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EVALUATION OF THE ANTIOXIDANT ACTIVITY OF THE CURUBA (*Passiflora Mollissima* Bailey) EXTRACTED FROM THE MUNICIPALITY OF PAMPLONA NORTE DE SANTANDER AND ITS APPLICATION IN A DAIRY PRODUCT TYPE ICE CREAM.

EVALUACIÓN DE LA ACTIVIDAD ANTIOXIDANTE DE LA CURUBA (*Passiflora Mollissima* Bailey) EXTRAÍDA DEL MUNICIPIO DE PAMPLONA NORTE DE SANTANDER Y SU APLICACIÓN EN UN PRODUCTO LÁCTEO TIPO HELADO.

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ABSTRACT

The antioxidant activity of the Curuba fruit (Passiflora Mollissima) from the municipality of Pamplona (Norte de Santander) – Colombia and the application in a food matrix was determined. The fruits were collected in the municipality of Pamplona – Norte de Santander (7°22′34"N 72°38′54"W). Antioxidant activity was determined by DPPH• and ABTS methods. The results of the antioxidant activity test showed that

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the pulp of Curuba, by the methods of DPPH• and ABTS.+ reached values of CI50 105.4±3.209 µg/mL and 61.4±2.19 µg/mL respectively. Therefore, the pulp of Passiflora Mollissima is considered promising for designing food products due to its high antioxidant activity.

Key words: Antioxidant activity, fruits, Curuba, shelf life.

RESUMEN

Se determinó la actividad antioxidante del fruto de Curuba (*Passiflora Mollissima)* proveniente del municipio de Pamplona (Norte de Santander) – Colombia y la aplicación en una matriz alimentaria. Los frutos fueron recolectados en el municipio de Pamplona – Norte de Santander (7°22′34″N 72°38′54″O). La actividad antioxidante fue determinada por los métodos DPPH• y ABTS. Los resultados de la prueba de actividad antioxidante mostraron que la pulpa de Curuba, por los métodos de DPPH$^{\bullet}$ y ABTS$^{\cdot+}$ alcanzaron valores de CI_{50} 105.4±3.209 µg/mL y 61.4±2.19 µg/mL respectivamente. Por lo tanto, la pulpa de *Passiflora Mollissima* es considerada como promisoria para diseñar productos alimenticios por su elevada actividad antioxidante.

Palabras clave: Actividad antioxidante, frutas, curuba, vida útil.

INTRODUCTION

In Colombia, due to its geographical location and privileges tropical climate, it has a wide variety of more than 400 native fruit species, of which a large number belong to the so-called exotic fruits, among them the curuba. This is a pre-Columbian fruit and there are

references to it in Inca iconography. It is cultivated in the Eastern mountain range, in the departments of Boyacá, Antioquia, Huila, Meta and Valle (Ministerio de Agricultura y Desarrollo Rural, 2019); likewise, the Spanish colonizers named the curuba flower as “the passion flower”, because of its resemblance to a crown of thorns and it is believed to be native to the Andes region, it is characterized by having lily-shaped petals and deep mauve color, its flowers are hermaphrodite and have no odor..

Globally, only a few countries are producers of curuba, among the Colombia, Brazil and Ecuador. Colombia is one of the largest exporters of this fruit in the world, exporting approximately 4566 kg net (Ministerio de Agricultura y Desarrollo Rural, 2019). In general, crops begin production at 12 to 14 months after planting and this will depend on the climatic conditions and the thermal floor in which they are planted (Rojano et al., 2012; Mantilla & Hernández, 2019).

The interest is growing among the population in consuming foods with beneficial nutritional properties for health, which contribute in such a way to reduce the risk of disease and maintain a healthy diet beneficial to health (Ochoa et al., 2014).

On the other hands, several research studies have suggested that the consumption of fruits and vegetables reduces oxidative stress and modifies the lipid profile, thereby reducing the risk of diseases caused by free radicals and high blood cholesterol (Uttara et al., 2009). The use of antioxidants of plant origin, such as phenolic acids, flavonoids, and tocopherols in the food industry is becoming increasingly important due to their anti-carcinogenic, anti-diabetic and other benefits attributed to human health. Some fruits have a high content of antioxidants, due to the fact that these molecules decrease or delay oxidations on different substrates, so their frequent consumption is important. Therefore, for several years there have been several studies on antioxidants of natural origin in some plant species such as fruits, vegetables, among others, so that they can be used in the food, cosmetic and medicinal industry due to the fact that they are presumed to help reduce health problems (Uttara et al., 2009; Zapata et al., 2013).

The objective of the research was to evaluate the antioxidant activity of curuba (*Passiflora*

Mollissima bailey) extracted from the municipality of Pamplona Norte de Santander and its application in an ice cream type dairy product.

MATERIALS AND METHODS

Harvest and preliminary treatments of the plant material.

Passiflora Mollissima fruits were harvested in the municipality of Pamplona (Coordinates 7°22′34″N 72°38′54″O) considering their quality and maturity index.

The selected fruits were washed by immersing them in distilled water and sodium hypochlorite at 100 ppm for 10 minutes in order to avoid the growth of microorganisms. Then, they were rinsed with abundant distilled water and exposed to air to be dried at room temperature.

Determination of chemical characteristics of the pulp.

The chemical characterization of the pulp was carried out and determined the nutrient content through the following tests described below: protein; which used the Kjeldahl method according to AOAC 955.04; ash; which used the direct method according to AOAC 924.05; moisture; which used the drying method at 100+2 ºC according to AOAC 925.09; fiber; by enzymatic gravimetric method; carbohydrates; fat; by Soxhlet method according to AOAC 936.1512 (Kuskoski et al., 2005).

Determination of antioxidant activity od pulp.

Method of the DPPH•-radical

The free radical scavenging activity DPPH• was determined using the method described by Silva *et al*. (2004) with some modifications. 75 µL of sample were added to 150 µL of a methanolic solution of DPPH• (100 ppm) end incubated at room temperature for 30 minutes, after which the disappearance of the DPPH•-radical was determined spectrophotometrically at 550 nm in a Multiskan Ex microplate reader (Thermoscientific). Ascorbic acid was used as a positive control for DPPH•-radical uptake (25 ppm). The IC_{50} was determined by evaluating various serial concentrations of the sample by linear regression analysis. The results were expressed as the mean ± E.S.M of the percentage of DPPH•-radical uptake

Diaz- Passo Yerlis [1], Puerta-De La Barrera Valeria [1], Granados-Conde Clemente [1], Pastrana-May Giovanna[1], León-Méndez Glicerio [2]*

relative to the control group. Percent inhibition (% Inh) was calculated using equation (1).

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

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 alcohol), respectively.

ABTS$^{\cdot+}$-radical method

The free radical activity of ABTS• was determined using the method describe by Re *et al.* with some modifications. The ABTS•-radical was formed after reaction of ABTS 3.5mM with 1.25 mM potassium persulfate (final concentration). Samples Will be incubated between 2 to 8°C and in the dark 16-24h. once the ABTS•-radical was formed, it was diluted with ethanol to an absorbance of 0.7± 0.05 at 734nm. To a volume of 190 µL of the dilution of the ABTS•-radical, 10 µL of the sample under study was added and incubated at room temperature for 5 minutes, after which the disappearance of the ABTS•-radical was determined spectrophotometrically at 734 nm in the Multiskan Ex microplate reader (Thermoscientific). Ascorbic acid was used as a positive control for ABTS•-radical uptake (4 ppm). The IC_{50} will be determined by evaluating serial sample concentrations by linear regression analysis. The results were expressed as the mean ± E.S.M of the percentage of ABTS•-radical uptake relative to the control group.

Formulation of the product

The formulation of the product (ice cream) and the natural antioxidant was elaborated, in which the concentrations of *Passiflora Mollissima* pulp to be added were varied, with the purpose of obtaining comparable results in the antioxidant capacity. Both the concentration and the antioxidant capacity of *Passiflora Mollissima* in the final product are important variables, as well as the shelf life of the frozen yogurt.

Statistical analysis

The tests were performed in triplicate in order to ensure reliable analytical results using GraphPad Prism 8 software. The results were expressed as mean ± EEM (standard error of the mean).

RESULTS AND DISCUSSION

In the results shown in Table 1, it can be observed that the Passiflora *Mollissima* Pulp evaluated has a low moisture content, with a value of 90.4 %; likewise, it has a high carbohydrate content (8.08 %) and a very low lipid content (Granados et al., 2021).

Fruits contain 0,1-1,5 % of nitrogen compounds, of which proteins account for 35-75 % of fruits; amino acids are also well represented. The fraction of soluble nitrogen compounds consists on average of 50 % free amino acids. All other nitrogen compounds are rather scarce. It should be noted that most of the protein fraction, which I subject to great changes depending on the type of fruit and its degree of ripeness, is composed of enzymes (Kuskoski et al., 2005). The amount of protein in fruits is low (Table 1).

Table 1. Chemical characterization of Passiflora *Mollissima* Pulp grown in the municipality of Pamplona (Norte de Santander).

Moisture	Ash	Protein	Fiber	Carbohydrates	Fats
90.4± 0.548	0.64± 0.055	0.74± 0.055	0.46± 0.055	8.08± 0.536	0.14± 0.055

The antioxidant activity of *Passiflora edulis pulp was evaluated by* DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods, and achieved CI_{50} values of 105.4±3.209 µg/mL and 61.4±2.19 µg/mL respectively. These results were expressed as antiradical activity or IC_{50}, which is defined as the concentration of the antioxidant that decreases the uptake of the radical to 50 % or the initial amount.

Another very common way to follow fatty acid oxidation is the measurement of thiobarbituric acid reactive species (TBARs), which are secondary products of lipid peroxidation and are widely used in the food industry, even more relevant, in this particular case, because of the use of spectrofluorimetric techniques that have a high sensitivity and specificity.

Rojano *et al.,* evaluated the free radical trapping capacity of *Passiflora mollissima* (Kunth) L. H. Bailey (curuba), from the region of Antioquia, Colombia, to trap reactive oxygen species. It showed that aqueous extracts of curuba are rich in polyphenols, especially tannins, flavonoids and phenolic acids. In addition, they have a high capacity

to trap various reactive oxygen species, specially the ROO• radical, with an ORAC value equal to 108164.9 mmol of Trolox/100 g of dried pulp, higher than most fruits and vegetables.

Therefore, the aqueous extract of curuba has a high nutraceutical potential, due to the content of polyphenols, which directly affect its capacity to trap radical reactive oxygen species.

Chaparro *et al.,* described the nutritional and antioxidant characteristics of long curuba. Indicating that this fruit is a source of vitamins A, C and niacin, minerals such as potassium, phosphorus, magnesium, sodium, chlorine, iron; it provides moderate amounts of carbohydrates and calories. The content of total carotenoids, phenols and flavonoids was 118.8 mg *β*-carotene 460.1 mg gallic acid and 1907.6 mg catechin/100 g, respectively. El value DPPH, FRAP and ORAC were 60843.1 µmol, 8520.3 µmol and 20754.9 µmol equivalents of Trolox/100 g of dried fruit, respectively. Concluding that the nutritional and antioxidant value of long curuba should be taken advantage of by the general population and as raw material by the agroindustry to benefit its production chain.

The changes in the TBAR index of yogurt containing different amounts of *Passiflora Mollissima* pulp and stored at 63ºC during 18 days are presented in Figure 1. Figure 1shows the effect of different concentrations of antioxidants (ascorbic acid as a control at 0.02%, *Passiflora Mollissima pulp*) upon the formation of thiobarbituric acid reactive substances (TBARS) in the ace cream samples.

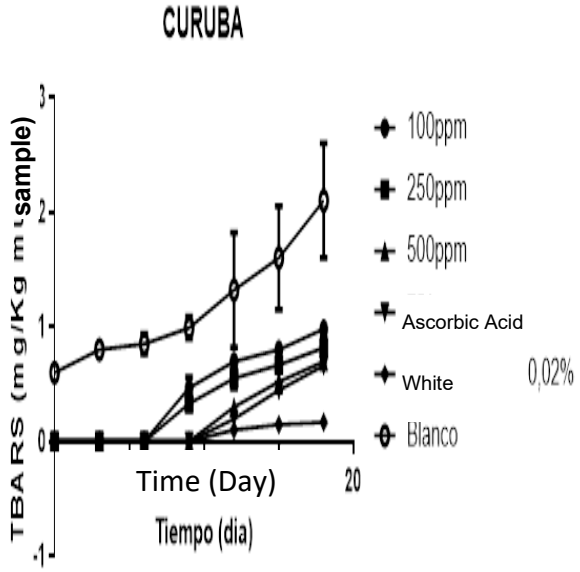

Figure 1. Changes in the TBAR index of ice cream containing different amounts of curuba fruit extract stored at 63 ºC during 18 days.

The antioxidant activity of food is manifested through the various elements that compose it, which act through various reductive mechanisms when interacting with reactive oxygen species (ERO) or other radicals. The evaluation of the antioxidant capacity of foods has gained great importance in the last few years, due to the wealth of information that can be obtained, which includes aspects such as resistance to oxidation, the quantitative contribution of compounds with antioxidant properties and the antioxidant impact that foods generate in the organism when they are consumed (Zapata et al., 2013).

Ochoa *et al.,* evaluated the antioxidant capacity of milk cream supplemented with 0.40; 0.60 and 0,80% P/P of Curuba extract and the oxidative stability during 25 days of storage at 4ºC. The presence of 0.40; 0.60 and 0.80% P/P of Curuba extract where reduce the production of malondialdehyde with respect to the blank in a 6, 15 and 22% respectively, the antioxidant power of the supplemented samples was superior to that of the blank. In the sensory evaluation, a difference was found between the milk cream with and without extract. It was observed that the curuba extract delays the oxidation process of the milk cream and may be associated with the antioxidant capacity of the product.

Passiflora Mollissima is considered a promising product for the design of food products due to its high antioxidant activity.

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