins, and resveratrol), depending on the type of waste, the cultivar and climatic and cultivation conditions (Ahmad & Ali Siahsar, 2011; Rockenbach et al., 2007, 2008; Pontes et al., 2010; Burin et al., 2010). Due to the functional properties of these residues, which are capable of acting on the metabolism and human physiology producing beneficial health effects, the extraction of these bioactive substances can provide many

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opportunities for adding value to food products contributing to the improvement of dietary pattern of the population and helping prevent diseases such as cancer, cardiovascular disease, Alzheimer's, and other degenerative diseases, besides decreasing the environmental impact and economic losses (Tangolar et al., 2009; Cetin et al., 2011; Deng et al., 2011; Lachman et al., 2013). However, for better understanding the use and benefits of this plant and its waste, it is necessary to know its chemical constituents and evaluate the risk/benefit ratio of their use through chemical, microbiological, and toxic potential analyses (Devesa-Rey et al., 2011).

The bioassay method using the micro crustacean *Artemia salina sp* has been used to evaluate the toxicity of plant products; this is simple and low cost method. Although not specific, this method has a good correlation with tumor cells and pesticidal activity (Leite et al., 2009). This bioassay allows the evaluation of overall toxicity, and therefore it is considered the preliminary study of compounds with potential biological activity. Studies on the lethality of *Artemia salina sp*. in plant extracts have been widely conducted (Araújo et al., 2010; Lachumy et al., 2010; Bussmann et al., 2011; Jiménez et al., 2011).

This paper aimed to characterize grape pomace in terms of chemical composition, mineral content, and functional properties and determine its microbiological quality and toxic potential to evaluate the feasibility of using it in food industries improving the nutritional value of food products and the quality of life of consumers.

2 Materials and methods

2.1 Grape samples' characterization, processing, and flour preparation

Samples of grape (*Vitis vinifera* L.) of the variety Benitaka were collected by the authors directly from the Vine Complex at Marrecas settlement (2011/2012 harvest season) in the municipality of São João do Piauí, Piauí state, Northeastern Brazil. After harvest, the grapes were transported in cool boxes to the city of Teresina, capital of the state of Piauí, to the Laboratory of the Federal Institute of Education, Science and Technology of Piauí, Teresina South Zone, where they were stored under refrigeration for 48 hours at 4°C until analysis.

Initially, the grapes were weighed and graded according to Normative Instruction Nº. 01 of 1 February 2000, the Ministry of Agriculture, Livestock and Supply (Brasil, 2000). The grapes used were from Group I (consisting of variety with seed), Subgroup Colorful, Class 2 (mass of clusters between 200g and 500g), Division 20 (berries with a caliber greater than or equal to 20mm and less than 22mm) and Extra Class (typical coloring, green stems, and no bunch deformation). Then grapes were then cleaned and pressed using an industrial depulper separating the pomace (skin and seeds) and pulp for the extraction of grape juice.

After extraction, the pomace was stored in polyethylene bags at −18 °C until processing. The pomace was dried in an air circulation oven (Tecnal, model TE-394 /L) at 60 °C for approximately 16 hours. The dried residue was triturated using a domestic blender (Walita) and a flour was obtained and sieved using a set of seven sieves (10, 30, 40, 60, 80, 100, and 200 mesh corresponding to openings: 2, 0.60, 0.42, 0.25, 0.18, 0.15, and 0.075 mm, respectively). The flour was packed in lidded polyethylene bottles until analysis. The flour was then subjected to chemical analysis for determination of vitamin C, anthocyanins, minerals, microbiological quality, and toxic potential. Subsequently, from the flour obtained, extractions using different solvents were performed 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 the difference in the mass of the sample before and after drying; the result was expressed in 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 (2008).

Lipids, protein, total dietary fiber, and total carbohydrate

Lipids were obtained by Soxhlet extraction using hexane as solvent under reflux for 6 hours, according to the analytical standards of the Adolfo Lutz Institute (2008). Protein was determined by the micro-Kjeldahl method using copper sulphate and selenium as catalysts of mineralization and boric acid as the receiver solution in the distillation of ammonia. Next, the sample was titrated with 0.1 N hydrochloric acid. The conversion factor of 6.25 was used to covert nitrogen into protein, as recommended by Association of Official Analytical Chemistry (1995). Total dietary fiber (TDF) 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

Pectin was determined following the Pearson method (Pearson, 1976) and consisted of the neutralization 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.

Fructose, glucose, and sucrose

Fructose, glucose, and sucrose were determined according to the method of Feinberg & Burgner (1992) based on the extraction of sugars from an aqueous medium and determining the levels of these sugars by high performance liquid chromatography.

Total energetic 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 Bioactive compounds

Soluble and insoluble dietary fiber

The values of soluble fiber (SDF) and insoluble 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 amyloglucosidase (*Sigma* A-9913). Based on this hydrolysate, the insoluble fiber content was determined by washing in water and acetone and the soluble fiber obtained from the filtrate by precipitation with ethanol 98% and filtration with ethanol and acetone.

Vitamin C

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.

Anthocyanins

The determination of anthocyanins was performed according to the method of Francis (1982). For the extraction, 1g of dehydrated pomace was homogenized with a solution of HCl (1.5 N) and ethanol 85%. After a period of 24 hours under refrigeration and absence of light, the extracts were filtered and read at 535nm using a Coleman 33D spectrophotometer. The results were expressed as mg of anthocyanins totais/100g sample and calculated using the formula: (absorbance x dilution factor)/98.2.

2.4 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 flame photometry using a flame 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.

2.5 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 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, 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 starch subgroup: flours, and powdered or flocked starch and cornmeal (Agência Nacional de Vigilância Sanitária, 2001).

2.6 Toxic potential using *Artemia salina sp.*

Preparation of extracts

From the grape pomace flour, extraction was performed using different solvents using the Soxhlet apparatus as described by the Adolfo Lutz Institute (2008), according to the analytical standards of the Adolfo Lutz Institute. 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 in a rotary evaporator (Fisatom, model 801) at a temperature of 50°C. After the process, the extracts were subjected to 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 gave rise to 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).

2.7 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, 2002).

3 Results and discussion

3.1 Characterization of grape pomace flour

After the pomace sieving process through a sieve using five different mesh openings, the flour particle size was standardized between 0.42 mm and 0.60 mm in diameter since the retention of larger amounts of flour occurred in the sieves between 30 and 40 "mesh". The yield relative to the initial raw material, it was found that 1 kg of grape marc fresh yields approximately 321g of processed flour, with yields close to 32%.

3.2 Physicochemical characterization

The results of the physicochemical analyses are shown in Table 1. In addition to low moisture (3.33 ± 0.04 g/100g), the pH value of the pomace grape flour was below neutral (3.82 ± 0.01), which led to greater stability hampering the development microorganisms because fungi generally prefer acidic pH (4.5-5.0) and bacteria prefer near neutral pH (6.5-7.0). The pH of grape residue flour are directly correlated with the pH of fresh grape. Investigating grapes of the variety Benitaka, Pinheiro et al. (2009) observed pH of 3.65, a value similar to that found in the present study.

The moisture content found in this study are lower than those found by Tangolar et al. (2009) in seeds of seven grape varieties (4.95 to 6.54 g/100g). According to Resolution N°. 263, dated 2005, 22 September, the National Agency for Sanitary Vigilance (2005), the maximum value of for flours should be 15%. As for acidity, the value found in this study was 0.64 ± 0.004 g of citric acid/100g. Acidity can determine the quality of the flour under study. With respect to wheat flour, the higher the acidity, the lower its quality, and when it is used as a raw material, it directly affects the final product.

Comparing the values observed in the present study with that of the pure wheat flour, maximum of 3% (Abud & Narain, 2009), an adequate pomace grape flour acidity is achieved. The higher acidity values correlate with more significant concentrations of tartaric and malic acids in the skins of grapes (Rizzon & Miele, 2001). The amount of total dietary fiber (46.17g/100g) was higher than that of the other nutrients, leading to the conclusion that it is as a major component of the raw material of this study, in quantitative terms. This value was higher than that found in studies that quantified the dietary fiber content of flour residues in grape, 25.62 and 31.66 g/100g (Bampi et al., 2010). However, it was higher when compared to the fiber content in green banana flour (17.98 g/100g) and wheat flour (2.3 g/100 g) (Universidade Estadual de Campinas, 2006). Fiber content is related not only to the quantitative but also qualitative aspect since grape pomace fibers are structurally different from those found in other cereals and other fruits since they are associated with polyphenols with antioxidant activity.

Table 1. Physicochemical analysis of grape pomace (*Vitis vinifera* L.) flour.

Parameters (% dry basis)	Results (Mean ±SD)
Moisture (g/100g)	3.33 ± 0.04
Ash (g/100g)	4.65 ± 0.05
Total Lipids (g/100g)	8.16 ± 0.01
Protein (g/100g)	8.49 ± 0.02
Carbohydrate (g/100g)	29.20
Pectin (g/100g)	3.92 ± 0.02
Frutose (g/100g)	8.91 ± 0.08
Glucose (g/100g)	7.95 ± 0.07
Total dietary fiber (g/100g)	46.17 ± 0.80
Total Calories (Kcal/100g)	224

With regard to the lipid content, the value found (8.16g/100g) was higher than that reported by Bampi et al. (2010) in flour grape residues (2.56 g/100g). The lipids of the grapes are mainly concentrated in its seeds and consist of about 90% monounsaturated fatty acids, known for their beneficial properties, particularly to the cardiovascular system (Rockenbach et al., 2010).

The amount of protein corresponded to 8.49 g/100g of sample analyzed. Higher values (11g/100g, 12g/100g, and 14g/100g) were found by Valiente et al. (1995), Llobera & Cañellas (2007) and Bravo & Saura-Calixto (1998) in grape residues.

With respect to the carbohydrates, they accounted for 29.20 g/100g sample, and fructose was present in the greatest amount (8.91 g/100 g), followed by glucose (7.95 g/100 g). There were no significant values for sucrose. Bampi et al. (2010) reported 52.56 g/100g values for carbohydrates in Japanese grape flour. Carbohydrates and proteins are essential for human health. Carbohydrates are the body's preferred energy source, and they provide fuel for the central nervous system and for the other organs of the human body. Proteins are required for the growth, development, regeneration and reconstruction of the body and are responsible for the production of antibodies, blood cells, hormones, and enzymes (Cetin et al., 2011).

3.3 Bioactive compounds

Vitamin C levels can be considered an index of nutritional quality of foods because its presence demonstrates that other nutrients were probably preserved since vitamin C is thermolabil. The amount of Vitamin C in grapes is 10.8 mg/100 g edible part, on average (Pinheiro et al., 2009). The grape pomace flour obtained had 26.25 mg ascorbic acid/100g (Table 2). This result was higher than that found by Souza et al. (2012) in the skin of grape *Vitis vinifera* L. (4.9 to 12.2 mg ascorbic acid/100g) and it is significant since the samples were oven heated.

Regarding the content of anthocyanins, the grape pomace flour obtained had 131mg/100g. This result was higher than that found by Sousa et al. (2011) in fruit waste, with values of 8.4 µg/100g in residues of *acerola,* and 3.2 µg/100g in guava

Table 2. Bioactive compounds in pomace grape (*Vitis vinifera* L.) flour.

Bioactive compounds	Results (Mean ±SD)
Vitamin C (mg ascorbic acid/100g)	26.25 ± 0.01
Total anthocyanins (mg/100g)	131 ± 0.4
Soluble dietary fiber (g/100g)	9.76 ± 0.03
Insoluble dietary fiber (g/100g)	36.40 ± 0.84

waste. Anthocyanin pigments are present in the grape skin, and their levels may vary from 30 to 750 mg/100g fruit, in agreement with the values found in this study (Bridle & Timberlake , 1997). Rockenbach et al. (2011) found higher values of anthocyanins, ranging from 385.93 to 934.67 mg/100g mg/100g, in red grape bagasse Pinheiro (2009) found values of 3.56 mg/100mL in grape juice at baseline and 1.43 mg/100mL after 210 days of storage. Levels of anthocyanins in grapes is associated not only with the portion of the fruit analyzed, but they can also be influenced by factors such as cultivar, cultivation method, climatic aspects, and physicochemical factors such as pH and temperature (Kato et al., 2012).

As for the total dietary fiber, the IDF 79% (36.4 g/100g) was higher than the SDF, 21% (9.76 g/100g). The IDF is predominantly composed of cellulose and hemicellulose and lignin (lesser amount). Pectin predominates in the SDF (Bravo & Saura-Calixto, 1998).

In this study, we found 3.92 ± 0.02 g/100g of pectin, a value close to a similar study (2.3-4.4 g/100g) (Bravo & Saura-Calixto, 1998). Higher values (6.2 g/100g) were found by Llobera & Canellas (2007) in grape by-products. According to Mildner-Szkudlarz et al. (2013), the proportion of SDF and IDF in the diet should be between 1:4 and 1:3. Soluble fiber reduces the risk of heart diseases and lowers levels of cholesterol triglycerides, and glucose in the blood (Mildner-Szkudlarz et al., 2013; Marlett et al., 2002). The insoluble fraction has a positive influence on the colon (Stampanoni, 1998). According to Schneeman (1987), 30-50% SDF and 50–70% IDF are considered to be well-balanced proportions for maximum health benefits. Thus, the flour obtained from grape pomace, providing 79% IDF and 21% SDF, is a good sources of DF. According to Pérez-Jiménez et al. (2008), grape DF significantly reduced the lipid profile and blood pressure, these effects were significantly greater than those caused by other DFs such as oat fiber or psyllium, probably owing to the combined effect of DF and antioxidants.

3.4 Minerals

According to the results of the mineral analysis shown in Table 3, iron, potassium, zinc, manganes, and calcium were present in higher concentrations. There were no significant values for copper. Potassium levels higher than those of sodium can lead to a mineral balance that favors hypertension control. A diet rich in potassium lowers blood pressure and consequently the risk of morbidity and mortality due to cardiovascular diseases; in addition, potassium intake can decrease urinary calcium excretion and consequently reduce the risk of developing osteoporosis (Cetin et al., 2011).

Table 3. Composition of minerals (mg/100g) in grape pomace (*Vitis vinifera L.)* flour.

Minerals	Results (Mean ±SD)
Calcium	0.44 ± 0.715
Magnesium	0.13 ± 0.255
Sodium	0.044 ± 0.056
Potassium	1.40 ± 0.313
Iron	18.08 ± 0.03
Manganese	0.817 ± 0.550
Phosphorus	0.183 ± 0.255
Sulfur	0.089 ± 0.336
Zinc	0.98 ± 0.702

Comparing with Dietary Reference Intakes (DRI) (National Academy of Sciences, 2011), the amount of iron found in this study (18.08 mg/100g) supplies the adult daily requirements for iron (8mg/day for men and 8 to 18mg/day for women). For zinc, the recommended daily intake is 11mg for men and 8 mg for women. The amount of zinc found was 0.98 mg/100g. These minerals are considered essential for the human body. Iron, among other functions, is associated with the production of blood cells, and zinc is essential for the immune system. Both nutrients are also considered potent antioxidants. Lachman et al. (2013), in a study on minerals present in grape seed found lower values of calcium (0.27 mg/100 g), iron (4.54 mg/100 g), and phosphorus (0.02 mg/100 g) and higher values of manganese (1.45 mg/100g). However, similar values were reported for magnesium (0.10 mg/100 g), sodium (0.042 mg/100g), and zinc (1.1 mg/100g).

Rizzon & Miele (2012), investigating minerals in grape juice, found higher values of sodium (0.067 mg/100 g), potassium (129.5 mg/100g), phosphorus (10.5 mg/100g), and magnesium (8.78 mg/100g), but lower values of iron (0.14 mg/100g).

3.5 Microbiological quality

The results shown in Table 4 indicate that the grape pomace flour has microbiological characteristics acceptable for human consumption, which is consistent with the standards recommended by the Resolution Nº. 12 of January 2, 2001, the National Agency for Sanitary Surveillance (2001). Possibly, the satisfactory results of microbiological parameters are due to the fact that the grape flour does not provide favorable conditions for microbial growth, such as low moisture and pH lower than 4.

3.6 Toxic potential using the larvae of *Artemia salina sp.*

Meyer et al. (1982) established the relationship between the degree of toxicity and median lethal dose, LC_{50} of plant extracts against larvae of *Artemia salina sp.*; values above 1000µg/mL are considered nontoxic. All extracts were evaluated against their toxicity to *Artemia salina sp.* and were considered nontoxic since all microcrustaceans remained alive. Nascimento et al. (2008) studied toxicity in three species of medicinal plants of the genus *Phyllanthus* and reported absence of toxicity only in the species *P.amarus*. Leite et al. (2009) found that hexane

Table 4. Microbiological Analysis of grape pomace (*Vitis vinifera* L.) flour.

Microorganism	Result	Tolerance*
Salmonella (in 25g)	Absent	Absent
Fecal Coliforms (MPN/g)**	<3	10^2
Bacillus cereus (CFU/g)***	<100	3×10^3

*According to Resolution No. 12 of January 2, 2001, the National Health Surveillance Agency (2001), food group 10, item a. **MPN/g = Most Probable Number per gram. ***CFU/g = Colony Forming Unit per gram.

extract from avocado seeds showed toxicity to *Artemia salina* (LC_{50} of 2.37mg/mL^{-1}).

The absence of toxicity may be an advantage to a possible use of this extract in the development of new herbal medicines and for human use.

4 Conclusions

This study is the first characterization of grape pomace grown in the state of Piaui. The results show that it is an important source of nutrients and compounds with functional properties. The grape pomace flour obtained showed low and pH below neutral, which can help prevent growth of pathogenic microorganisms. The amount of total dietary fiber is quantitatively greater compared to that of carbohydrates, proteins, and lipids, indicating that this residue could be included in the daily diet as a source of fiber and food supplement. Regarding compounds with functional properties, the results show that grape pomace may be a potential source of bioactive compounds, especially higher concentration of insoluble fibers in relation to the soluble fraction, and significant amounts of vitamin C and anthocyanins. As for minerals, iron, potassium, zinc, calcium, and manganese are present in higher concentrations. Furthermore, the results of this study suggest that the flour produced from grape pomace, which is environmentally appropriate and easy to obtain, may be a potential food ingredient in the daily diet or as a nutritional supplement. The microbiological and toxicity bioassays show that the grape pomace evaluated was not contaminated and it was therefore considered and were considered. It is suggested, however, that further studies should be conducted on this residue, for example, to evaluate the presence of other bioactive compounds, including the evaluation of the antioxidant activity of the phenolic compounds and fatty acid composition in the seeds of this residue.

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