








**EVALUATION OF THE ANTIOXIDANT ACTIVITY OF GULUPA EXTRACT (*Passiflora edulis* f. *edulis*) AND ITS EVALUATION IN THE PREPARATION OF AN ICED YOGURT**

**EVALUACIÓN DE LA ACTIVIDAD ANTIOXIDANTE DEL EXTRACTO DE GULUPA (*Passiflora edulis* f. *edulis*) Y SU EVALUACIÓN EN LA ELABORACIÓN UN YOGURT HELADO**

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**ABSTRACT**

The antioxidant activity of the fruit of gulupa (*Passiflora edulis*) 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 the gulupa pulp, by the methods of DPPH• and ABTS.+ reached values of CI50 90±0.707µg/mL and 59.4±0.894 µg/mL respectively. Therefore, the pulp of *Passiflora edulis* is considered

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promising for designing food products due to its high antioxidant activity.

**Key words:** Antioxidant activity, fruits, gulupa, shelf life.

## RESUMEN

Se determinó la actividad antioxidante del fruto de gulupa (*Passiflora edulis*) 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 gulupa, por los métodos de DPPH• y ABTS<sup>+</sup> alcanzaron valores de  $CI_{50}$   $90 \pm 0.707 \mu\text{g/mL}$  y  $59.4 \pm 0.894 \mu\text{g/mL}$  respectivamente. Por lo tanto, la pulpa de *Passiflora edulis* es considerada como promisoría para diseñar productos alimenticios por su elevada actividad antioxidante.

**Palabras clave:** Actividad antioxidante, frutas, gulupa, vida útil.

## INTRODUCTION

The gulupa (*Passiflora edulis* f. *edulis* Sims) is a fruit native to the Brazilian Amazon and mainly diversification centers located throughout the Andean region; this is the

third most exported fruit from Colombia (after bananas and avocados) (Franco et al., 2014; Cámara de Comercio Bogotá, 2015), In addition to improving the cardiovascular

system, it is known to be rich in antioxidants, as it prevents clogging of the arteries. It is also an exotic fruit of the passion fruit family, very similar, but with a purple skin and a sweet taste (perfect balance of acidity and sweetness, like a cross between passion fruit and granadilla (De armas, 2022). The cultivation of passionflower in Colombia is very important because it represents an important line in the fruit sector, there is a great diversity of possibilities and it offers a wide range for international markets. Exotic fruits have established themselves as a great line in Colombia's exporting future (De la Espriella *et al.*, 2023).

Understanding the diversity and genetic structure of plant species with high potential, such as gulupa, is of utmost importance, as it allows the implementation of effective strategies for its conservation and improvement; in Colombia, studies carried out to analyze this fruit are scarce (*P. edulis*) and therefore, there is a need to carry out several studies in order to develop strategies for the use and improvement of exotic fruit (Latorre Araque, y Villamizar Quiñones, 2019, Guevara-Cuasapud y Gómez-Barrera, 2020; De armas, 2022).

The addition of antioxidants to a fruit frozen yogurt is done with the aim of slowing oxidation processes, decreasing the possibility of generation of toxic compounds, decreasing the loss of nutritional value caused by the degradation of essential fatty acids and by the destruction of vitamins A, E and D, in addition to the fact that these natural compounds reinforce the activity of endogenous antioxidant systems, providing extra protection for oxidative stress (Granados *et al.*, 2012; Ayola, *et al.*, 2019; Tarón Dunoyer *et al.*, 2022).

The interest is growing faster in the consumption of foods with high antioxidant power, since it provides greater benefits to the consumer, as well as contributing to minimize the risks of suffering diseases. Several studies agree that the inclusion of natural antioxidants in foods has benefits for human health, since they protect cellular components such as DNA, proteins and lipids from attack by oxygen-reactive substances (Uttara *et al.*, 2009). The objective of the research was to evaluate the antioxidant activity of gulupa in a yogurt-type ice cream.



## MATERIALS Y METHODS

### ***Collection and preliminary treatments of the plant material.***

The fruits of *Passiflora edulis* were collected 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 min 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 carried out, to which the nutrient content was determined by means of the tests described below: protein, using the Kjeldahl method according to AOAC 955.04; ash; by the direct method according to AOAC 924.05; moisture; by the drying method at 100±2 °C according to AOAC 925.09; fiber; by the enzymatic gravimetric method; carbohydrates; fat; by the Soxhlet method

according to AOAC 936.1512 (Kuskoski *et al.*, 2005).

### ***Determination of antioxidant activity of pulp.***

#### **DPPH• radical method**

The free radical scavenging activity DPPH• was determined by 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) and incubated at room temperature for 30 min, 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<sub>50</sub> 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 relative to the control group. Percent inhibition (% Inh) was calculated using equation (1).

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

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

### **ABTS<sup>•+</sup> radical method**

The free radical ABTS<sup>•+</sup> scavenging activity was determined using the method described by Re *et al.* with some modifications. The ABTS<sup>•+</sup> radical was formed after reaction of 3.5mM ABTS with 1.25 mM potassium persulfate (final concentration). Samples will be incubated between 2 to 8°C and in the dark during 16-24h. Once the ABTS<sup>•+</sup> radical was formed, it was diluted with ethanol to an absorbance of  $0.7 \pm 0.05$  at 734nm. To a volume of 190  $\mu$ L of the dilution of the ABTS<sup>•+</sup> radical, 10  $\mu$ L of the sample under study was added and incubated at room temperature for 5 minutes, after which the disappearance of the ABTS<sup>•+</sup> radical was determined spectrophotometrically at 734 nm in the Multiskan Ex microplate reader (Thermoscientific). Ascorbic acid was used as a positive control for ABTS<sup>•+</sup> radical uptake (4 ppm). The  $IC_{50}$  was determined by evaluating serial sample concentrations by

linear regression analysis. The results were expressed as the mean  $\pm$  E.S.M of the percentage of ABTS<sup>•+</sup> radical uptake relative to the control group.

### **Formulation of the product**

The formulation of the product (frozen yogurt) and the natural antioxidant was elaborated, in which the concentrations of *Passiflora edulis* pulp to be added were varied in order to obtain comparable results in the antioxidant capacity.

Both the concentration and the antioxidant capacity of *Passiflora edulis* in the final product are important variables, as well as the shelf life of the frozen yogurt.

### **Statistical analysis**

Assays were performed in triplicate to ensure reliable analytical results using GraphPad Prism 8 software. Results were expressed as mean  $\pm$  EEM (standard error of the mean).

## **RESULTS AND DISCUSSION**

The results in Table 1 show that the *Passiflora edulis* pulp evaluated has a low moisture content, with a value of 81.40%; it also has a high carbohydrate content (17.33 %) 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 is 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 edulis* pulp grown in the municipality of Pamplona (Norte de Santander).

Moisture	Ashes	Proteins	Fiber	Carbohydrates	Fats
81.4± 0.548	0.19± 0.022	0.96± 0.055	0.18± 0.045	17.33± 0.514	0.12±0.045

The antioxidant activity of *Passiflora edulis* pulp was evaluated by DPPH<sup>•</sup> and ABTS<sup>•+</sup>, methods, reaching values of CI<sub>50</sub> 90±0.707µg/mL and 59.4±0.894 µg/mL respectively. These results were expressed as antiradical activity or IC<sub>50</sub>, which is defined as the concentration of the antioxidant that decreases the uptake of the radical to 50 % of the initial amount.

Another very common way to follow fatty acid oxidation is the measurement of

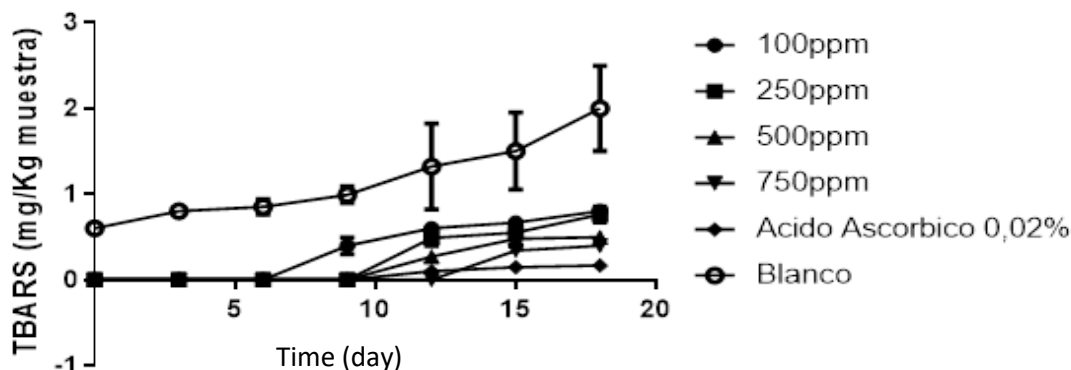
thiobarbituric acid reactive species (TBARs), which are secondary products of lipid peroxidation and are widely used in the food industry, even more relevant, in this particular case, because of the use of spectrofluorimetric techniques that have high sensitivity and specificity.

Franco *et al.*, indicated that the antioxidant activity of gulupa juice may be due to the ascorbic acid and carotenoid contents. It is important to define the time of consumption

after harvesting the fruit, in order to maximize its value as a nutraceutical food. These aspects are useful to strengthen the position of gulupa in the export market.

Therefore, the antioxidant activity is mainly due to its content of natural compounds such as polyphenols, flavonoids and vitamin C, which are known for their ability to neutralize free radicals in the body. These free radicals can cause cell damage and contribute to premature aging and various diseases.

The changes in the TBAR index of frozen yogurt containing different amounts of *P. edulis* pulp and stored at 63°C for 18 days are presented in Figure 1. Figure 1 shows the effect of different concentrations of antioxidants (ascorbic acid as control at 0.02%, *P. edulis* pulp) on the formation of thiobarbituric acid reactive substances (TBARS) in the frozen yogurt samples.



**Figure 1.** Changes in the TBAR index of frozen yogurt, containing different amounts of gulupa fruit extract, stored at 63 °C for 18 days.

The antioxidant activity of a food is manifested through the various elements that compose it, which act through various reducing mechanisms when interacting with

reactive oxygen species (ERO) or other radicals. The evaluation of the antioxidant capacity of foods has gained great importance in recent 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 body when consumed (Zapata et al., 2013).

*Passiflora edulis* pulp is considered promising for designing food products due to its high antioxidant activity.

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