

BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF FRUITS

**VIRGÍNIA MIRTES DE ALCÂNTARA SILVA
NEWTON CARLOS SANTOS
VICTOR HERBERT DE ALCÂNTARA RIBEIRO**

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Virgínia Mirtes de Alcântara Silva
Newton Carlos Santos
Victor Herbert de Alcântara Ribeiro

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The organizers

Presentation

Brazil is among the 17 megadiverse countries (a term coined by Russell Alan Mittermeier, in 1997, to refer to the countries that harbor the highest levels of biodiversity and endemic species) however, it is the most prominent for containing approximately 20% of all world diversity.

When it comes to agriculture, the country also stands out worldwide, in this case with the Horticulture species (understood as the branch of agriculture that studies the techniques of production and use of trees, shrubs and herbs, through their flowers, leaves, fruits and tubers).

This scientific work deals specifically with fruit and vegetables, including native ones, which place Brazil as the third largest fruit producer in the world, behind China and India (ANDRADE, 2014), with 65% of this production being consumed in the country itself and 35% abroad (EMBRAPA, 2021), while in vegetables, according to Waldemar Pires de Camargo Filho and Felipe Pires de Camargo, in 2015, it ranked sixth in world production.

According to the IBGE (2021), Brazil has great responsibility at the global level, as expressed in the sustainable development goals (SDGs), established by the UN and supported by the country, noting that the year 2021 was chosen by the UN as the International Year of Fruits and Vegetables.

Such facts would fully justify the realization of this publication, which covers research in Horticulture with due food safety.

There is a lot to be done and this work is an example of what we can do, and human beings have awakened their interest in healthier food, especially in recent decades, demonstrating common sense through growing interest in the chemical composition and antioxidant properties of fruits and vegetables and vegetables. This "new" behavior food currently plays a central role in the prevention and treatment of disease (ALESSANDRA ROSSI et al., 2008).

Scientific research has shown a negative association between fruit and vegetable consumption and the incidence of diseases, whose positive effects are attributed to the presence of nutrients (eg, vitamins A, C and E, and phenolic content) (ENGLBERGER et al., 2009; FLORES et al., 2012).

There is a need to highlight the fruits for representing the largest natural reserves of antioxidants, for their richness in bioactive compounds, responsible for flavor, color, odor and oxidative stability. Such bioactive compounds modulate metabolic processes, in addition to influencing cellular activities, with their antioxidant, anti-cancer, anti-inflammatory and anti-allergic properties, causing beneficial physiological effects, preventing or reducing the risk of numerous diseases (BERNARDI et al., 2019).

In fruit, the country offers new products with functional foods, using various fruits (e.g. oranges, guavas, grapes, watermelons, grapefruit, cherries, apples, bananas, lemons, melons, etc.) that provide a range of nutrients and phytochemical compounds (phenolics, flavonoids and carotenoids), vitamins (vitamin C, folate and provitamin A), minerals (potassium, calcium and magnesium) in addition to essential fibers. Red fruits (blackberries, blueberries, cranberries, raspberries and strawberries) receive special attention in research to prevent chronic diseases. Among the studied vitamins appear water-soluble Vitamin C (ascorbic acid), acting as the first defense against free radicals (BOMFIM, et al., 2017).

Fruits can be consumed fresh or in processed products such as juices, nectars, jellies, purees, flours, and fermented beverages - wines and beers etc. (BRAZIL, 2015). For the case of beers, several fruit trees are used (eg cherries, raspberry, peach, apricot, grape, plum, orange and apple), where the antioxidant activity, the content of total polyphenols and flavonoids are considerably higher in the cherry species, grapes, plum and orange, respectively (PEREIRA, 2009). Regarding the use of fruit by-products (ex: peels, seeds, stalks and bagasse) there is great potential for its bioactive compounds.

In addition to the application of phenolic compounds as a replacement or complement to food additives (vitamins, mineral salts and others), the industry has a great challenge regarding the most suitable extraction method for its isolation.

The use of unconventional methods for the extraction of these compounds meet the concepts of green chemistry (ANASTAS & WILLIAMSON, 1996), using biodegradable solvents, with low energy consumption. There are also promising alternatives such as fermentation, physical techniques and enrichment through residues increasing the bioactive compounds in the product.

Thinking about these aspects and the importance of the techniques used, that is, drying, lyophilization and pasteurization used in different fruits, in order to preserve their nutrients and bioactive compounds. The selected fruits were apples (*Malus domestica*), jaboticaba (*Myrciaria cauliflora*), achachariu (*Garcinia humilis*), physalis (*Physalis angulate*)

and mandacaru (*Cereus jamacaru*). The work is increasingly contributing to the technological and productive development of the country in the definitive implementation of an efficient and clean model of food production for the world.

I consider this an essential work, since research on nutrition and antioxidant actions in horticulture, especially with fruits, is still infrequent, and this work comes to contribute and minimize such scarcity.

Dr. Renato Ferraz de Arruda Veiga

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Chapter I

ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPOUNDS OF LYOPHILLED JABUTICABA (*Myrciaria cauliflora*) PEELS

Virgínia Mirtes de Alcântara Silva
Newton Carlos Santos
Raphael Lucas Jacinto Almeida
Victor Herbert de Alcântara Ribeiro
Gabriel Monteiro da Silva
Jaderson Felipe Santos Dantas
Fabrcia Santos Andrade

Introduction

Jaboticaba (*Myrciaria cauliflora*) is a native fruit of South America, typically cultivated in Brazil. It is a fleshy fruit with an intense violet skin and a sweet white pulp. Although the bark is not widely consumed, polyphenols such as ellagic acids, quercetin and anthocyanins are bark constituents representing a potential antioxidant action (SOUZA et al., 2016).

The food industry generates a large amount of waste, the main ones being the husks, seeds, and seeds of fruits, and these can be used as raw material, thus adding value to this material that would be discarded (MACHADO et al., 2013). According to Padua et al. (2017) an alternative for the reuse of jaboticaba residues can be the elaboration of flour, which can be used for the preparation and enrichment of food.

The drying process involves reducing the water content of the product until it reaches a safe level, which can be applied to ensure the preservation of the physiological and physicochemical quality of the product to be stored for a long period of time (SANTOS et al., 2019).

Freeze-drying is one of the best drying methods, as it makes it possible to maintain the organoleptic and nutritional properties of foods. The method consists of freezing the product followed by dehydration, which occurs through the sublimation process, providing a reduction in the water content and consequently minimizing the occurrence of most reactions that cause product degradation (VIVAS et al., 2019).

In this context, aiming to reduce the environmental impacts caused by the inappropriate disposal of these wastes to the environment and to add greater nutritional and commercial value to jaboticaba barks. This work aims to freeze-dry the jaboticaba bark and characterize the powder obtained in terms of physicochemical parameters and bioactive compounds, thus evaluating their effect on its composition.

Methodology

For the development of this work, jabotica fruits (*Myrciaria cauliflora*) acquired in the local commerce in the city of Campina Grande, Paraíba, were used. Then, they were transported to the Food Engineering Laboratory of the Federal University of Campina Grande - UFCG. The fruits were selected, washed and sanitized with sodium hypochlorite in solution (200 mg.L⁻¹ of free chlorine). The jaboticaba fractions were separated (pulp, husk, and seed) by manual depulping and their husks were submitted to lyophilization processes.



Figure 1. Jaboticaba used in the research.

Freeze drying of peels

Jaboticaba peels were initially subjected to slow freezing in a freezer for 48 h at a temperature of -18°C. After freezing, the husks were transferred to a benchtop lyophilizer (Terroni, LS 3000) and subjected to a temperature of -50°C for 48 h.

Obtaining powder from jaboticaba bark

The crushing of jaboticaba peels after lyophilization was done using an industrial blender in a time of 3 minutes, under agitation. The samples were placed in laminated packages under the protection of light, until the moment of analysis, to maintain the properties of the samples.

Physicochemical characterizations

The fresh jaboticaba husks and powder obtained by freeze-drying were characterized according to the following physical-chemical parameters: moisture content was determined by drying in an oven at 105°C until constant weight; ash content was determined by muffle incineration; the total protein content was quantified by the Micro-Kjeldahl method, which consisted of the determination of total nitrogen according to the methodology described by Brasil (2008); the lipid content was quantified by the modified method of Blig and Dyer (1959); crude fiber content following the method described by Ven Soest (1967); and the total carbohydrate content was calculated by difference to obtain 100% of the total composition (FAO, 2003).

Water activity (A_w) was determined using the Decagon® Aqualab CX-2T device at 25°C; the content of total soluble solids (TSS) expressed in (°Brix), pH with direct reading in the digital pH meter, the total acidity determined by titration, according to Brasil (2008). To determine the reducing sugar content, the methodology described by Miller (1969) was followed.

Determination of bioactive compounds and antioxidant activity

In the fresh jaboticaba bark and the powder collected by freeze-drying, the following bioactive compounds were determined: the total flavonoids and anthocyanins followed the pH or method described by Francis (1982); total phenolic compounds compounds were quantified using the Folin-Ciocalteu method described by Waterhouse (2006), using gallic acid as a standard; and the antioxidant activity by the ABTS+ method was determined by the

method proposed by Re et al. (1999), with modifications made by Rufino et al. (2007), expressed in ($\mu\text{mol Trolox g}^{-1}$).

Results

Table 1 shows the results obtained in the analysis are physicochemical for the peel blemish in nature and lyophilized.

Table 1. Physicochemical analysis of fresh and dehydrated jabuticaba bark

Parameters	<i>Jabuticaba Bark</i>	
	<i>In natura</i>	Lyophilized
Moisture (%)	83.14 ± 0.14	10.57 ± 0.32
Ashes (%)	0.794 ± 0.21	3.41 ± 0.23
Lipids (%)	0.81 ± 0.91	1.94 ± 0.14
Protein (%)	7.62 ± 1.02	6.67 ± 0.87
Raw Fiber (%)	1.37 ± 1.23	4.93 ± 0.69
Carbohydrates (%)	6.27 ± 0.56	72.48 ± 0.66
Water activity (a_w)	0.991 ± 0.01	0.302 ± 0.02
pH	4.21 ± 0.02	2.94 ± 0.05
Acidity (% citric acid)	1.31 ± 0.16	2.01 ± 0.57
Reducing sugars (%)	8.74 ± 0.81	23.52 ± 0.36
Total soluble solids (°Brix)	16.0 ± 0.01	27.89 ± 0.02

The moisture value found for fresh jabuticaba bark was 83.14% and for freeze-dried bark powder was 10.57%. This low content is in accordance with the requirement of the Brazilian legislation for the standard of identity and quality of flour, which recommends values in the range of 5 to 15% (ANVISA, 2005). Flour with moisture content above 15% tends to form lumps, which can harm the production of pasta by continuous process (FERNANDES et al., 2008). In the work carried out by Leite-Legatti et al. (2012) found a value of 15.33% in jabuticaba bark flour.

The ash percentages ranged from 0.794 to 3.41%, reflecting in a higher concentration of mineral residue after incineration, where for the freeze-dried husks there was a gain of 2.616%. However, these values obtained were consistent with those determined by authors such as Ascheri et al. (2006), when studying fermented (1.35%) and fresh (3.49%) jabuticaba bagasse, dried in a circulation oven at 60°C and Costa et al. (2007) for bark powder (2.03%) and pineapple bagasse (2.15%). Leite-Legatti et al. (2012) found higher values, 4.23% for jabuticaba bark dried in a forced air oven and 3.52% for freeze-dried bark, respectively. As

well as the value found by Ferreira et al. (2012) of 3.89% for jabuticaba bark flour obtained by drying in an oven with forced air circulation at a temperature of 60°C.

According to Lima et al. (2008), jabuticaba bark can be considered a source of minerals such as iron, potassium, magnesium, manganese, phosphorus, calcium, and copper, which play vital roles in cell metabolism (FELIPE et al., 2008).

The values found in the present work for the lipid content in fresh and freeze-dried jabuticaba bark were 0.81 and 1.94%, respectively. Values close to those of the present study were obtained by Lage et al. (2014) for dry bark (1.59%) and by Leite-Legatti et al. (2012) of 1.72% for the freeze-dried bark, which are higher than the values obtained by Gurak et al. (2014), of 0.83%. According to Russo et al. (2012), the low lipid content of jabuticaba bark may bring benefits to human health, as a large part of the population consumes high-fat diets, and that this fact is contributing to the emergence of non-communicable chronic diseases, such as obesity.

The raw peel had a protein concentration of 7.62%, it can be seen in Table 1 that the peel dehydration process caused protein degradation of up to 0.95%. Close values were obtained by Ferreira et al. (2012) of 5.23%, Appelt et al. (2015) and Alves et al. (2013), 6.40 and 6.06%, respectively, for jabuticaba bark flours.

Leite-Legatti et al. (2012) obtained the value of 4.89% protein for the lyophilized product and stored at -80°C. According to Brasil (2012), a product can be considered a protein source when it presents at least 6 g of protein in 100 g or 100 mL. Thus, it can be considered that jabuticaba bark flour is a source of protein.

Although fibers do not have nutritional value, their determination is also considered important in food products, since their structures and characteristics play different physiological functions in the gastrointestinal tract (CECCHI, 2007).

The content of Crude Fiber (FB) in fresh jabuticaba bark was 1.37% and freeze-dried bark presented a value of 4.93%. Faria et al. (2016) found a higher value for the fresh bark of 1.84% and a lower value for the freeze-dried bark, 3.89% of crude fiber.

The lyophilized jabuticaba bark powder had carbohydrate (72.48%) as the main macronutrient. These values are higher than those found by Ferreira et al. (2012) 58.70% and that of Ascheri et al. (2006) 56.06%. Since the fiber content is included in the total of carbohydrates, which may be overestimating its value.

The fresh jabuticaba bark had a high-water activity value (0.991). There was a reduction when they were subjected to the lyophilization process, in which it presented a

value of 0.302. For this parameter when comparing the two drying methods, there were statistically significant differences.

According to Franco and Ladgraf (2008), water activity is water that is difficult to remove by conventional drying processes, as they are linked to macromolecules, not being free to act as a solvent or to participate in chemical reactions, and thus not used by microorganisms for cell multiplication.

The pH obtained for in natura peel was 4.21, Lima et al. (2008) in their studies found a pH of 3.47 for the fresh jabuticaba bark of the Paulista variety and 3.39 for the Sabará variety. The freeze-dried husks had a value of 2.94. Miliagato et al. (2007) reported that for the whole fruit of jabolão dried in an air circulation oven at 45°C, the pH found was 4.09.

While for the total titratable acidity (TT) the value obtained in the present work for in natura peel was 1.38 g of citric acid/100g of fresh sample. Lima et al. (2008) found for the fresh jabuticaba bark the values for the Paulista and Sabará variety of 1.37 and 1.67 g of citric acid/100g of fresh sample. Higher value for this parameter was obtained for freeze-dried flour (2.01 g citric acid/100g sample).

Total soluble solids (TSS) represent the content of soluble sugars, organic acids, and other minor constituents (SEYMOUR et al., 2012). The concentration of these solids is one of the most important variables to measure fruit quality.

The amount of total soluble solids found for fresh jabuticaba bark was 16 °Brix. Salomão et al. (2018) characterized the fresh fruit of several varieties and the values obtained were in the range of 9.1 to 17.6°Brix, for the variety Sabará the content was 14°Brix. It is noticeable that for the freeze-dried jabuticaba peel powder, the soluble solids value (27.89°Brix) was higher than that of the fresh fruit.

The reducing sugar content found in this work for in natura peel was 8.74% and 23.52% for the freeze-dried powder. Thus, it is suggested that the higher the sugar content, the greater the hygroscopicity of the product. This aspect is of practical importance, especially about the conditions for marketing these powdered products.

Table 2 shows the mean values obtained for bioactive compounds and antioxidant activity present in fresh and lyophilized jabuticaba bark.

Table 2. Bioactive compounds from fresh and dehydrated jabuticaba bark

Parameters	<i>Jabuticaba bark</i>	
	<i>In natura</i>	Lyophilized
Anthocyanins (mg/100g)	31.45 ± 1.12	99.23 ± 0.97
Flavonoids (mg/100g)	60.31 ± 1.79	123.67 ± 3.49
Total phenolic compounds (mg GAE/100g)	1497.87 ± 8 96	1054.63 ± 14.28
Antioxidant Activity (ABTS+) (µmol Trolox/g)	325.98 ± 13.21	287.98 ± 9.44

Regarding the anthocyanin values, it can be seen in Table 2 that the in natura bark had a content of 31.45 mg/100g. According to Maria do Socorro (2010), the value obtained for anthocyanins for the whole fresh fruit of jabuticaba was 58.1 mg/100g, a value higher than that obtained in the present study. And a higher concentration of this compound was obtained in the freeze-dried bark powder, which had a content of 99.23 mg/100g. Wu et al. (2006) analyzed the values of anthocyanins for well-known tropical fruits such as: strawberries contains 21 mg/100g, red grapes 27 mg/100g, red raspberries 92 mg/100g, cherries 122 mg/100g, blackberries 245 mg/100g and cultivated blueberries 387 mg/100g, these values are in the same range as found for jabuticaba, a not so conventional fruit.

According to Kähkönen et al. (2001), anthocyanins are composed of bright colors responsible for a large part of the red, blue, and purple color of the fruits, being abundant mainly in red fruits such as blueberries and black currants.

The fresh jabuticaba bark had 60.31 mg/100g of flavonoids, values that were lower than those found by Maria do Socorro et al. (2010) who obtained 147.0 mg/100g for jabuticaba (peel + pulp) in natura. For jambolão, camu-camu and uvaia the values found were in the range of 70.9; 20.1 and 17.5mg/100g, respectively, thus being lower than the levels obtained for the powder prepared in the present study, which was 123.67 mg/100g (lyophilized). The contents of total flavonols are higher in the bark than in its pulp, because of this result, it is suggested to use the jabuticaba bark as a potential source of flavonoids.

According to Gallego et al. (2007), the antioxidant capacity of flavonoids confers a therapeutic potential for the treatment of cardiovascular diseases, including ulcers and cancer of the gastrointestinal tract.

Regarding the contents of total phenolic compounds, 1497.87 mgGAE/100 g was obtained in the fresh jabuticaba bark. It is noteworthy that the lyophilization process degraded these compounds, with a value of 1054.63 mgGAE/100 g. In the study by Leite-

Legatti et al. (2012) with fresh jaboticaba peel and the whole fruit of *M. jaboticaba* presented 11400 and 3215 mgGAE/100g, respectively.

The antioxidant action of a compound is directly related to the bioactive components present and depends on the chemical structure and concentrations of these phytochemicals in the food (MAGALHÃES et al., 2008). Jaboticaba had a high content of total phenolic compounds and, consequently, high antioxidant activity (REYNERTSON et al., 2014).

The values obtained for the antioxidant activity by the ABTS method in the present work for *in natura* bark was 325.98 $\mu\text{mol Trolox/g}$, and the freeze-dried bark powder presented a value of 287.98 $\mu\text{mol Trolox/g}$, being noticeable that the drying method directly influenced the antioxidant power of the product.

Conclusion

Given the results presented, it is seen that the use of jaboticaba bark *in natura* and after drying in the form of flour are viable for the development of new products, to minimize the disposal of waste produced by the agrifood industry.

Jaboticaba bark flour is a source of phenolic compounds with antioxidant activity and fiber and can be considered a functional food.

Comparing the drying techniques used, lyophilization stood out as it preserved more the physical chemical parameters and bioactive compounds.

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Chapter II

BIOACTIVE COMPOUNDS AND INSTRUMENTAL COLOR IN FUJI APPLE (*Malus domestica*) PEELS

Virgínia Mirtes de Alcântara Silva
Newton Carlos Santos
Raphael Lucas Jacinto Almeida
Victor Herbert de Alcântara Ribeiro
Gabriel Monteiro da Silva
Jaderson Felipe Santos Dantas
Fabrcia Santos Andrade

Introduction

Apple is one of the four most consumed fruits in the world. This fruit can be consumed mainly in natura, or in other forms that involve technological processes, that is, in the form of concentrated juice, vinegar, dehydrated and in fermented beverages, such as cider.

In addition, apple by-products, including peel and bagasse, account for approximately 30% of the original fruit (GULSUNOGLU et al., 2020). This residue is formed by all parts of the apple (epicarp, mesocarp and endocarp), and its chemical composition includes water (75-87%), carboxylic acids, sugars, fibers and phenolic compounds in variable amounts (NOGUEIRA & WOSIACKI, 2012).

Its by-products have the potential of bioactive ingredients that can be used in the development of new supplied foods, nutraceutical supplements and food additives. An efficient alternative is the production of flour from agro-industrial by-products to be incorporated into the composition of various types of food such as biscuits, cakes and breads.

It is possible to obtain a fiber concentrate from apple pomace, which could be used in various products, such as dairy products, beverages, jellies, cereal bars, among others (PROZ, 2017).

In addition to being a fruit rich in phenols, pectic substances and cellulose, which together with lignin constitute dietary fiber (AIRES, 2016).

According to Khanizadeh et al. (2008) apples are an excellent source of several phenolic compounds and have high total antioxidant capacity. The antioxidant properties of apples are highly dependent on their variety, agricultural practices, climate, storage, and processing conditions. Apples more abundant in phenolic compounds consequently tend to have greater antioxidant activity (KALINOWSKA et al., 2014).

Apple fruits have a wide variety and balanced composition, being moderately energetic and well-proportioned in sugar and acid, giving it a pleasant flavor. The chemical composition of apples varies according to cultivar, production region and horticultural practices (PIRES et al., 2017).

The harvest of apples in Brazil takes place during the summer and a large part of the production is stored to be made available to consumers throughout the year. Therefore, it is essential to harvest at the ideal time for the fruits to preserve their quality and reduce production losses during and after storage. Apples harvested before the ideal harvest point (immature), despite showing good post-harvest conservation of some quality aspects, present undesirable characteristics such as smaller size, little color, flavor and aroma and greater susceptibility to some physiological disturbances. On the other hand, apples harvested at more advanced stages of maturation are more susceptible to the occurrence of mechanical damage, rot, and some physiological disturbances (MAGRIN et al., 2017).

The accumulation of anthocyanins, pigments that give apples the reddish color, is greater on days with high luminosity and cold nights, in the weeks that precede the beginning of ripening and harvesting. Therefore, bicolor apple harvests tend to be delayed when weather conditions are unfavorable to the accumulation of anthocyanins. This procedure implies harvesting more ripe apples, with higher ethylene production rate, lower pulp firmness, higher incidence of stalk cracks and lower storage potential (ARGENTA et al., 2015).

In this context, the present study aims to determine the content of total phenolic compounds, total anthocyanins, total flavonoids, total chlorophyll, and the instrumental color of the fuji apple peel.

Methodology

Raw material

The raw material used in the development of this work consisted of fruits of the Fuji apple tree (*Malus communis*) (Fig.1.1). In Figure 1.2 we can see the Fuji apple and its morphology. acquired in the city of Campina Grande–PB. Then, they were transported to the Food Engineering Laboratory of the Federal University of Campina Grande - UFCG, and the selection steps were carried out (whole fruits, without lesions and with completely red skins), cleaning and sanitization in a sodium hypochlorite solution (200 mg L⁻¹ of free chlorine).



Figure 1.1. Apples used in the research.

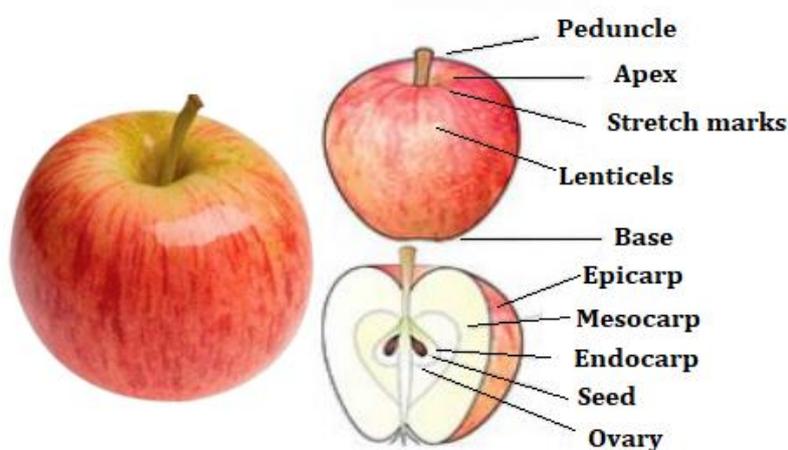


Figure 1.2 Apple Fuji variety. Source: Borges Filho (2018).

Determination of total phenolic compounds

Total phenolic compounds were quantified by the Folin-Ciocalteu method described by Waterhouse (2006), using gallic acid as a standard. The calculations performed for the determination of phenolic compounds were based on a standard curve with gallic acid, and

readings were made in a spectrophotometer at 765 nm, with results expressed in mg/100 g of gallic acid.

Determination of anthocyanins and flavonoids

The method used to read the total anthocyanins and total flavonoids present in the apple peel was the single pH method described by Francis (1982). The method consists of carrying out a quantitative transfer of an aliquot of the concentrated extract to a recipient and then this aliquot is diluted with an amount of Ethanol – HCl solution at 1.5 mol. L⁻¹, thus having a volume of diluted extract.v

Determination of total chlorophyll

The chlorofle contents of the apple peel were quantified after their extraction using 80% acetone and calcium carbonate according to Lichtenthaler (1987).

Determination of instrumental color

The color parameters were determined using the Mini Scan Hunter Lab XE Plus spectrophotometer (Reston, VA, USA), in the Cielab color system. Which allowed obtaining the parameters: L* (luminosity); a* (transition from green (-a*) to red (+a*)); and b* (transition from blue color (-b*) to yellow color (+b*)).

Results

The apple peel had a high content of total phenolic compounds (359.29 mg.100g⁻¹ of gallic acid) (Table 1). Morais et al. (2019) when analyzing apple peels in the ripe maturation stage, obtained a total phenolic content of 212.12 mg.100g⁻¹ of gallic acid, using hydroalcoholic extract. Lima et al. (2017) obtained total phenolic compounds content of 87.3 mg.100g⁻¹ of gallic acid for kikan fruits. Teixeira et al. (2016) when quantifying total phenols with ethanol extract of the fruit *Physalis Peruviana* L, it presented an amount equivalent to 149.3 mgGAE.100g⁻¹.

Table 1. Bioactive compounds from fresh fuji apple peel

Bioactive compounds	Apple (<i>in natura</i>)
Total phenolic compounds (mg 100g ⁻¹ of gallic acid)	359,29 ± 19,21
Total anthocyanins (mg/100g)	14,52 ± 0,58
Total flavonoids (mg/100g)	1,62 ± 0,15
Total chlorophyll (mg/100g)	0,0564 ± 0,001

Saleem e Saeed (2019) when quantifying the content of total phenolic compounds present in orange, lemon, and banana peels, using different solvents, obtained values ranging from 12.4 to 21.21 mg/g extract; 16.29 to 29.72 mg/g extract; 8.6 to 15.6 mg/g extract, respectively. Anthocyanins are relatively unstable pigments, and their greatest stability occurs under acidic conditions. The fresh apple peel had an anthocyanin content of 14.52 mg/100g. For the same variety in a study Kokalj et al. (2019) obtained anthocyanin content of 66.5 mg/kg. This difference may be related to several external factors, particularly light, as the formation of anthocyanins is mainly affected by this factor.

According to Fennema et al. (2010), although most of the yellow color of foods is attributed to the presence of carotenoids, this color in some foods is attributed to the presence of non-anthocyanin flavonoids. In that study, the apple peel had a content of 1.62 mg/100g for flavonoids. Moreira et al. (2018) obtained 2.88 mg/100g of flavonoids in kiwi fruit in natura. Among flavonols, quercetin glycosides are almost exclusively found in apple peel (Awad et al., 2000; Kokalj et al., 2019). According to Bi et al. (2014) the main phenolics found in apples are flavonols, anthocyanins, phenolic acids and dihydrochalcones.

Low concentrations of chlorophyll were obtained for the skins (0.0564 mg/100g). Chlorophyll pigments are highly appreciated as functional components in fruits and vegetables, both for their green coloring properties and for their health benefits for human consumption derived from their biological properties. (DOMÍNGUEZ et al., 2016).

Table 2. Shows the mean values obtained for the chromatic coordinates

Colour	Apple (<i>in natura</i>)
L*	49,09 ± 1,52
a*	32,72 ± 1,13
b*	24,60 ± 2,45

The evaluation of the color coordinates, through the values of L*, a* and b* assume relevant importance for fresh fruits. It is noteworthy that these coordinates were obtained when the whole fruit was analyzed, that is, with skins. Therefore, the L* coordinate is a parameter that can vary from zero (black) to 100 (white), with a value of 49.09. Lower values for this coordinate were obtained by Silva et al. (2019) in mandacaru fruits at two stages of maturation that ranged from 31.94 to 36.74.

As for the intensity of a* (positive values represent red), a value of 32.72 was obtained, and for the intensity of b* (positive values represent yellow), a value of 24.60 was obtained. These results reflected the reddish yellow color, which is a characteristic of this fruit.

According to Dar et al. (2019) the apple peel color is one of the most important factors that determine the acceptance of the apple market. In general, red cultivars are the most preferred. However, consumer preferences vary from country to country and region to region.

Conclusions

According to the results of the present study, it was possible to fulfill that a fuji apple peel has a high content of total phenolic compounds and the presence of flavonoids and anthocyanins.

Furthermore, the results of the color analysis can be used in agribusiness to predict the degree of ripeness of the fruit and, consequently, the choice of the technological process and the storage conditions of the fruit.

Thus, check whether a colorimetry represents a relevant area for the quality control and standardization of food products in the industry, as the results of its analysis are used in the food industry to predict the color of the finished products, which depends on the degree of maturation of the the result of the choice of the technological process and storage conditions.

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Chapter III

CENTRAL COMPOSITION AND BIOACTIVE COMPOUNDS OF FRUIT PHYSALIS (*P. peruviana*) FRESH AND DEHYDRATED

Virgínia Mirtes de Alcântara Silva
Newton Carlos Santos
Raphael Lucas Jacinto Almeida
Victor Herbert de Alcântara Ribeiro
Gabriel Monteiro da Silva
Jaderson Felipe Santos Dantas
Fabrcia Santos Andrade

Introduction

The genus *Physalis* includes about 100 species and belongs to the Solanaceae family, characterized as a group of high economic importance. These species are applied in several segments, such as human food (*P. peruviana*), production of pharmaceutical substances (*P. angulata*) and ornamentation (*P. alkekengi*).

Edible physalis (*P. peruviana*) is the species that stands out in Brazil, it is defined as a small fruit that has a color that varies between yellow and orange, has a sweet flavor and is rich in several nutrients such as vitamin A and C, iron, and phosphorus, in addition to alkaloids, flavonoids, carotenoids and bioactive compounds, which are considered as functional compounds (MUNIZ et al., 2015; SOUZA et al., 2017).

The high moisture content present in the composition of physalis is the main factor responsible for the growth of microorganisms and the occurrence of biochemical reactions, consequently causing its rapid deterioration. To minimize this problem, food conservation techniques are applied, such as drying, whose main objective is to reduce the moisture

content, enabling safe storage for a long period of time (LANDIM et al., 2016; OLIVARES et al., 2017).

However, some studies found losses of thermosensitive compounds after fruit processing, due to the application of conventional drying methods, which are generally associated with reduced nutritional, functional and sensory quality of products (OLIVARES et al., 2017).

In this context, this study aims to characterize and evaluate the influence of temperature on its proximate composition and bioactive compounds of physalis fruits.

Methodology

Raw material and sanitation

The physalis fruits (*P. peruviana*.) were purchased in the local trade in the city of Campina Grande, Paraíba, Brazil. Then, they were transported to the Food Engineering Laboratory of the Federal University of Campina Grande – UFCG. They were selected, washed in running water, sanitized, and sanitized in chlorinated water (100 ppm) for 15 minutes.



Figure 1. *Physalis* fruits es used in the research.

Fruit dehydration

The fruits were subjected to a drying process at temperatures of 40, 50, 60 and 70°C in an air circulation oven with a speed of 1.5 m s⁻¹, until reaching constant mass.

Centesimal composition

In natura and dehydrated physalis fruits were centesimally characterized according to the following centesimal parameters: moisture by drying in an oven at 105°C to constant weight; Ashes by incineration in a muffle; The total protein content was quantified by the Micro-Kjeldahl method, which consisted of the determination of total nitrogen according to the methodology described by Brasil (2008); and lipids by the modified method of Blig and Dyer (1959); The total carbohydrate content was calculated by difference to obtain 100% of the total composition (FAO, 2003).

Bioactive compounds

The content of total anthocyanins and flavonoids followed the single pH method described by Francis (1982). The method consists of a quantitative transfer of an aliquot of the concentrated extract to a recipient and then this aliquot is diluted with an amount of Ethanol - HCl solution at 1.5 mol.L⁻¹, thus having a volume of diluted extract. The quantification of total carotenoids (lycopene) followed the methodology described by Davies (1976).

Total phenolic compounds were quantified using the Folin-Ciocalteu method described by Waterhouse (2006), using gallic acid as a standard. The calculations performed for the determination of phenolic compounds were based on a standard curve with gallic acid, and the readings performed in a spectrophotometer at 765 nm, with the results expressed in mg.100g⁻¹ of gallic acid.

Antioxidant activity

Antioxidant activity by the ABTS+ method was determined by the method proposed by Re et al. (1999), with modifications made by Rufino et al. (2007), expressed in (μmol Trolox g⁻¹), while the antioxidant activity by DPPH was made according to the methodology described by Rufino et al. (2007), with adaptations, presenting the final result in g of sample/g of DPPH captured (EC50). For both analyses, distilled water was used as extractive solvent.

Statistical analysis

The experimental data were analyzed in triplicate and the results were submitted to the analysis of variance of a single factor (ANOVA) of 5% probability and the significant qualitative responses were submitted to the Tukey test, adopting the same level of 5% of

significance. For the development of statistical analysis, the software Assistat 7.7 (SILVA & AZEVEDO, 2016) was used.

Results

Proximate composition, bioactive compounds and antioxidant activity of fresh fruit

In the fruits analyzed *in natura* (Table 1), about 88.57% of its composition is water. Pereda et al. (2019), found a humidity of 79.11% for physalis *in natura*. As well as Hassanien (2011), who observed fruit moisture around 78.9%. In addition to moisture, higher levels of ash and lipids were observed in this study, when compared to the values found by Pereda et al. (2019), whose values were 0.81% of ash and 0.39% of lipids.

Puente et al. (2011) and Hassanien (2011), both reported ash contents of 1.0%. However, fruit quality can change due to pre-harvest factors, such as: temperature, relative humidity, light, soil texture, wind and rain (MATTIUZ, 2007).

Table 1. Proximate composition and bioactive compounds of physalis *in natura*

Parameters	<i>in natura</i>
Humidity ¹ (%)	88,57 ± 4,12
Ashes (%)	2,87 ± 0,54
Lipids (%)	1,08 ± 0,35
Protein (%)	0,84 ± 0,21
Total carbohydrates (%)	6,64 ± 0,64
Anthocyanins (mg/100g)	0,77 ± 0,07
Flavonoids (mg/100g)	20,74 ± 4,12
Total carotenoids (µg/g de licopeno)	4,51 ± 2,15
Total phenolic compounds (mgGAE/100g)	208,93 ± 22,13
Antioxidant activity by ABTS ⁺ (µmol Trolox g ⁻¹)	4,68 ± 0,97
Antioxidant activity by DPPH (% sequestration)	87,47 ± 2,47

Note: 1 wet base; *in natura*: fresh fruit.

The protein content presented was lower than that found by Pereda et al. (2019) (1.35%). However, according to Puente et al. (2011), the protein content of *Physalis peruviana* L can vary from 0.3 to 1.90%. The carbohydrate content, on the other hand, was lower than that found in the studies carried out by Hemalatha et al. (2018), Pereda et al.

(2019) and Hassanien (2011), whose values were 13.10, 14.22 and 19.6%, respectively. This variation may occur due to the variation of factors such as climatic aspects of the place of production and the ripening stage of the fruits at the time of analysis.

Anthocyanins are relatively unstable pigments, and their greatest stability occurs under acidic conditions. The physalis in natura presented anthocyanin content of 0.77 mg.100g⁻¹. The chemical knowledge of anthocyanins can be used to minimize degradation through the proper selection of specific anthocyanin processes and pigments for the intended applications. The main factors governing the degradation of anthocyanins are pH, temperature and oxygen concentration (FENNEMA et al., 2010).

According to Fennema et al. (2010), although most of the yellow color of foods is attributed to the presence of carotenoids, this color in some foods is attributed to the presence of non-anthocyanin flavonoids. In that study, Physalis in natura had a content of 20.74 mg.100g⁻¹ for flavonoids. In the studies carried out by Licodiedoff et al. (2013a), flavonoid concentrations of 78.64 (µg Rutin/mL), 4.67 (µg Myricetin/mL) and 2.38 (µg Kaempferol/mL) have been reported for Physalis peruviana RS. El-Beltagi et al. (2019) reported a total flavonoid concentration of 6.39 (mg Quercetin/g DW), which shows a variation in values according to the analytical method, in addition to the influence of intrinsic and extrinsic factors that can influence the composition of the fruits.

The content of carotenoids in this study was higher than those reported by Cárcamo-Medina et al. (2019), which reported lycopene contents of 2.22 (mg/g juice) for fresh Physalis peruviana juice, and 3.75 to 2.63 (mg/g juice) for juices subjected to heat treatment. Machado et al. (2019) in their studies with Physalis pulp obtained 6.99 µg/g of carotenoids.

According to Fennema et al. (2010), carotenoids are sensitive to light, excessive heat and exposure to acids. This sensitivity makes them very vulnerable during their processing and storage, which means that several precautions must be taken to minimize their losses.

As for the content of phenolic compounds in this study, a higher concentration was observed when compared to the study by Silva et al. (2016) with physalis fruit species in a subtropical area, found a value of 93.57 (mg GAE.100 g⁻¹) for the species P. peruviana, and those presented by El-Beltagi et al. (2019), with a value of 125.44 (mg GAE.100 g⁻¹). Machado et al. (2019) in their studies on the stability of the Physalis pulp obtained 60.87 mgGAE.100 g⁻¹ for the phenolic compounds

The antioxidant activity, by the ABTS method for the in natura pulp of physalis was 4.68 (µmol Trolox.g⁻¹), lower than that reported by Licodiedoff et al. (2013b) in the study the

antioxidant activity of fruits at the beginning and end of the ripening stage, finding values between 7.88 to 8.07 ($\mu\text{M trolox.g}^{-1}$ fruit) for small fruits and 7.49 to 7.81 ($\mu\text{M trolox g}^{-1}$ fruit) for large fruit. Valdenegro et al. (2012) showed that the antioxidant activity of physalis can range from 5.24 to 8.67 ($\mu\text{M trolox g}^{-1}$ fruit) depending on the stage of maturation and storage time.

As for the antioxidant activity by the DPPH method, López et al. (2013), for *Physalis peruviana* found values of 53.97 Mm TE.100 g^{-1} for fresh fruit by the DPPH method. Meanwhile, Chang et al. (2008) reported values between 13.17 and 94.64 (% kidnapping). The value of 87.47 (% sequestration) found in this study is within the percentage range observed in the literature for the DPPH method.

Curi et al. (2017) carried out the characterization of different species of Native American physalis and obtained for *physalis peruviana* 75.06 (% sequestration) for antioxidant capacity by DPPH. Teixeira Jury et al. (2016) evaluated the content of total polyphenols and the antioxidant capacity of the ethanol extracts of the fruits *Physalis peruviana* L. obtained 100 (% sequestration) for the antioxidant activity by DPPH.

Proximate composition, bioactive compounds and antioxidant activity of dehydrated fruits. The effect of drying temperature applied to physalis fruits are shown in Table 2.

Table 2. Proximate composition and bioactive compounds of physalis after drying at different temperatures

Parameters	40°C	50°C	60°C	70°C
Humidity ¹ (%)	10,89±0,12 ^a	9,87±0,16 ^b	9,57±0,20 ^c	8,58±0,15 ^d
Ashes (%)	2,84±0,44 ^b	2,91±0,64 ^a	2,77±0,39 ^c	2,57±0,84 ^d
Lipids (%)	1,10±0,25 ^a	1,18±0,35 ^b	0,99±0,18 ^c	0,88±0,47 ^d
Protein (%)	0,80 ± 0,47 ^a	0,76 ± 0,29 ^b	0,71 ± 0,16 ^{bc}	0,69 ± 0,78 ^c
Total carbohydrates (%)	84,37 ± 0,34 ^b	85,28 ± 0,46 ^b	85,96 ± 0,27 ^b	87,28 ± 0,65 ^a
Anthocyanins (mg/100g)	1,45±0,15 ^a	1,12±0,22 ^a	0,59±0,23 ^b	0,31±0,19 ^c
Flavonoids (mg/100g)	32,52±2,89 ^a	30,56±2,87 ^b	20,12±5,14 ^c	10,12±4,16 ^d
Total carotenoids (µg/g de licopeno)	8,57±1,12 ^a	6,54±0,98 ^b	3,41±1,10 ^c	1,65±0,45 ^d
Total phenolic compounds (mgGAE/100g)	249,58±25,14 ^a	223,89±22,41 ^b	198,15±15,70 ^c	97,25±35,26 ^d
Antioxidant activity by ABTS ⁺ (µmol Trolox g ⁻¹)	5,18±0,45 ^a	4,89±0,32 ^b	3,75±0,25 ^c	2,11±0,49 ^d
Antioxidant activity by DPPH (% sequestration)	89,02±2,45 ^a	80,07±3,78 ^b	45,12±5,48 ^c	12,98±1,15 ^d

Note: a, b, c, d Equal superscript letters on the same line do not show a significant difference at the 5% probability level.

Note that the lowest moisture content was found in dried fruits at temperature 70°C, showing a statistically significant difference when comparing the values obtained with each

other. However, according to Ferrão et al. (2019) the increase in drying temperature resulted in greater efficiency in removing water during the process, but it can cause changes in color, flavor and texture that result in unique properties in the product. Santos et al. (2019) when drying peach slices, they obtained a moisture content of 9.22% for slices subjected to 80°C.

The increase in the drying temperature from 40 to 70°C resulted in a variation of up to 0.34% in the ash content, with the highest percentage being observed at the temperature of 50°C (2.91%), statistically for all temperatures applied to this same parameter show a significant difference at the 5% probability level. Lower values were obtained by Santos et al. (2017) for the Camapu fruit (1.98%).

There was a reduction in the lipid content as the drying temperature increased, where the lower lipid content (0.88%) was consequently obtained for the higher applied temperature (80°C). Values higher than those in the present study were observed by Meneses et al. (2018), in which they obtained 1.61% of lipids for dehydrated mango residues at 55°C. Regarding the protein content, the fruits subjected to a temperature of 60°C showed no statistical differences in relation to fruits subjected to temperatures of 50 and 70°C, in addition, the increase in temperature caused a reduction of up to 0.11% in the protein content. Barros et al. (2019) also observed in their studies a reduction in protein content as the drying temperature increased.

Total carbohydrates were determined by difference based on the other constituents and therefore moisture reduction automatically led to a small increase of up to 2.91% in carbohydrate content between applied temperatures. However, only the value obtained at the temperature of 70°C (87.28%) was significantly different between the other treatments.

All bioactive compounds analyzed (anthocyanins, flavonoids, total carotenoids, phenolic compounds) and antioxidant activity presented degradation with the increase in drying temperature from 40 to 70°C. Likewise, the results obtained also showed statistically significant differences at the 5% probability level ($p < 0.05$).

However, when compared to fresh fruit, temperatures of 40 and 50°C, showed a better conservation of all bioactive components and the antioxidant activity by the DPPH method showed greater activity when compared to the ABTS+ method. A reduction in bioactive compounds was also observed by López et al. (2013), in the study of the effect of dehydration temperature on the physicochemical properties and antioxidant capacity of blackberry (*Physalis peruviana L.*) as the drying temperature increased.

Conclusion

It was observed that the temperature applied during the drying process directly influenced the proximate composition and bioactive compounds of physalis. It was verified that the increase in temperature caused a reduction in moisture, ash and lipids and that the temperatures of 40 and 50°C provided a better preservation of all bioactive components and antioxidant activity.

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Chapter IV

INFLUENCE OF PASTEURIZATION ON THE DETERMINATION OF PHENOLIC COMPOUNDS AND ON THE ANTIOXIDANT ACTIVITY OF THE PULP AND PEEL OF MANDACARU FRUIT (*Cereus jamacaru*)

Virgínia Mirtes de Alcântara Silva

Newton Carlos Santos

Raphael Lucas Jacinto Almeida

Victor Herbert de Alcântara Ribeiro

Gabriel Monteiro da Silva

Jaderson Felipe Santos Dantas

Fabília Santos Andrade

Introduction

Mandacaru (*Cereus jamacaru*) is a native species of the Caatinga vegetation, belonging to the Cactaceae family. In Brazil the species is present throughout the Northeast from the states of Piauí, Paraíba, Ceará, Sergipe, Rio Grande do Norte, Alagoas, Pernambuco, Bahia, and northern Minas Gerais (ANDERSON, 2001; PRADO, 2003).

It develops in rocky soils, together with other species of cactus, forms the typical landscape of the semi-arid region of the Northeast (SILVA & ALVES, 2009; SANTOS NETO et al., 2019). Mandacaru can reach up to 10 meters in height and have a trunk that can reach 60 cm in diameter, with many vertical stalks, forming a compact top. In the dry season, the stem is cut, and the thorns burned to be used as feed for livestock, due to the ability to store large amounts of water (ZARA et al., 2012; MOREIRA et al., 2018).

Exotic fruits cultivated and exploited rationally can be characterized for their use as foods and/or functional ingredients (RUFINO, 2008). Exotic fruits have several bioactivities

and health benefits, which has been drawing attention for its effects antioxidants, anti-inflammatory, antimicrobials and anticancer, and help in prevention and treatment of chronic diseases (LI et al., 2016).

In recent decades there has been a growing interest in research on natural bioactive compounds (NBCs) from exotic fruits, mainly due to their various medicinal properties and potential application in the food, chemical and pharmaceutical industries.

Some research were carried out to identify the medicinal properties of its extracts, evaluating the antioxidant, antiproliferative and antibacterial actions in the treatment of several diseases, kidney, liver, respiratory, stomach and sinuses for the development of new drugs. (DUTRA et al., 2018; MOTA et al., 2019).

Industrial and economic importance has been attributed to plants of this species, where their shoots are used for the extraction of complex heteropolysaccharides and gum. Heteropolysaccharides are used in industrial wastewater purification processes and the gum is used in the cosmetics and food industry (TAVARES et al., 2013). Mandacaru fruit is perishable, has a short shelf life, representing an obstacle to its fresh commercialization. It is recommended that it undergo processing, so that it can reach more distant consumer markets and supply its products year-round (SILVA et al., 2019).

Therefore, finding alternative processing techniques to produce food has become a research trend. Simultaneously, the properties of these foods can change during their processing (WANG et al., 2020). These effects depend on temperature and time. Traditionally, relatively low temperatures were used to prevent unwanted changes in food products, and this required the use of a relatively long contact time to reach the desired level of inactivation. Modern industrial pasteurization processes that require high productivity from the treatment plant, however, involve the use of high temperatures and short contact time conditions (LAU et al., 2020).

In this context, the present work aims to apply the pasteurization process on the pulp and peel of the mandacaru fruit and evaluate its influence on the total phenolic compounds and on the antioxidant activity.

Methodology

Raw material and sanitation

Mandacaru fruits (*Cereus jamacaru*) were harvested in the municipality of Fagundes-PB during the harvest period. Then, they were transported to the Food Engineering

Laboratory of the Federal University of Campina Grande - UFCG, and the selection steps were carried out (whole fruits, without lesions and with completely red skins), cleaning and sanitization in a sodium hypochlorite solution (200 mg L⁻¹ of free chlorine).



Figure 1. Mandacaru fruit (fruit, skin, and seed). Source: Mizrahi (2014).

Pasteurization application

By manual pulping, the mandacaru fruit fractions were separated into pulp and peel. Then, the fractions obtained were processed in a domestic blender and placed separately in glass containers where they were pasteurized by immersion in water at 65°C for 30 minutes, following the procedures described by Almeida et al. (2020a).

Analysis of bioactive compounds and antioxidant activity

The pulp and in natura and pasteurized peels were determined and the content of total phenolic compounds was quantified using aqueous extract from the Folin-Ciocalteu method described by Waterhouse (2006), using gallic acid as standard; the antioxidant activity by the ABTS+ method was determined by the method proposed by Re et al. (1999), with modifications made by Rufino et al. (2007), expressed in ($\mu\text{mol Trolox g}^{-1}$); and the determination of the antioxidant activity by DPPH free radical capture was carried out following the methodology described by Rufino et al. (2010) and the results were expressed in g/g of DPPH.

Statistical analysis

The experimental data were analyzed in triplicate and the results were submitted to the analysis of variance of a single factor (ANOVA) of 5% probability and the significant qualitative responses were submitted to the Tukey test, adopting the same level of 5% of significance. For the development of statistical analysis, the software Assistat 7.7 (SILVA & AZEVEDO, 2016) was used.

Results

Table 1 shows the values obtained for total phenolic compounds and antioxidant activity of unpasteurized (in natura) and pasteurized mandacaru fruit pulp.

Table 1. Total phenolic compounds and antioxidant activity of fresh and pasteurized Mandacaru fruit pulp

Parameters	Pulp	
	<i>In natura</i>	Pasteurized
Total phenolic compounds (mgGAE/100g)	29,15 ± 2,32 ^a	32,69 ± 2,02 ^a
Antioxidant activity (ABTS+) (µmol Trolox/g)	9,71 ± 0,52 ^a	7,55 ± 0,38 ^b
Antioxidant activity (DPPH) (g/g de DPPH)	6,93 ± 0,86 ^a	5,70 ± 0,53 ^a

Note: Equal superscript lowercase letters on the same line do not differ significantly between treatments ($P > 0.05$); ABTS+ - Antioxidant activity due to the ABTS+ radical scavenging capacity; DPPH - Antioxidant activity due to the DPPH radical scavenging capacity. Source: Own (2020).

The in natura pulp that was not subjected to heat treatment had a phenolic content of 29.15 mgGAE/100g. This value is close to that obtained by Moreira et al. (2018) who also analyzed the pulp of the mandacaru fruit, obtained 28.35 mgGAE/100g for the total phenolic compounds. After application of the heat treatment, an increase in the content of total phenolic compounds in the pulp was observed, 32.69 mgGAE/100g. However, statistically this increase did not show a significant difference at the 5% probability level.

According to Silva et al. (2019) there is a positive correlation between the decrease in the incidence of chronic degenerative diseases and the increased consumption of plant foods, due to the bioactive properties related to these foods, the antioxidant capacity of compounds present in fruits, leaves and others stands out. edible parts of the vegetable, which is generally

attributed to phenolic compounds (PATIL et al., 2009; GORDON et al., 2011; GIRONÉS-VILAPLANA et al., 2014; NIMALARATNE & WU, 2015)

The antioxidant activity of the in natura pulp due to the ABTS+ radical scavenging capacity obtained a value of 9.71 $\mu\text{mol Trolox/g}$. Lower values were reported by Melo et al. (2017) that obtained antioxidant activity of 5.83 $\mu\text{mol Trolox/g}$ for Mandacaru fruit pulp in the fully red ripening stage, harvested in the Mesoregion of Agreste Paraibano and 4.93 $\mu\text{mol Trolox/g}$ for Mandacaru fruit pulp at the stage of fully red maturation harvested in the Mesoregion of Curimataú Paraibano. The thermal pasteurization process reduced the antioxidant activity to 7.55 $\mu\text{mol Trolox/g}$, this degradation being statistically significant ($P > 0.05$).

Regarding the antioxidant activity by the DPPH radical scavenging capacity, in natura pulp presented a value of 6.93 g/g of DPPH and pasteurized pulp presented a value of 5.70 g/g of DPPH, however, this reduction was not statistically significant.

Almeida et al. (2020) when applying the pasteurization process to juá pulp, they observed non-significant reductions in antioxidant activity by the ABTS+ method, reducing from 35.33 $\mu\text{mol Trolox/g}$ to 10.83 $\mu\text{mol Trolox/g}$ and by the DPPH method, reducing from 33.23 g $\mu\text{g/g DPPH}$ to 27.94 g/g DPPH.

Table 2 presents the values obtained for the total phenolic compounds and antioxidant activity of unpasteurized (in natura) and pasteurized Mandacaru fruit peel.

Table 2. Total phenolic compounds and antioxidant activity of fresh and pasteurized Mandacaru rind

Parameters	Shell	
	<i>In natura</i>	Pasteurized
Total phenolic compounds (mgGAE/100g)	275,98 \pm 15,36 ^a	319,15 \pm 11,73 ^b
Antioxidant activity (ABTS+) ($\mu\text{mol Trolox/g}$)	11,62 \pm 1,34 ^a	9,04 \pm 0,76 ^b
Antioxidant activity (DPPH) (g/g de DPPH)	8,46 \pm 0,90 ^a	7,15 \pm 0,62 ^a

Note: Equal superscript lowercase letters on the same line do not differ significantly between treatments ($P > 0.05$); ABTS+ - Antioxidant activity due to the ABTS+ radical scavenging capacity; DPPH - Antioxidant activity due to the DPPH radical scavenging capacity. Source: Own (2020).

The in natura rind of the Mandacaru fruit presented 275.98 mgGAE/100g for the total phenolic compounds. Values higher than those in the present study were obtained by Santos

(2018) who obtained 326.78 mgGAE/100g for the mandacaru fruit peel and by Almeida et al. (2020b) obtained a value of 1497.87 mgGAE/100g in jabuticaba bark in natura. The pasteurization process promoted an increase in the content of these compounds to 319.15 mgGAE/100g, which was statistically significant.

The antioxidant activity of the in natura peel due to the ABTS+ radical capture capacity obtained a value of 11.62 $\mu\text{mol Trolox/g}$, which is higher than that present in its raw pulp (Table 1). Melo et al. (2017) obtained antioxidant activity of 11.39 $\mu\text{mol Trolox/g}$ for Mandacaru fruit rind in the ripening stage with the beginning of red pigmentation, harvested in the Mesoregion of Agreste Paraibano and 8.31 $\mu\text{mol Trolox/g}$ for Mandacaru rind in maturation stage with onset of red pigmentation collected in the Mesoregion of Curimataú Paraibano. Pasteurization promoted a statistically significant reduction in antioxidant activity to 9.04 $\mu\text{mol Trolox/g}$.

The antioxidant activity by DPPH radical scavenging capacity for in natura bark was 8.46 g/g of DPPH, this value slightly lower than the obtained ABTS+ radical scavenging capacity. The value obtained for pasteurized peel 7.15 g/g of DPPH does not show statistically significant difference.

Final considerations

The highest values of bioactive compounds were found for a Mandacaru fruit rind.

There is a concentration of phenolic compounds for the mandacaru peel and degradation of the antioxidant activity due to the pasteurization process for both analyzed fractions, except for the analysis of DPPH for one pulp.

Pasteurization kinetics can be performed by obtaining the thermophysical parameters and evaluating this treatment during the storage of the fractions.

Thus, the fruit and its derivatives are sources of compounds with health benefits and technological functions, being an emerging fruit with high potential for application in food, supplements and pharmacological formulations.

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Chapter V

USE OF DIFFERENT EXTRACTS IN THE DETERMINATION OF BIOACTIVE COMPOUNDS IN ACHACHAIRU (*Garcinia humilis Vahl*)

Virgínia Mirtes de Alcântara Silva

Newton Carlos Santos

Raphael Lucas Jacinto Almeida

Victor Herbert de Alcântara Ribeiro

Gabriel Monteiro da Silva

Jaderson Felipe Santos Dantas

Fabrcia Santos Andrade

Introduction

The fruits of the achachairu are globose-oblong, externally yellow orange, with a thick, smooth, firm, and resistant skin. The pulp, not adherent to the skin, is white, succulent and with a mucilaginous texture, representing 1/3 of the average mass of the fruit. The flavor is very pleasant and balanced sweet-sour, with a total soluble solids content of 15°Brix and pH 4.1 (BARBOSA et al., 2008).

After processing, by-products are generated, which often do not have a specific destination, becoming environmental contaminants and, consequently, generating operating costs for companies, as they need treatment for disposal. Among the most found agro-industrial residues, husks and seeds stand out. After processing the fruits for the elaboration of juices and pulps, 40% of residues are obtained. Currently, studies are quantifying bioactive compounds (phenolic compounds) with antioxidant capacity of these types of materials, to assign them an application (INFANTE et al., 2013).

Phenolic compounds are secondary metabolites widely distributed in all higher plants. They include simple phenols, benzoic and cinnamic acids, coumarins, tannins, lignins, lignans

and flavonoids. Currently there is great interest in these substances due to their antioxidant potential and the association between their consumption and the prevention of various diseases (HAMINIUK et al., 2012; LATTANZIO, 2013; JIMÉNEZ-VELÁZQUEZ et al., 2020).

According to Ketnawa et al. (2020) recently, increased understanding of the importance of fruits and vegetables in the human diet to boost nutrition and well-being. Epidemiological studies indicate that regular consumption of fruits and vegetables has positive effects on human health and can prevent chronic diseases (MANAGA et al., 2018).

In this context, aiming to reuse and add value to the achachairu bark, the present study aims to carry out the physicochemical characterization and use solvents of different polarities to determine the total phenolic compounds, total tannins, and the antioxidant capacity of the achachairu bark in nature.

Methodology

Acquisition of fruits

The achachairus (*Garcinia humilis*) were purchased from local stores, transported in coolers to the Food Engineering Laboratory of the Federal University of Campina Grande UFCG, where they were washed in a 200-ppm sodium hypochlorite solution for 15 min and then rinsed in running water. With the help of a household knife, the fractions: peel, pulp and seed of the fruits were separated.



Figure 1. Achachairu fruit (fruit, skin, and seed) used in the research.

Physicochemical characterization

The in natura peels were characterized in triplicates according to the following parameters: moisture content, ash, proteins, lipids according to the methodology of Instituto Adolfo Lutz (BRASIL, 2008). The total carbohydrate content was calculated by difference to obtain 100% of the total composition (FAO, 2003). The water activity of the samples was

determined in Aqualab 3TE (Decagon, Devices USA) at room temperature (25°C). Vitamin C content was determined through the reaction of ascorbic acid with 2,6-dichlorophenol indophenol (DCFI), as described by Brasil (2008), and the results were expressed in mg ascorbic acid/100g sample.

Preparation of extracts

To analyze the extraction of bioactive compounds from the achachairu bark, two solvents of different polarities were used, water and 70% ethanol. To determine the total phenolic compounds, extracts were prepared by diluting 1g of sample in 50 mL of distilled water/70% ethanol and left to rest for 30 min. In the analysis of antioxidant activity by ABTS+, DPPH and total tannins, 5g of the sample was weighed, 50 mL of water/ethanol 70% was added and taken to the shaker at a temperature of 25°C and a speed of 100 rpm for 2 hours. It was then filtered on quantitative paper and transferred to a 100 mL Becker, after which it was taken to the forced air circulation oven at a temperature of 40°C, until the solvent had completely evaporated (water/ethanol 70%) (PANSERA et al., 2003) with adaptations.

Determination of bioactive compounds

The total phenolic compounds were quantified using the Folin-Ciocalteu method described by Waterhouse (2006), using gallic acid as standard with the results expressed in mg gallic acid equivalent (GAE) 100g⁻¹ of sample. Antioxidant activity by the ABTS+ method was determined by the method proposed by Re et al. (1999), with modifications made by Rufino et al. (2007) the absorbance was measured at 734 nm after 6 minutes of reaction, using ethyl alcohol as blank. The evaluation of the ability to scavenge the DPPH free radical (2,2-diphenyl-1-picrylhydrazyl) was carried out according to the methodology described by Williams et al. (1995) adapted by Borgini and Torres (2009). The absorbance measurement was performed at a wavelength of 517 nm. Percent discoloration was calculated according to Equation 1.

$$\%DPPH_{\text{discoloration}} = 1 - \left[\frac{(Abs_{\text{sample}} - Abs_{\text{white}})}{Abs_{\text{control}}} \right] \times 100 \quad (\text{Eq.1})$$

To determine the total tannins, a dilution with 125 mg of extract and 250 mL of distilled water/70% ethanol was prepared. To obtain the linear analytical curves, a stock

solution with a concentration of 0.005 – 0.01 g mL⁻¹ of tannic acid, diluted in water, was used. A blank was used for each reading. From the equation obtained from the linear analytical curves, the total tannin contents in the sample were calculated, according to the study by (PANSERA et al., 2003) with adaptations.

Statistical analysis

The results of the analyzes for the different extracts were submitted to statistical treatment using a completely randomized design with a comparison test of means, using the Assistat software version 7.7 beta (SILVA & AZEVEDO, 2009).

Results and Discussion

Proximate composition

Table 1 shows the means and standard deviations obtained for the physicochemical characteristics of the in natura achachairu bark.

Table 1. Physicochemical characterization of fresh achachairu hull

Parameters	Achachairu hull <i>in natura</i>
Water content ¹ (%)	78,70 ± 0,67
Water activity (a _w)	0,91 ± 0,004
Ashes (%)	0,85 ± 0,06
Proteins (%)	1,08 ± 0,09
Lipids (%)	0,75 ± 0,008
Carbohydrates (%)	18,62 ± 0,5
Vitamin C (mg of ascorbic acid/100g sample)	5,13 ± 0,07

Note: ¹wet base.

Through Table 1, the fresh achachairu bark has a high-water content (78.70%) and high-water activity (0.91). Similar values for water content were observed by Almeida et al. (2018) in jabuticaba bark (81.22%), by Galdino et al. (2016) for water activity in prickly pear (0.976) and by Santos et al. (2020) in grapefruit peel (82.18%) and (0.995), respectively. According to Gava et al. (2008), products with water activity greater than 0.90 are classified as high moisture products. This fact, according to Meneses et al. (2018) directly influences the quality and microbiological stability of foods, causing rapid degradation. Therefore, it is

necessary to apply conservation techniques such as convective drying, to reduce the water content and water activity, in order to prolong the shelf life of the product.

Regarding the ash content, the bark had a value of 0.85%, higher than that observed by Souza et al. (2018) in the araçá-boi fruit peels (0.08-0.38%) and lower than that observed by Resende et al. (2019) in the breadfruit peel (7.85%). According to Eke-Ejiofor and Onyeso (2019), the ash content of a food provides an indication of the mineral composition of the food sample.

A protein content of 1.08% was observed, a value lower than that observed by Tome et al. (2019) (1.94%) in which they stated that the achachairu peel has a higher protein content than the other fractions of the fruit, such as the pulp and stone. It was also verified a low lipid content (0.75%), a similar value obtained by Farias et al. (2016) in jabuticaba bark (0.67%). 5.13 mg of ascorbic acid/100g sample in the achachairu bark were quantified, similar values were determined by Virgolin et al. (2017) in fresh pulp of achachairu (5.60%), abiu (3.06%) and unlucky (5.60%). Higher values were determined by Barros et al. (2012) on tangerine peels (47.6%). The carbohydrate content obtained was 18.62%, similar values were observed by Tome et al. (2019) (15.81%), who found that the peel of the achachairu has a higher percentage of carbohydrates in its composition, when compared to the pulp and seeds of the fruit.

Bioactive compounds

In Figure 2, the averages obtained for the determination of bioactive compounds present in the in natura achachairu bark are expressed, for two solvents of different polarities (water and ethanol).

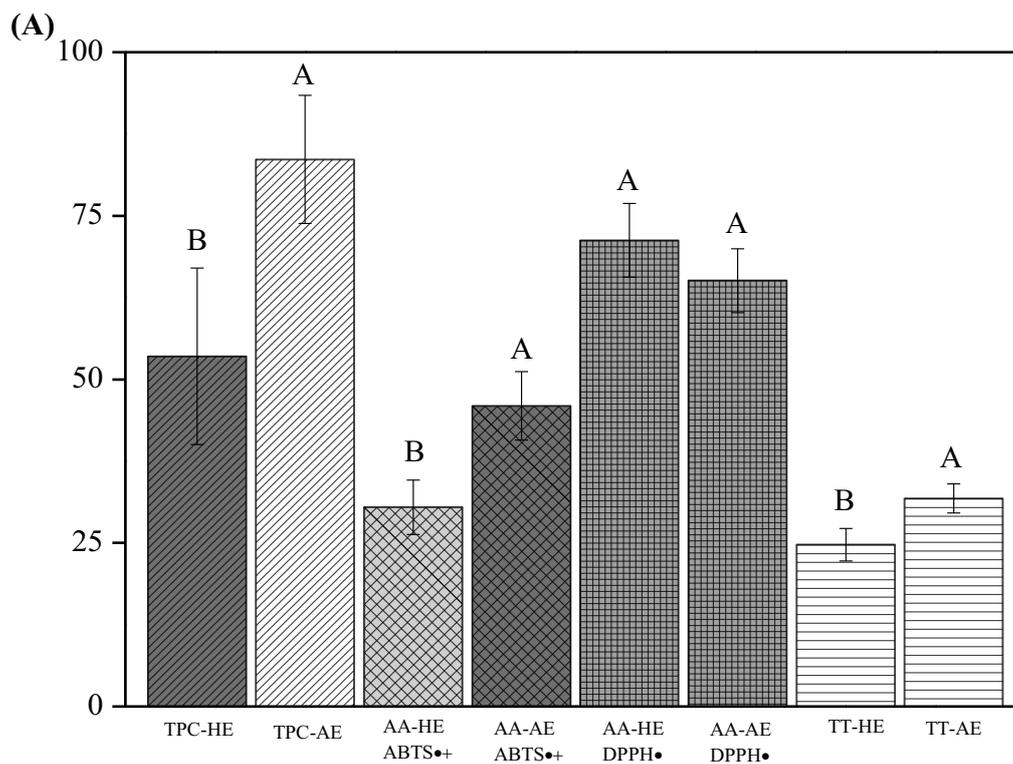


Figure 2. determination of bioactive compound extractions.

Note: Equal superscript capital letters do not differ significantly between analyzed solvents ($P > 0.05$). TPC: Total phenolic compounds expressed in mg GAE $100g^{-1}$, GAE - Gallic Acid Equivalent, HE - Hydroalcoholic Extract, AE - Aqueous Extract, AA - ABTS•+ Antioxidant Activity expressed in $\mu\text{mol Trolox } g^{-1}$, AA - DPPH• Antioxidant Activity expressed as percentage of discoloration, TT - Total Tannins expressed as Tannic Acid Equivalent mL^{-1} .

In Figure 2, it is possible to analyze the effect of solvent extraction in relation to the bioactive compounds present in the in natura achachairu bark. For the parameters of total phenolic compounds, antioxidant activity by ABTS•+ and total tannins, the aqueous extract (AE) had higher extraction power, due to its polarity, while for the antioxidant activity by DPPH• it was not possible to notice significant differences. The results are consistent with the studies by Zang et al. (2017) who found in fresh mangosteen (*G. mangostana*) a degree of discoloration of 50.79% in the methanolic extract, a value lower than that obtained in the present study for the two solvents used.

Tannins are considered antinutrients due to their adverse effect on protein digestibility and may be present in greater amounts during the initial period of fruit growth (TESSMER et al., 2014). They are phenolic substances soluble in water, so there were significant differences between the analyzed peels in relation to the solvent used in the extraction of these compounds, with higher values for the aqueous extract. For the others it

was not noticeable to verify a defined profile for which solvent would be the best in the extraction. This significant difference between the phytochemical content of the extracts suggests the influence of the solvent used for extraction. As they present different degrees of polymerization, phenolics are extracted according to their solubility in pure or diluted organic solvent (LEONG et al., 2007).

According to Pellegrini et al. (2007) and Melo et al. (2008), the solubility in a certain solvent is a peculiar characteristic of the phytochemical, which justifies the lack of a universal extraction procedure due to the structural diversity and sensitivity of the phenolic compounds to the extraction conditions.

Conclusion

According to the results obtained, it is verified that the use of different extracts to determine the bioactive compounds of the achachariu bark are efficient, identifying the presence of a high content of total phenolic compounds, total tannins, and antioxidant capacity.

Fruit peels represent a very rich by-product and sources of bioactive compounds and generally have higher nutrient content than their respective pulps, representing a raw material for the development of new nutraceutical and/or pharmaceutical products.

Thus, the identification and quantification of compounds of interest in fruit co-products are important to prove its potential benefits.

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Authors Curriculum

Virgínia Mirtes de Alcântara Silva: Degree in Biological Sciences from the University of Vale do Acaraú-CE in 2010, PhD and Master in Engineering and Natural Resources Management from the Federal University of Campina Grande - UFCG, Specialist in Geoenvironment and Semi-Arid Water Resources from the University State of Paraíba - UEPB, Specialist in Geography and Environmental Management by the Integrated University of Patos - FIP. Working in the areas of Remote Sensing and GIS, Desertification, Ecological Restoration, Recovery of Degraded Areas and Climatological Characteristics of Brazil, Extreme Rain Events and Environmental Disasters. PhD in Engineering and Management of Natural Resources working in Cryoconservation of Genetic Resources of Plant Origin, with an emphasis on the area of Heat and Mass Transfer linked to Refrigeration and Drying of Food (drying, osmotic dehydration, lyophilization, enrichment of foods and new foods products). She is part of the editorial board of the EPTEC publisher. Reviewer of the African Journal of Plant Science; African Journal of Food Science; Journal of Development and Agricultural Economics, Journal of Plant Breeding and Crop Science, Journal of Horticulture and Forestry (JHF), Journal of Pharmaceutical Research International, Journal of Medicinal Plants Research and African Journal of Agricultural Research, Member of the International Research Network at Climate Resilient Development - RIPPEDRC- Climate Resilience Network, created in 2019, as an international partnership, approved by the CNPQ/Araucaria Foundation notice: PI 06/2018 Researcher Links Program (United Kingdom), which provided for the organization of the Workshop Brazil- United Kingdom on Financing Climate-Resilient Urban Development, September 9-13, 2019.
virginia.mirtes2015@gmail.com; virginia.m.alcantara@gmail.com

Newton Carlos Santos: Bachelor's Degree in Industrial Chemistry from the State University of Paraíba (UEPB); Professional Internship at ASA Industries e Commerce - Vitamilho, working in the Quality Control sector; Specialist in Food Science and Technology by the Federal Institute of Rio Grande do Norte (IFRN); Master in Agricultural Engineering from the Federal University of Campina Grande (UFCG), working in the area of Heat and Mass Transfer with an emphasis on Refrigeration and Drying of Food. He is currently a PhD student in Chemical Engineering at the Federal University of Rio Grande do Norte (UFRN).
newtonquimicoindustrial@gmail.com

Victor Herbert de Alcântara Ribeiro: Graduated in Agroecology from the State University of Paraíba (2016). Master in Agricultural Sciences (Agroecology) from the Center for Human, Social and Agrarian Sciences of the Federal University of Paraíba - Campus III Bananeiras. Doctoral candidate in Engineering and Natural Resources Management with emphasis in Heat and Mass Transfer related to Refrigeration and Drying of Food (drying, osmotic dehydration, lyophilization, enrichment of food and new products (development of feed for fish with residues) from algaroba *Prosopis juliflora* (Sw) DC) It also works with topics related to biofertilizers, organomineral substances and the culture of sesame (*Sesamum indicum* L.).
victor_herbert@hotmail.com

Raphael Lucas Jacinto Almeida: Bachelor's Degree in Industrial Chemistry from the State University of Paraíba (2017), where he worked in the area of microbiology and production of probiotic milk drink and Degree in Chemistry from Cruzeiro do Sul (2021), Professional Internship (2017) held in the Industry of Bebidas de Garanhuns - Jatobá Ltda, working in the Quality Control and Microbiology sectors; Specialist (2019) in Food Science and Technology by the Federal Institute of Rio Grande do Norte; Master in Chemical Engineering (2019) from the Federal University of Campina Grande, with experience in the

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Biotechnology area with an emphasis on enzymatic hydrolysis, starch extraction and processing of agro-industrial residues. He is currently a PhD student in Chemical Engineering at the Federal University of Rio Grande do Norte, with a project on hydrothermal and enzymatic modification of starch.

[**raphaelqindustrial@gmail.com**](mailto:raphaelqindustrial@gmail.com)

Jaderson Felipe Santos Dantas: *Graduated in Dentistry at Universidade Potiguar (2020), where he was monitor of the disciplines: Oral Diagnosis and Oral Rehabilitation Clinic I and II. He is currently a Master's Student in the Postgraduate Program in Dental Sciences at the Federal University of Rio Grande do Norte with a concentration in Dental Clinics. He is interested in topics related to herbal medicine, development of new products and dental materials and oral rehabilitation.*

[**jaderson.dantas@gmail.com**](mailto:jaderson.dantas@gmail.com)

Gabriel Monteiro da Silva: *Bachelor's Degree in Industrial Chemistry from the State University of Paraíba (2021), where he was a Scientific Initiation Scholar in the period (2015/2021) working in the area of functional dairy product development and food analysis. He is currently a Master's Student in the Postgraduate Program in Agricultural Engineering at the Federal University of Campina Grande with an area of concentration in: Processing and Storage of Agricultural Products. development, functional dairy product development and food analysis.*

[**silvagm839@gmail.com**](mailto:silvagm839@gmail.com)

Fabrcia Santos Andrade: *Graduated in Industrial Chemistry from the State University of Paraíba (2020), worked in the Institutional Program for Scientific Initiation Scholarships in Agricultural Sciences and Food Technology. from 2016 to 2020, working in processing and reuse of agricultural waste with experience in the areas of osmotic dehydration, drying using the oven method, elaboration of powdered pulp by foam layer and food storage. Currently and Master's Student of the Postgraduate Program in Agricultural Engineering at the Federal University of Campina Grande in the area of Processing and Storage of Agricultural Products.*

[**fabricia.santos.andrade@hotmail.com**](mailto:fabricia.santos.andrade@hotmail.com)

The Year 2021 has been designated by the UN as the International Year of Fruits and Vegetables. And contributing to this sense, we carried out this work, which represents a synergistic effort by a team of researchers. In recent decades there has been a growing interest in research into the chemical composition of fruits, vegetables, and vegetables, they contain high levels of phytochemicals that have anti-inflammatory effects, which are still poorly researched. Although fruits are important for food, research on their antioxidant and nutritional contribution to food systems is still limited. Due to the relevance of bioactive compounds and antioxidant activities, we chose several techniques, among them we highlight drying, lyophilization and pasteurization used in different fruits, peels, and pulps. The fruits selected for this work were apple (*Malus domestica*), jaboticaba (*Myrciaria cauliflora*), achachariu (*Garcinia humilis*), physalis (*Physalis angulata*) and mandacaru (*Cereus jamacaru*). This work aims to contribute to the country's technological and productive development in the definitive implementation of an efficient and clean model of food production for the world, in line with the goals established by the 2030 Agenda determined by the United Nations.

