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DEVELOPMENT OF MICROCAPSULES FROM THE CAIMITO FRUIT CULTIVATED IN THE DEPARTMENT OF SUCRE TO USE ITS ANTIOXIDANT CAPACITY.

De la Espriella Angarita, S.[1*]; Granados Conde, C.[1*]; León Mendez, D.[2]; Leon Mendez, G.[3]; Pajaro Castro, N[4].

[1]University of Cartagena, Faculty of Engineering, Research in Engineering, Innovation, Quality Food and Health Group (INCAS).
[2]Corporación Universitaria Rafael Núñez, Programa de Tecnología en Estética y Cosmetología, GITEC, Cartagena, Colombia.
[3] Fundacion Universitaria Tecnologico Comfenalco, Faculty of Engineering, Research Group CIPTEC, Cartagena, Bolívar, Colombia.
[4]Medical and Pharmaceutical Sciences Group, School of Health Sciences, Department of Medicine, University of Sucre, Sincelejo, 700003, Sucre, Colombia.

*cgranadosc@unicartagena.edu.co; sespriellaa@unicartagena.edu.co

Abstract

Introduction: The caimito (*Chrysophylum cainito* L.) belongs to the family of the *Sapotaceae*, presents an exquisite flavor and is commonly consumed as fresh fruit. Objective: Develop microcapsules from the Caimito fruit cultivated in the department of Sucre to use its antioxidant capacity. Materials and method: The Caimito fruits, which were acquired in the municipality of San Onofre (Sucre), were evaluated bromatologically. Likewise, its antioxidant capacity was measured using the DPPH and ABTS method. Finally, microencapsulated from this fruit was developed through maltodextrin used as an encapsulator for the use of its antioxidant activity in the food and cosmetics industry. Conclusions: With the development of new products such as the microcapsules of the caimito fruit, different consumption alternatives can be offered to preserve the nutritional properties of the fruit and enjoy these foods at other times of the year.

Keywords: Caimito, antioxidant activity, microencapsulated.

Introduction

Oxidative damage in the human body plays an important causal role in the initiation and progression of diseases related to some neurodegenerative and cancers caused by free radicals and reactive oxygen species. The natural antioxidants of the fruits provide a measure of protection that retards the process of oxidative damage. Recent studies have shown that many flavonoids and polyphenols are significantly related to the total antioxidant activity of many fruits. The fruits are rich in flavonoids content and it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids each day [1].

Apart from its biological properties, natural antioxidants are also of interest in cosmetics, pharmaceuticals and especially in the food industries, as they can also be used as substitutes for synthetic antioxidants, providing protection against oxidative degradation of free radicals [3].

Chrysophyllum Warbler L., commonly known as Caimito, is a species belonging to the family of Sapotáceas native in Central America and Mexico [2], widely known in other tropical countries of America and the Asian continent (Hawaii and the Philippines). In these countries it is highly valued for its forestry, ornamental and medicinal value. The fruit is a smooth berry, not climacteric, with fleshy and sweet pulp; pinkish red or cream white color, with high antioxidant capacity and high nutritional and health potential. However, this fruit is produced only in the market of some regions on a very limited scale [4].

In the Department of Sucre, this fruit is consumed in fresh and is one of the most common homemade fruit trees but despite its potential, its cultivation is limited to family orchards at certain times of the year, especially from june to november [5] [17]. Therefore, taking into account its nutritional importance and the need to dispose of this fruit at all times, the focus of the research has shifted to the use of its antioxidant properties through the development of microcapsules of caimito in order to preserve their properties and, in turn, to offer another alternative of consumption and to facilitate the distribution of the same to other regions of Colombia.

Methods

The Caimito (*Chrysophyllum Warbler* L.) was purchased in the municipality of San Onofre, located in the northeast of the Department of Sucre (9°43'59'' north latitude and 75°31'59'' west longitude). The fruits were collected in maturity of consumption, which were determined by the color of the shell (75% purple) [6] and were selected fresh, whole and no type of mechanical or microbiological deterioration.

Stage 1. Obtaining and bromatological characterization of the pulp of caimito (*Chrysophyllum Cainito L.)*

Initially, the previously selected fresh material was chosen and the cleaning process was carried out with running water to remove dust, soil and dirt in general, then it was disinfected with citrosan at 2.5%. Next, the fruits were taken and the pulp was extracted [6]. Afterwards, the chemical characterization of the pulp was carried out, to which the nutrient and micronutrients content was determined by the tests described below: protein; the Kjeldahl method was used according to AOAC 955.04; Ashes, the direct method was used according to AOAC 924.05; moisture, the drying method was used at 100 + 2 ° C according to AOAC 925.09. [7]; fiber, the Gravimetric enzymatic method was used; carbohydrates, the method was used by difference [8]; fat, the Soxhlet method was used according to AOAC 936.15 [9]; total energy, the method was used by calculation [8].

Stage 2. Evaluation of the antioxidant capacity of the pulp of caimito (*Chrysophyllum Cainito L.)*

Determination of total phenols

The content of total phenols was determined by the colorimetric method of Folin-CIOCALTEU, a mixture phosphowolframic y phosphomolybdíc acids was used as reagent in basic medium, which are reduced by oxidizing phenolic compounds, resulting in blue oxides of Tungsten (W_8O_{23}) and molybdenum

(Mo_8O_{23}). A pattern curve was constructed using the standard Gallic acid between 50 – 500µg/mL. The corresponding extract was diluted to a concentration at which the content of phenols will be within the range of the standard curve. The results were expressed as mg of gallic acid/250 mL of sample. Absorbance readings were performed at 760 nm in a visible UV spectrophotometer Thermo Scientific™ GENESYS 10S [11 -13].

Método del radical DPPH

The activity getter of free radicals DPPH was determined using the method described by Silva et al. with some modifications [14-15]. 75 µL of sample were added to 150 µL of a methanolic solution of DPPH• (100 µg / mL) and incubated at room temperature for 30 min, after which the disappearance of the DPPH radical was spectrophotometrically determined• at 550 nm in the reader of microplates Multiskan Ex (Thermoscientific). Ascorbic acid was used as a positive control of DPPH radical uptake (25 µg / mL). The IC50 was determined by evaluating several serial concentrations of the sample by linear regression analysis. The results were expressed as the mean ± the standard error of the mean (ESM) of the percentage of uptake of the DPPH radical• relative to the control group. The inhibition percentage (% Inh) was calculated using equation (1).

$$\%\, Inhibition = \frac{(A_0 - A_f)}{A_0} * 100 \text{ (Equation 1)}$$

Where A_0 and A_f are the absorbance values of the blank (DPPH solution in alcohol) and the sample (DPPH solution plus antioxidant dissolved in ethanol), respectively.

Method of the ABTS radical

The capturing activity of the ABTS free radical was determined using the method described by Re *et al* with some modifications [22]. The ABTS radical was formed after a reaction of 3.5 mM of ABTS with 1.25 mM of potassium persulfate (final concentration). The samples were incubated between 2-8 ℃ and in the dark for 16-24 h. Once the ABTS radical was formed, it was diluted with ethanol until obtaining an absorbance of 0.7 ± 0.05 at 734 nm. To a volume of 190 µL of the dilution of the ABTS radical 10 µL of the sample was added and incubated at room temperature for 5 minutes; after this time elapsed, the disappearance of the ABTS radical at 734 nm in the Multiskan Ex (Thermo-scientific) microplate reader was spectrophotometrically determined. Ascorbic acid (4 µg / mL) was used as a positive control of uptake of ABTS radicals [14].

Stage 3. Development of Microcapsules from the pulp of Caimito (*Chrysophyllum Cainito L.*)

Microencapsulation by spray drying of pulp of caimito using maltodextrin as an encapsulating agent.

Before starting the microencapsulation stage by spray drying, a pulp-maltodextrin mixture was prepared as a wall agent. The percentage of total solids oscillated by 25%. A Spray dryer type Mini Spray Dryer B-290 was used with a feeding speed of 10mL/min [16]. The powder obtained was collected in airtight jars, suitably labeled, the plates were weighed and stored in a desiccator at room temperature [10.16].

Stage 4. Bromatological characterization and evaluation of the antioxidant capacity of microcapsules of caimito.

At this stage, the bromatological characterization of the Caimito macroparticles was carried out, following the procedures described in stage 1 for the bromatological characterization of the fruit of caimito and in stage 2 in order to evaluate the antioxidant capacity.

Stage 5. Statistical analysis

The tests were carried out in triplicate in order to guarantee reliable analytical results using the GraphPad Prism 5 program. The results were expressed as the mean ± ESM (standard error of the mean). Significant differences were determined by Student's T analysis with values of p <0.05 with significant differences.

Results

The results associated with the chemical composition of the caimito fruit cultivated in the

municipality of San Onofre are shown in table 1, where it can be observed that it is a fruit that has a high moisture content (80,44 g/100g) and a considerable carbohydrate content (17,95 g/100g), as indicated by Zambrano et al. (2013) this fruit being purple has more flavor and is richer in sugar [24] compared to green caimito. As for the ash content (2,20%), values similar to that indicated by Hernández *et al.* 2008 were presented [5] and in turn, the content of protein, fats, moisture, fiber and potassium were below what was reported by them in their study.

In relation to micronutrients, it can be observed that the caimito presents a good source of phosphorus (18,64 mg/100g) and calcium (22,80 mg/100g). Similarly, the amount of iron (0,92 mg/100g) and ascorbic acid (12,40 mg/100g) containing the fruit is higher than that reported by FUNIBER, being 0,5 mg/100g and 10 mg/100g [26] (Table 1).

Therefore, it is considered that the great variation in these characters makes it possible to search for materials with a higher proportion of pulp and nutrients and, in this way, to increase their potential use as fresh or processed fruit [5].

As for the antioxidant capacity, the complexity of the mixture of compounds in an extract, often requires special conditions to be able to measure it.

For this reason, there are several methods for the determination of this capacity [21], therefore, the use of several techniques to evaluate the antioxidant capacity allows to obtain greater degree of information with greater precision of the behavior of the extracts. The antioxidant capacity was evaluated by two methods: DPPH and ABTS and its results were expressed as activity antiradicalaria or IC50, which is defined as the inhibitory concentration of 50% of the initial reagent. In this study, it was found that the fruit of caimito presented a higher value obtained by the method DPPH (116 IC50) compared with the ABTS method (63,82 IC50), however, the method of discoloration of the ABTS cation-radical is applicable to lipophilic and Hydrophilic antioxidants which allows it to be implemented for systems both aqueous and lipophilic [22] and in turn, it is very soluble in water and chemically stable, instead, the DPPH can only be dissolved in organic environment so it measures preferably the antioxidant capacity of little polar or non-polar compounds [25].

With reference to total phenols, it was observed that the result is higher (15,28 mg AG / 100mg) than indicated by Tuesta et al. (2014), in which their study showed a content of 7,81 mg AG/100 mg, which are responsible for the antioxidant potential in this fruit [21].

When analyzing table 2, it is important to note that the moisture content decreased significantly compared to the content of the fruit going from 80,44 g/100 g to 1,93g/100g, however, the carbohydrate content increased by 17,95g/100g to 95,12g/100g, which is attributed to the drying process by Spray Drying and the addition of maltodextrin as an encapsulant due to the fact that it is a polysaccharide, which is introduced into the matrix or polymeric system with the objective of preventing the loss of nutrients, protect them from the reaction with other compounds present or prevent them from suffering oxidation reactions, acting as packaging on a microscopic scale under controlled conditions of the obtained powder product of caimito [10,23]. As for the rest, nutrients and micronutrients, such as: the content of proteins, ashes, fats, fiber, potassium, calcium, iron, phosphorus and ascorbic acid, it can be seen that the values were lower than that obtained in the pulp of fruit of caimito. However, the objective of obtaining a powder product that conserves the nutrients of the fruit but at the same time, its conservation time, was greater and it could be available at all times.

Discussion

The spray drying is dehydration process usually employed to protect labile compounds from deleterious environmental conditions. The particles powder obtained showed a spherical structure with regular surfaces which can be desirable for the stability of trapped substances as well as to control the release of some ingredients [19]. Powder chemical characterization caimito was carried out considering parameters such as moisture, protein, carbohydrates, fiber, ash, vitamin C, calcium, iron, potassium and phosphorus. When both air temperatures (inlet and outlet) increased, the

moisture decreased too (1,93 %) as a result of the dehydration process and mass transfer [27,28].

With regard to the antioxidant capacity assessed by the two methods (DPPH and ABTS), it can be observed that compared with the data obtained in the fruit, these were superior, which reflects that the antioxidant activity was decreased, because there is an inversely proportional relationship indicating that the higher IC50 less antiradicalaria activity. However, it is important to note that the total phenol content remains present in 64,52% in the powder product compared to the value obtained in the fruit.

With the development of new products such as the microcapsules of the caimito fruit, different consumption alternatives can be offered to preserve the nutritional properties of the fruit and enjoy these foods at other times of the year. In the same way, both the caimito fruit and the powdered product proved to have a moderate antioxidant capacity, which would be interesting to highlight for greater utility in the field of food and cosmetics.

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Table 1. Bromatological characterization of pulp of caimito (*Chrysophyllum cainito L.*)

Parameter	Composition (X ± ESM)
Protein (g/100g)**	1,24 ± 0,024
Ashes (g/100g)	2,20 ± 0,092
Moisture (g/100g)	80,44 ± 0,204
Fiber (g/100g)	1,20 ± 0,029
Carbohydrates (g/100g)***	17,95 ± 0,204
Fats (g/100g)	0,37 ± 0,030
Potassium (mg/100g)	3,06 ± 0,040
Calcium (mg/100g)	22,80 ± 0,200
Iron (mg/100g)	0,92 ± 0,037
Phosphorus (mg/100g)	18,64 ± 0,186
Ascorbic acid (mg/100g)	12,40 ± 0,245
DPPH ppm (IC_{50})	116,00 ±0,632
ABTS ppm (IC_{50})	63,82 ± 0,612
Total phenols (mg AG/100mg)	15,28 ± 0,086

* Data on dry basis. (± ESM): Mean value ± standard error of the mean.
** N x 25
*** Carbohydrates, which was calculated by difference.
The means with different letter are significantly different at $p < 0.05$

Table 2. Bromatological characterization of the microcapsules of caimito (*Chrysophyllum cainito L.*)

Parameter	Composition (X ± ESM)
Protein (g/100g)**	0,97 ± 0,010
Ashes (g/100g)	1,96 ± 0,040
Moisture (g/100g)	1,93 ± 0,014
Fiber (g/100g)	0,05 ± 0,002
Carbohydrates (g/100g)***	95,12 ± 0,041
Fats (g/100g)	0,01 ± 0,002
Potassium (mg/100g)	2,12 ± 0,037
Calcium (mg/100g)	18,34 ± 0,121
Iron (mg/100g)	0,54 ± 0,024
Phosphorus (mg/100g)	14,38 ± 0,58
Ascorbic acid (mg/100g)	5,20 ± 3,84
DPPH ppm (IC_{50})	120,60 ±0,245
ABTS ppm (IC_{50})	68,82 ± 0,594
Total phenols (mg AG/100mg)	9,86 ± 0,051

* Data on dry basis. (± ESM): Mean value ± standard error of the mean.
** N x 25
*** Carbohydrates, which was calculated by difference.
The means with different letter are significantly different at $p < 0.05$.