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PROXIMATE CHEMICAL ANALYSIS, PHYSICOCHEMICAL AND ENZYMATIC CHARACTERIZATION, BIOACTIVE COMPOUNDS, AND ANTIOXIDANT CAPACITY OF BONETE (*JACARATIA MEXICANA*)

[1*]Carlos Hernán Herrera Méndez, [2]María Vargas y Vargas, [3]José María Tamayo Manrique, [4]Gustavo Rivera Solís and [5]Emir Alejandro Hernández Gómez

[1]Departamento de Ingeniería Agroindustrial, Universidad de Guanajuato, México.

[2]Tecnológico Nacional de México/ IT Mérida, México.

[3]Centro de Bachillerato Tecnológico Industrial y de Servicios, Mérida, México.

[4]Instituto de Investigaciones Silvio Zavala, Universidad Modelo, Mérida, México.

[5]Tecnológico Nacional de México/ IT Mérida, México.

*Corresponding Author

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ABSTRACT

Bonete (*Jacaratia mexicana*) is a tree that grows in southeastern Mexico (Yucatán). The nutritional contribution and potential health benefits of its fruits, particularly of this species growing in this specific region, are unknown. Proximate analysis, physicochemical and enzymatic characterization, as well as the bioactive compound content and antioxidant activity of the fruit pulp, were performed. Proximate analysis and physicochemical characterization were carried out using the methods described by the Association of Official Analytical Chemists (AOAC). Phenolic compounds and antioxidant activity were quantified using the Folin–Ciocalteu method and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay. The yield was 244.2 g relative to the whole fruit. The macronutrient content, expressed as a percentage, was 1.84 % protein, 5% lipids, and 7.72% carbohydrates. The physicochemical characteristic of acidity was 0.231 %, with a pH value of 5.07, which makes the food slightly alkaline, an advantage for gastric balance (acidity). The activity of two enzymes was characterized: polyphenol oxidase (browning) and pectin methylesterase (fruit softening). The total polyphenol content (672.29 mg gallic acid equivalents/100 g pulp) indicates that this fruit has a high value, and the values of the other active compounds analyzed suggest that this fruit may have high functional potential in individuals with various health conditions.

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Keywords: Characterization, Components, Macronutrients, Polyphenols, Pulp

1. INTRODUCTION

Bonete (*Jacaratia mexicana*) belongs to the *Caricaceae* family (Arias et al., 2012). In Mexico, it is known by several names, including papaya de monte, papaya orejona, and papaya cimarrón, as well as 'k'umché' in the Mayan language (Yucatán, Mexico), which is the term used for the bonete studied in this research. It is a long-lived tree of the canopy in tropical deciduous forests and occasionally of the subcanopy in tropical semi-evergreen forests. The trunk reaches up to 12 m in height and has a narrow crown of thick branches. From the seedling stage, five superficial roots and one vertical root become established. The fruits ripen over weeks or months during the rainy season. They do not change from their green color, nor do they fall, until they begin to decompose. Considered a direct ancestor of the papaya, this tree's populations are currently at risk of disappearing from their native habitat. It has historically been used by local populations for timber and medicinal purposes (Zulueta et al., 2015).

1.1 Description of Fruits and Seeds

Stems unarmed (lacking spines or thorns), up to 1 m in diameter; bark brown; fourth-order branchlets 5 to 7 mm in diameter, with transverse semicircular leaf scars approximately 4 mm in diameter. Apical leaves on fourth-order branchlets; petiole 3 to 7 cm long x 1 to 1.5 cm in diameter; petiolules 1 to 3 mm long; leaflets 4 to 8 cm long and 3.7 cm wide, obovate, cuneate base, acuminate apex, entire margin. Berry-type fruit, up to 30 cm long and 13 cm in diameter, pendulous, ovoid or conical; peduncles 3 to 13 cm long; 5 ribs up to 4 cm high, projected at the base 1.3 to 4 cm; base concave or truncate; pericarp greenish-red or yellow at maturity; seeds 4 to 8 mm long, 2 to 5 mm in diameter, ovoid or subglobose in shape.

1.2 Uses

Given the limited information, the fruits are primarily used for local consumption in the state of Yucatán.

From the K'umché tree (calabash tree) or Yucatecan bonete, as it is known in Mayan communities, practically everything is used:

- In tender fruits, the pulp can be consumed as soup or, when ripe, as a sweet preserve.
- The pulp can be eaten alone or used to prepare a beverage.
- Oil can be extracted from the seeds, and they can also be consumed as a snack.
- Its fruit offers a type of latex or resin.

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The ripe pulp contains enzymes that have digestive properties similar to, though more potent than, papain, facilitating digestion and relieving stomach pain and inflammation. These enzymes also have analgesic properties. In some communities, it is used as an alternative in traditional natural medicine: its peel can be boiled and applied to a scorpion sting, as it acts effectively against the arthropod's venom (Bacap, 2017).

1.3 Distribution

It is a species with a wide distribution range in Mexico, distributed along both the Pacific and Gulf of Mexico slopes. On the Pacific slope, it encompasses the states of Nayarit, Jalisco, Colima, Michoacán, Guerrero, Oaxaca, and Chiapas. On the Gulf of Mexico side, it has been recorded in the states of Veracruz, Tabasco, Campeche, and Yucatán. It has also been recorded in the states of Quintana Roo, Guanajuato, Puebla, and Morelos. Outside of Mexico, it has been reported in Nicaragua and El Salvador.

2. MATERIALS AND METHODS

The bonete fruits were collected in Tizimín, Yucatán, located 150 km from the city of Mérida, Yucatán. All fruits were transported to the Instituto Tecnológico de Mérida, to the Food Enzymology Laboratory, for subsequent characterization, analysis, and enzymatic testing.

2.1 Fruit yield

Fruit yield was determined by calculating the proportion of each part (pulp, seed, and peel) relative to the total weight of the whole fruit.

2.2 Proximate composition

Proximate composition was determined using AOAC (1998) methods: moisture content (Method 925.09); ash (Method 923.03); crude fat (Method 920.39); crude protein with 6.25 as the protein conversion factor (Method 954.01); and total carbohydrates by difference.

2.3 Physicochemical characterization

2.3.1 Determination of °Brix

The determination of the degrees Brix contained in each of the fruits was measured using a refractometer, with a 1:10 ratio to obtain greater sensitivity and specificity during sample reading.

2.3.2 Determination of Acidity

This analytical technique is used to determine the acidity content in raw materials and processed and unprocessed food products. Three grams of sample are weighed and homogenized in 10 mL of distilled water. Subsequently, it is titrated or neutralized with a base (OH-). As the base is added,

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the amount of free protons in the solution decreases, as they bind with OH- groups to form water. By continuing to add OH- groups, a point will be reached at which no free protons remain, indicating the endpoint of the titration.

2.3.3 Determination of pH

This analytical technique is used to determine pH in raw materials and processed and unprocessed food products. It is based on the electrometric measurement of the hydrogen ion activity present in a product sample using a pH meter (potentiometer). A representative sample of 2 to 3 grams is taken and diluted in 20 mL of water to allow an ideal pH reading.

2.4 Enzymatic characterization

2.4.1 Determination of polyphenol oxidase (PPO) activity

The method of Aranda (2004) was used, with pyrocatechol as substrate. The determination of PPO activity was carried out by spectrophotometric determination, based on the accumulation of the product, O-quinones, which have an absorption band at approximately 420 nm. In a test tube, 2.8 mL of the corresponding substrate (substrate solution in 0.2 M phosphate buffer) and 0.2 mL of enzymatic extract were placed. The mixture was incubated in a bath at 30 °C with shaking at 200 rpm for 30 min. During this time, readings were taken every 5 min. Time zero was the reading taken immediately after adding the enzyme to the substrate.

2.4.2 Determination of pectin methylesterase (PME) enzymatic activity

The analysis was carried out using the PME enzyme extraction and quantification method (Hagerman, 1986). 4.5 g of fruit pulp were taken and mixed with 15 mL of 8.8 % w/v NaCl solution. They were homogenized, then the mixture was centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant (enzymatic extract) was placed and adjusted to pH 7.5 with 0.1 N NaOH. An aliquot of 100 µL was taken from the enzymatic extract and 200 µL of distilled water, 100 µL of 0.01 % bromothymol blue solution, and 1000 µL of 0.01 % pectin solution adjusted to pH 7.5 with 0.1 N NaOH were added. Absorbance was read at a wavelength of 620 nm for a time of 3 min. The difference between the absorbance at time 0 and at 3 min represented the absorbance differential that expresses enzymatic activity in µmol of galacturonic acid per min.

2.5 Determination of bioactive compounds and antioxidant activity

2.5.1 Total anthocyanin content

To obtain the total anthocyanin concentration, the pH differential method reported by Rapisarda (2000) was used. The pH differential method allows the estimation of total anthocyanin content by

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using buffer systems, a bleaching agent (bisulfite), and measurement with a UV-visible spectrophotometer.

2.5.2 Total flavonoid content

The total flavonoid content was determined according to Maksimovic (2005). A 0.1 mL aliquot of the ethanolic extract was taken and added to a 25 mL volumetric flask. To this, 0.5 mL of aluminum chloride solution (5 % in methanol) was added, and the volume was completed with methanol, homogenizing at the end. The reaction mixture was allowed to stand for 30 minutes in the dark, allowing the formation of the yellow-colored complex. Measurement was carried out at 415 nm using a spectrophotometer, with a blank solution prepared using 0.1 mL of ethyl alcohol (96%) instead of the test solution, maintaining the same conditions and reaction time. All samples were analyzed in triplicate.

2.5.3 Vitamin C

To quantify the content of ascorbic acid, an aliquot of the sample was titrated with a 2,6-dichloroindophenol solution until the pink color persisted for more than 5 seconds. The 2,6-dichloroindiphenol solution was standardized with an ascorbic acid solution. The results are expressed as mg of ascorbic acid/100 g of fruit weight.

2.5.4 Total polyphenol content

The concentration of total phenols was measured using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). The quantification was carried out by plotting a calibration curve of gallic acid at concentrations of 0.05-0.5 mg/ml. The results are expressed as equivalent milligrams of gallic acid (mg EAG/100 g of fruit weight).

2.5.5 ABTS method (2,2'-azino-bis (3-ethylbenzothiazoline)-6 ammonium sulfonate)

This method evaluates the ability to trap radicals present in the medium. The blue-green cationic radical ABTS•+, a stable compound that is soluble in methanol, is generated by the interaction of ABTS with potassium persulfate. The method consists in evaluating the antioxidant capacity of the sample as a function of its ability to reduce the concentration of the ABTS•+ radical (Re et al., 1999). In the present study, the absorbance of 2.97 ml of cationic radical, mixed with 0.03 ml of sample, was measured at 734 nm.

The results are expressed as percentage of inhibition. Where:

% of inhibition = (initial absorbance - final absorbance)/initial absorbance) * 100

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3. RESULTS AND DISCUSSION

3.1 Fruit yield

During the handling of the raw material, it was observed that the interior of the bonete fruit is very similar to that of papaya, although the pulp differs slightly in its characteristic color from papaya and in the amount of seeds. The pulp content yield was 244.2 ± 3.6171, representing an average of 63.8 % (Table 1), which makes bonete an acceptable fruit. It was found that the weight of the seeds and the peel were similar, and the pulp has a somewhat sticky texture to the touch.

Table 1: Yield of bonete fruit (*Jacaratia mexicana*)

Fruit yield (g)			
Fruit (g)	**Seeds**	**Peel**	**Pulp**
382.4 ± 5.8949	66.2 ± 1.6822	72 ± 3.6055	244.2 ± 3.6171
100 %	17.3 ± 0.4645 %	18.8 ± 0.9374 %	63.8 ± 0.9517 %

Source: Own elaboration

3.2 Proximate composition

As shown in Table 2, the bromatological analyses indicate that bonete (*Jacaratia mexicana*) contains 84.41 ± 1.60 % moisture. This high moisture content can be considered a natural mechanism of the fruit for transporting nutrients and dissolving the various substances that give it its characteristics. The ash content shows that this fruit can be a natural source of minerals, containing 0.60 ± 0.27 % inorganic compounds. The protein content (1.84 ± 0.11 %) places this fruit at a slightly higher level than banana (1.1 %), apricot (1.4 %), and raspberry (1.2 %) (Domínguez et al., 1992), and minimally below avocado (2 %) and blackberries (2 %) (Rahmi et al., 2026). The fruit also presents a fat content (5 ± 2.64 %). In relation to total carbohydrates by difference (7.72 %), being a fruit very similar to papaya, it can be considered to contain mainly glucose, sucrose, and fructose (Zhou and Paull, 2001).

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Table 2: Bromatological analysis of bonete pulp (*Jacaratia mexicana*)

Characteristic	Average (%)
Moisture	84.41 ± 1.60
Ash	0.60 ± 0.27
Proteins	1.84 ± 0.11
Fats	5.00 ± 2.64
Total carbohydrates by difference	7.72

Source: Own elaboration

3.3 Physicochemical characterization

Table 3 presents the results of the physicochemical analyses performed on bonete pulp (*Jacaratia mexicana*).

Table 3: Physicochemical analyses of bonete pulp (*Jacaratia mexicana*)

Characteristic	Average (%)
°Brix	17 ± 1
pH	5.07 ± 0.023
Acid (%)	0.231 ± 0.013

Source: Own elaboration

The pH of 5.07 ± 0.023 observed in this investigation is similar to that found in papaya, which may be due to the low content of organic acids, making it close to neutrality (Ettien et al., 2023). According to Matsuane (2023), the total soluble solids found in bonete fruit (17 ± 1) are high compared to papaya (a similar fruit); knowing the soluble solids content is important in determining harvest time, organoleptic quality, and potential commercial value. The acidity of the pulp, based on ascorbic acid, was 0.231 ± 0.013 %, which is an indicator that the pulp of the fruit is slightly alkaline, allowing its enzymes to relieve stomach acidity (Herdiana, 2023).

3.4 Enzymatic characterization

3.4.1 Polyphenol oxidase (PPO) activity

Upon measuring polyphenol oxidase (PPO) activity, it is observed that it is present in an almost constant form starting at 10 minutes and that at 30 minutes a decrease is observed (Figure 1), which may indicate that the substrate is already in low concentrations after that time. It should be mentioned that this analysis was carried out at room temperature in a water bath at 30 °C.

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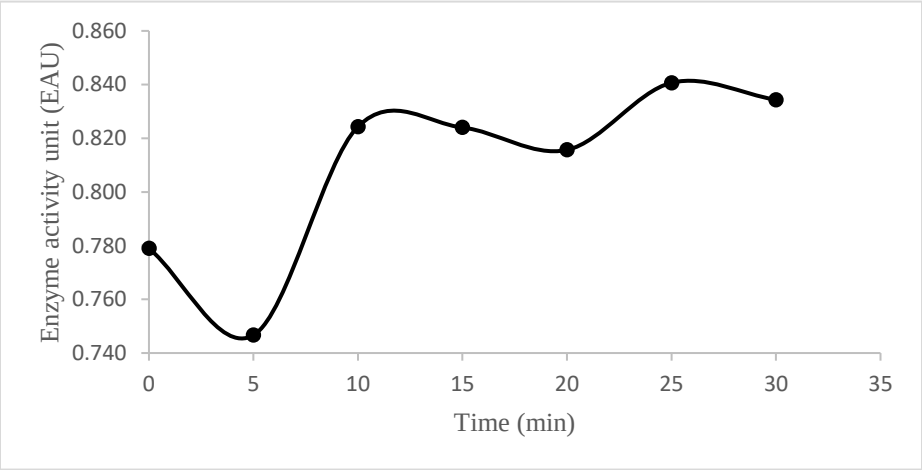

Source: Own elaboration

Figure 1: Analysis of polyphenol oxidase (PPO) enzymatic activity present in bonete pulp (*Jacaratia mexicana*)

Browning is one of the main causes of quality and nutritional value loss in fruit and vegetable preservation. This phenomenon can be considered as an initial enzymatic oxidation of phenols to slightly colored quinones. These are subject to subsequent reactions, catalyzed enzymatically or not, which lead to the formation of pigments and the loss of nutritional value due to the reaction itself (Rouet et al., 2003). The enzymes responsible for these reactions are oxidoreductases and present different activities, such as phenolases, tyrosinases, catecholases, and polyphenol oxidases, and can be inactivated by heat or oxygen removal and by the combined use of chemical antibrowning agents with heat treatment (Vamos et al., 1981).

3.4.2 Pectin methylesterase (PME) activity

Measurement of pectin methylesterase (PME) activity is fundamental for the agricultural and food sectors. It determines fruit firmness, serves as a guide for storage protocols, and conditions the texture and turbidity stability of processed products such as juices and jams (Handa et al., 1996). Pectin methylesterase activity is observed in a range of 620 nm; when the enzyme interacts with its substrate, the spectrum is read, obtaining a result of 0.587 ± 0.0025 at minute zero, and at minute 3 no change in activity is observed (Table 4).

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Table 4: Analysis of pectin methylesterase (PME) enzymatic activity present in bonete pulp (*Jacaratia mexicana*)

	Time (min)	
	0	3
Enzyme unit activity (EAU)	0.587± 0.0025	0.587±0.0025

3.5 Bioactive compounds and antioxidant capacity

Table 5 shows the values found in bonete pulp in relation to various active compounds and free radical scavenging.

Table 5: Bioactive compounds and antioxidant capacity present in bonete pulp (*Jacaratia mexicana*)

ANTHOCYANINS (mg cyanidin/100 g pulp)	**FLAVONOIDS** (mg quercetin/100 g pulp)	**VITAMIN C** (mg ascorbic acid/100 g pulp)	**POLYPHENOLS** (mg gallic acid/100 g pulp)	**% FREE RADICAL SCAVENGING**
2.48 ± 0.187	1.041± 0.441	22.32 ± 0.4	672.29 ± 15.063	16.06 ± 0.824

According to the results found, bonete presented a concentration of 2.48 ± 0.187 anthocyanins. Anthocyanins constitute one of the most extensive and important groups of water-soluble pigments in most fruits. They are primarily responsible for the varied pigmentation, ranging from orange to red, through purple and blue. Anthocyanins present in fruits are found in glycosylated forms. Anthocyanins are ingested mainly through the consumption of fruits, vegetables, and red wines. The daily intake of anthocyanins per person ranges between several milligrams and hundreds of milligrams. Anthocyanins protect against various diseases, particularly cardiovascular diseases and some types of cancer. Furthermore, visual acuity can be notably improved through the administration of anthocyanin pigments to humans (Horbowicz et al., 2008). Regarding flavonoids (1.041 ± 0.441), there is a positive correlation with respect to the polyphenol content found in the pulp, which indicates that the majority of the polyphenol content is composed of the different flavonoids present in the fruit; likewise, this fruit can be a good source of flavonoids in the diet. Regarding vitamin C content (22.32 ± 0.4), bonete was found to be high compared to other fruits: banana (3.3 mg), grape (4 mg), pineapple (6.4 mg), peach (8.4 mg), fig (12.2 mg) (Veerasamy et

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al., 2020), which indicates that this fruit can be a powerful antioxidant since ascorbic acid can donate a hydrogen atom and form stable free radicals.

Regarding polyphenols (672.29 ± 15.063), a high value, these are considered the most important antioxidant compounds in the diet, containing hydroxyl functional groups on aromatic rings. They are a large family consisting of a very diverse set of molecules, from the simplest (such as an aromatic ring with one or more hydroxyl groups) to the most complex (such as high molecular weight polymers). These compounds have not only been attributed antioxidant properties; there are also reports of anti-inflammatory and antimicrobial activity, among others (Chen et al., 2015).

The percentage of free radical scavenging (16.06 ± 0.824) was determined by the ABTS method. The value obtained is low despite the low selectivity of ABTS, which reacts with any hydroxylated aromatic compound, regardless of its actual antioxidant potential (Roginsky and Lissi, 2005), which is why there is so much variation in antioxidant capacity measurements. Different studies have been found where both the amounts of reagents and samples vary, as well as the type of handling and measurement of the samples. Therefore, comparing the results obtained from these measurements with another research is complicated.

4. CONCLUSIONS

In the area of Mexico where this study was conducted (Yucatán), this tree and fruit, bonete (*Jacaratia mexicana*), is endangered because it is a fruit very little known by new generations, although older people know its medicinal properties and in times of famine and scarcity there are historical indications that it was added to tortilla dough to improve nutritional condition.

This study highlights some of the fundamental characteristics of the pulp of this fruit, such as its yield, physicochemical and sensory attributes, some enzymatic activities, active compounds, and its antioxidant capacity. It was observed that this little-known or appreciated food presents elements that can add value not only to the pulp itself but also can serve as a food matrix for derived products. The general characterization of the pulp of this fruit may represent an important source of general health benefits (stomach and intestinal health, preventing neurodegenerative diseases, reducing the risk of cancer, alleviating menopause symptoms, and improving cardiovascular health).

Bonete pulp (*Jacaratia mexicana*) presents bioactive compounds such as vitamin C, anthocyanins, flavonoids, total polyphenol content, as well as free radical inhibition. With these results, the inclusion of this food in the daily diet can be suggested. The biological activity of polyphenols is related to their antioxidant character, due to their ability to trap metals, inhibit lipoxygenase enzyme activity, and act as free radical scavengers. According to Vasco et al. (2008) and Zapata (2014), fruits can be classified into three categories according to their phenolic content: low (<100

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mg GAE/100g), medium (100-500 mg GAE/100g), and high (>500 mg GAE/100g). According to this classification, bonete (*Jacaratia mexicana*) can be classified as high.

This research makes an initial contribution to fruit knowledge through the study of different analyses with emphasis on bioactive compounds and antioxidant activity in native fruits of the region that have been little or no studied at all. However, studies will be conducted on sensory properties and the extraction of more compounds from this fruit to help determine its use in the food and/or pharmaceutical industry, knowing that the added value of these traditional foods can also contribute to nutrition programs.

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