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EVALUATION OF THE ANTIOXIDANT POTENTIAL OF SPRAY-DRIED MICROENCAPSULATED EXTRACT AND ITS FORMULATION OF MACLEANIA RUPESTRIS (KUNTH) A.C. SM.

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ABSTRACT
Ecuador has a high diversity of native plants, whose fruits provide a large amount of nutrients that help improve the quality of nutrition and contribute to food security and food sovereignty. Macleania rupestris (Kunth) A.C. Sm. (hualicón) is a species of the Ericaceae family used as a food source in rural areas; it has edible fruits that are little exploited and its trade is incipient. The objective of the research was to evaluate the antioxidant potential of the microencapsulated extract by spray drying and the formulation of an instant powdered drink. The fruits were collected in Santiago de Quero-Tungurahua-Ecuador, the polyphenol content was determined by the Folin-Cioacalteau method, anthocyanins and its antioxidant activity by DPPH. The results of the microencapsulated extract of M. rupestris with maltodextrin achieved an efficiency of 82.03 %, reaching 362.01 mg AGE, 272.82 mg cyanidin-3-glucoside and 251.73 µmol Trolox 100 g^{-1}. Finally, when the instant beverage was reconstituted, it obtained 50 mg anthocyanins L^{-1}, products that complied with the physicochemical and microbiological requirements established by Ecuadorian regulations, as well as reaching an acceptability greater than 95 %. This study offers a new alternative for the incorporation of antioxidant compounds from the fruit of M. rupestris in the food industry.

Keywords: anthocyanins, antioxidant, Ericaceae, maltodextrin, microencapsulation.

INTRODUCTION
In Ecuador there is a great variety of wild fruits that provide a large amount of nutrients and bioactive compounds that help the body function. According to De la Torre et al. [1], there are 4591 food species of which 89% are native. The Ericaceae family is one of the most significant in terms of the flora of the Andean forests and paramos [2]-[5]. In reference to [6], he states that over the years, the nutritional potential of *Ericacea*e has been studied more frequently, and the high content of antioxidants and vitamins, which benefits the diet. *M. rupestris* is one of the native species of the Andes of Ecuador [7]. *M. rupestris* has several names, in Ecuador it is known as chamburo, chaquilulo, joyapa, hualicón, joyapa blanca, chupa lulu, among others; in Colombia as uva camarona [10], and in Venezuela as cacagüito [11]. This endemic species is distributed throughout countries such as Nicaragua, Costa Rica, Panama, Venezuela, Colombia, Ecuador, and Peru [10], between 2200 and 3500 masl, and is found in forests and humid and rainy moorlands, in the wild [12]. The fruits of hualicón are frequently consumed in rural communities of the Sierra, mainly by children and adults during their grazing tasks [8].

Plazas [9] mentions that *Macleania rupestris* (Kunth) A. C. Sm. "hualicón" is rich in flavonoids, tannins and anthocyanins, however, there are few studies that show this.

The antioxidant activity of foods, especially fruits, depends on the concentration of natural antioxidants present, which are responsible for protecting the cell against free radicals or oxidative damage, the same that generate degenerative diseases in the body [10], [11].

One of them are polyphenols, which are secondary metabolites with an important role in the food, pharmaceutical and cosmetic industry [12], due to their anti-inflammatory and antioxidant properties, among others [13]. Likewise, they help to combat cardiovascular diseases, neurodegenerative diseases, osteoporosis [14], [15] and the development of several types of cancer [16]. Anthocyanins are non-nitrogenous plant compounds widely distributed in nature [17], having higher concentration of antiocyanins in fruits, flowers and leaves, which are the ones that give the color variations such as orange, red, blue and violet, being identified as natural dyes used in the

food industry [18], [19]. Also an impact on the sensory properties of food, as well as on human consumption and health. One of the important roles is the extraction of these compounds for the improvement of visual acuity, also, the reduction of coronary heart disease, anti-inflammatory, anticancer, antitumor and antidiabetic effects [20].

Microencapsulation is a technological process based on coating solid, liquid and even gaseous materials with an encapsulating agent to obtain particles of 5 to 100 µm [21], [22], which allow preserving the metabolite for a longer time, so that several factors do not degrade it, such as humidity, light, oxygen, microorganisms, heat and contaminants [23], and thus improve the nutritional and sensory characteristics when the encapsulation is released [24].

In the food industry, its use is common, since it is a low-cost and easy to implement technology, as well as in the cosmetic, chemical, pharmaceutical and agrochemical industries [25], [26]. Microcapsules maintain the quality and stability of the encapsulated substances, such as colorants, fat, flavorings, vitamins, oils and aromas, being released when added to foods, products or after ingestion [27].

This study evaluates the antioxidant potential of the microencapsulated spray-dried extract and its formulation of *Macleania rupestris* (Kunth) A.C. Sm.

MATERIALS AND METHODS

The fruits were collected in Santiago de Quero, Tungurahua, Ecuador. The extraction and microencapsulation of bioactive compounds from *M. rupestris* was carried out in the laboratories of the Facultad de Ciencia e Ingeniería en Alimentos y Biotecnología of the Universidad Técnica de Ambato.

A. Obtaining the Extraction of Bioactive Compounds

The hualicón fruits were then dehydrated at a temperature of 60 ºC for 52 hours and ground to a fine particle size.

To obtain the extract, they were mixed in a ratio of 1:20 (plant material/solvent volume of 85:15), the solvents were ethanol and HCl, with an extraction time of 1 hour at a temperature of 70 ºC [28], followed by filtration and total solids concentrate at the rotary evaporator.

B. Obtaining Microencapsulation

Once the extract was obtained, 20 % of the concentrate and 80 % of maltodextrin were mixed; while the concentration of the solution was 30 %. For microencapsulation, the Mini Spray Dryer (BUCHI-B290) was used, with an inlet temperature of 150 ºC and outlet temperature of 90 ºC [29].

C. Microencapsulation Efficiency

The efficiency of the microencapsulation with the polymer was verified by a ratio of 1/10 of the microencapsulate and distilled water (H_2O), similarly, the same amounts of microencapsulate and ethanol (ETOH), stirred for 10 minutes and a 1/100 dilution was performed for the measurement of the absorbances at 535 nm in UV-VIS spectrophotometer (Fisher Scientific accuSkan GO) [30].

$$\%E.E = \frac{[H_2O] - [ETOH]}{[H_2O]}$$

Equation 1. Percentage Microencapsulation Efficiency

% E.E: Microencapsulation efficiency, percentage by mass (%)

[H_2O]: Total concentration present in the microcapsules (mg L^{-1})

[ETOH]: Surface concentration present in the microcapsules (mg L^{-1})

D. Determination of Anthocyanin Content

The absorbance reading at 535 nm was performed in a UV-VIS spectrophotometer (Fisher Scientific accuSkan GO) and ethanol was used as blank [28].

$$C = \frac{A}{\varepsilon} * \frac{V}{1000} * MW * \frac{1}{samplewt} * 10^6$$

Equation 2. Anthocyanin Content

C: Anthocyanin concentration (mg 100 g^{-1})

A: Absorbance

ε: Molar absorptivity of the compound cyanidin 3-glucoside

V: Total volume of anthocyanin extract (mL)

MW: Molecular weight of cyanidin 3 glucoside (g mol^{-1})

SAMPLE WT: Sample weight (g)

E. Quantification of Total Polyphenols by the Folin-Cioacalteau Method.

In a 5 mL volumetric ball, 0.1 mL of liquid plant extract (0.1 g of the microencapsulated with 4 mL of distilled water) and 0.1 mL of Folin-Cioacalteau reagent was added, allowing it to react for 3 minutes, and 2 mL of sodium carbonate solution (75 g L^{-1}) was added, gauging with distilled water, and allowed to stand for 1 hour to measure the absorbance at 750 nm in the UV-VIS spectrophotometer (Fisher Scientific accuSkan GO). For the calibration curve, concentrations were prepared with gallic acid from 50 to 200 ppm [31].

F. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (FTIR-ATR)

It was performed on the liquid extract of anthocyanins of the fruit under study, the microencapsulated and maltodextrin, for a comparison of the absorption bands of these samples, the Spectrum software (PerkinElmer) was used.

G. Evaluation of Antioxidant Activity in Vitro

The 2,2-diphenyl-1-picrylhydrazyl method also known as DPPH was used [32].

It was started by preparing the Trolox 500 µmol L^{-1} solution (0.0129 g Trolox in 50 ml of 96 % ethanol and 50 ml of distilled water). Trolox concentrations from 50 to 500 µmol L^{-1} were used for the calibration curve. The DPPH solution was concentrated to 150 µmol L^{-1} (0.059 g DPPH in 100 mL of 80 % methanol). These solutions were packaged in amber bottles for sun protection and kept refrigerated. When dilutions of the samples were made, a total of 20 µL of the sample was placed in the 96-well plate at 180 µL of the prepared DPPH solution, the absorbance obtained is called Absorbance of the sample (Am).

Control absorbance (Ac) is the result of placing 20 µL of water:ethanol 50:50 plus 180 µL of DPPH solution 150 µmol L^{-1} in the 96-well plate.

The absorbance of the blank (Ab) is the reading of 20 µL of water with 180 µL of methanol.

The microplate was shaken for 1 min and allowed to stand 40 min in the dark.

Absorbance readings were performed using a UV-VIS spectrophotometer (Fisher Scientific accuSkan GO) at 515 nm. The results were calculated using Equation 4.

$$DPPH = \left[1 - \left(\frac{A_m - A_b}{A_c - A_b}\right)\right] * 100$$

Equation 4. Evaluation of DPPH antioxidant activity

A_m**:** Sample absorbance

A_b**:** Absorbance of the reagent blank

A_c**:** Control absorbance

H. Preparation of an instant powdered beverage enriched with microencapsulated anthocyanins from *Macleania rupestris* (Kunth) A.C. Sm.

Sucrose, acidulants (citric acid, malic acid), flavoring (grape identical flavor), sweeteners, anticompactant, ascorbic acid and as the main active ingredient, *Macleania rupestris* anthocyanin microencapsulation were mixed.

I. Physicochemical and Microbiological Analysis of the Powdered Mixture

The analyses were carried out in accordance with Ecuadorian Standard INEN 2471 [33], which refers to powdered mixtures for preparing soft drinks or instant beverages. The percentage of moisture and pH were determined. Total and fecal coliforms, molds and yeasts. The microbiological analyses were carried out in the Laboratorio de Control y Análisis de Alimentos LACONAL of the Universidad Técnica de Ambato.

J. Anthocyanin Content

The same methodology was used for the extract and microencapsulation.

K. Sensory Analysis of Powdered Mix and Reconstituted Product

A tasting with a 5-point hedonic scale was carried out with 12 panelists in the Tasting Room of the Facultad de Ciencia e Ingeniería en Alimentos y Biotecnología, the samples being: the instant powdered beverage and the reconstituted beverage.

RESULTS AND DISCUSSION

A. Obtaining the Liquid Plant Extract

Considering the optimal conditions for the extraction of bioactive compounds from the fruits of *M. rupestris,* the liquid extract concentrate resulted 18.45 % total solids and 1.05 pH, obtaining a dark red color as it is in the case of pH less than 2 [28].

B. Microencapsulation of anthocyanins by spray-drying

Using the spray-drying microencapsulation technique, a fine powder of bioactive compounds was obtained from hualicón. A technique commonly employed in the food industry, which preserves one or several active compounds within a stable protective substance to produce encapsulation of various sizes with functional properties [34].

Maltodextrin DE 10 is a white and hygroscopic powder polysaccharide, within its physical characteristics is to be very soluble in water which offers great protection to sensitive ingredients [34]. The efficiency of microencapsulation with maltodextrin showed 82%, with a slight difference with the highest percentage of efficiency of microencapsulation of capulin with maltodextrin, with 85% efficiency, at an air inlet temperature of 150 ºC [35]. In reference to Moreno [36], in his research on the microencapsulated extract of red bean, he obtained approximately 71 %, indicating that this is due to the viscosity of the polymeric matrix, in addition to the fact that during the process, dust accumulated on the wall of the cyclone and the chamber.

C. Infrared Spectroscopy Analysis (FTIR-ATR)

Fourier transform infrared spectroscopy with attenuated total reflectance sampling mode allowed the identification and comparison of functional groups from very small samples [37].

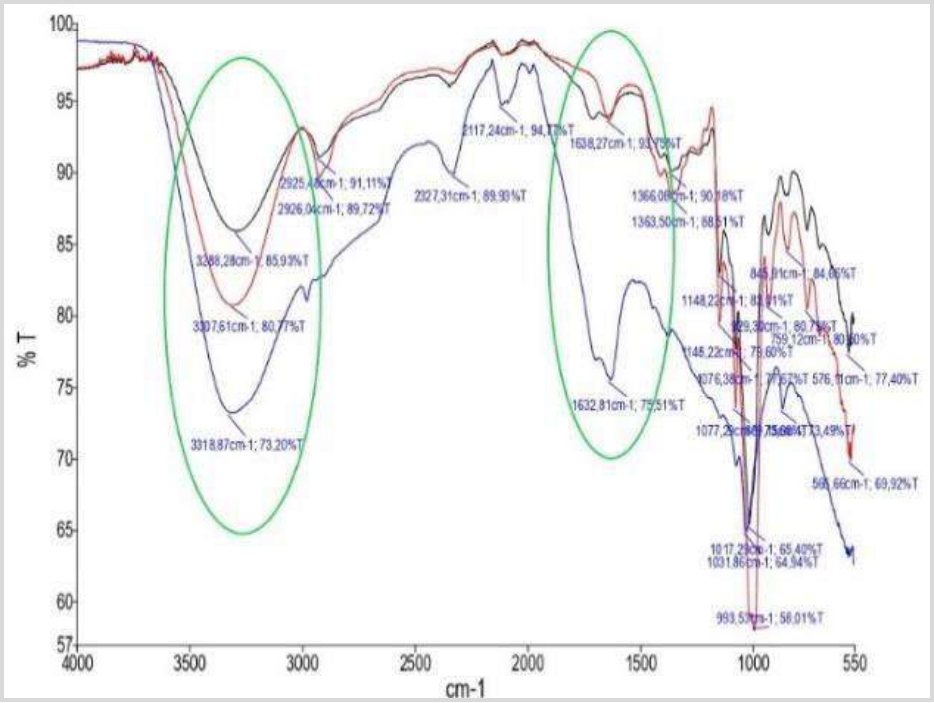

Figure 1. Infrared spectroscopy of extract, maltodextrin and microencapsulated extract of hualicón.

As shown in Figure 1, the extract without microencapsulation (blue color) has a characteristic band at 3318 cm^{-1}, which corresponds to the presence of the OH functional group (alcohols and phenols), which are found in a spectral range of 3200 to 3500 cm^{-1} and at 1632 cm^{-1}, corresponds to the aromatic rings [37], which corroborates the presence of anthocyanins in the extract of *Macleania rupestris.* In this context, Taco [38] reported, similar results of alcohols and phenols group at a band of 3399 cm^{-1} and aromatic type in a spectrum of 1642 and 1463 cm^{-1}, with C=C tension in extracts of mulberry (*Vaccinium floribundum).*

Upon microencapsulation, the absorption bands of the microencapsulate (black color) lost their intensity because the maltodextrin, as an encapsulating agent, is coating the active ingredient, which means that the microencapsulation process has been effective.

D. Evaluation of the Antioxidant Potential of *M. Rupestris* Extract and Microencapsulation.

Table I: Antioxidant potential of *M. rupestris* extract and microencapsulated bioactive compounds.

Analysis	Value obtained	Unit
Total polyphenols	1704.60	mg AGE* 100 g^{-1} extract
	362.01	mg AGE* 100 g^{-1} microencapsulated
Anthocyanins	1467.20	mg cyanidin-3-glucoside 100 g^{-1} extract
	272.82	mg cyanidin-3-glucoside 100 g^{-1} microencapsulated
Antioxidant activity	743.48	µmol Trolox 100 g^{-1} extract
	251.73	µmol Trolox 100 g^{-1} microencapsulated

*AGE: Gallic acid equivalent

The study shows the presence of the polyphenolic structure (anthocyanins, total phenols and flavonoids) by infrared spectroscopy. The content of phenolic compounds in the extract is 1704.60 mg AGE 100 g^{-1}, a lower

value than that reported in the work by [39] with a value of 6890 mg AGE 100 g^{-1} in ripe fruits, carried out by extraction by maceration with 96% ethanol, which is a big difference, likewise, another point to consider is the climatic conditions of each geographical location [40].

E. Anthocyanins (Cyanidin-3-Glucoside) of the Extract

Anthocyanins are responsible for the different colors of fruits, such as *Macleania rupestris*, which has shades according to its degree of maturity, as highlighted in [41], stage 1: green color and stage 5 corresponds to black color.

In the quantification of anthocyanins, Pacho and Valverde [42], indicate that the anthocyanin pigment in greater proportion is cyanidin 3-glucoside in *Macleania rupestris*, as well as [38] in the fruit of *Vaccinium floribundum* also of the Ericaceae family.

The anthocyanin content calculated at maturity stage 5 of *M. rupestris* was 1467.20 mg 100 g^{-1}, which depends on the maturity stage and genetics of the fruit, emphasizing that the key factor for the synthesis of anthocyanins is light, which, as radiation increases, there will be greater synthesis of phenolic compounds [43].

Comparing with data published in Ecuador, it has a certain similarity to mortiño, with a content of 1383 mg 100 g^{-1} at a horticultural stage of maturity and a blackish color [41].

Similarly, red fruits, cherries and grapes, are also rich in anthocyanins, standing out for being antioxidant and for their effect on health, being anti-inflammatory, cardioprotective, neuroprotective and also prevents carcinogenesis [44].

F. Antioxidant Activity of the Extract by DPPH Method of the Extract

The extract concentrated in anthocyanins presented an inhibition value of 61.96 %, which means that with this percentage it is possible that the concentrations are neutralized by the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), therefore, the percentage of inhibition and the antioxidant potential are high [39]. The percentage obtained is similar to the ethanolic extract of blueberry, with an inhibition percentage of about 65.90 %, noting a reduction according to the extraction solvent [45].

The antioxidant activity corresponds to the equivalent concentration of Trolox of 743.48 µmol 100 mL^{-1} of extract obtaining an IC_{50} 30.03 µg mL^{-1}, value below the IC_{50} of the blueberry (47.29±2 µg mL^{-1}) [46], reason why the hualicón only needs 30.03 µg mL^{-1} to inhibit 50 % of the free radicals.

The result of the mean inhibitory concentration (IC_{50}) of hualicon shows that the higher the antioxidant potential, the lower the IC_{50}, as stated in [47], so *M. rupestris* has a high content of phenolic compounds.

G. Antioxidant Potential of Microencapsulated *M. Rupestris* Extract.

When maltodextrin was used, the compounds were preserved in low concentrations of anthocyanins, although, berries, being part of the Ericaceae family, are a great dietary source of bioactive compounds, due to their antioxidant characteristics, which are relevant for plant growth and as a protector against environmental stress, injuries and infections [48].

The microencapsulated presented a concentration of polyphenols of 362.01 mg AGE 100 g^{-1}, in terms of anthocyanins 272.82 mg cyanidin-3-glucoside 100 g^{-1}, in relation to the percentage of DPPH radical inhibition of 48.24 % for a concentration of 251.73 µmol Trolox 100 g^{-1}. The antioxidant activity of the microencapsulated anthocyanins denotes a low content, perhaps because of not being completely dissolved when making the preparations of the sample to analyze, remaining inside most of the anthocyanins in the microcapsules, since the polymeric matrix is made by components, which create a barrier from covalent bonds with hydrophobic and hydrophilic properties [25], which allow extending the shelf life and preventing volatile losses of the compounds of interest [49].

H. Enrichment of an Instant Powdered Beverage with Anthocyanins from *Macleania Rupestris*

Nowadays, people seek to consume food products made with natural ingredients, and anthocyanins of vegetable origin are an option that has become of great interest, since they promote the reduction of cardiovascular, cancer, diabetic, visual and cognitive improvement diseases, among others. For this reason, it was decided to enrich a powdered beverage, being the active ingredient the anthocyanins of *M. rupestris* (hualicón), which were extracted and microencapsulated.

I. Determination of the Anthocyanin Content of an Instant Drink Powder

The reconstituted beverage obtained a concentration of 50 mg cyanidin-3-glucoside L^{-1}, so each member of a family consisting of 8 members will drink approximately 12.5 mg of anthocyanins per glass, as suggested by Wu et al. [50], since the daily intake of anthocyanins is ~11.6 ± 1.1 mg per day (WWEIA, NHANES 2007-2008, cited by Sebastian et al. [51]).

J. Physicochemical and Microbiological Characterization of Instant Beverage Powder Mixture.

The characterization of a food product, in this specific case of a powdered beverage, made it possible to guarantee quality control of the finished product, so that consumers obtain a safe and innocuous product. Therefore, it is necessary to evaluate certain physicochemical, microbiological and sensory criteria to ensure its stability, in addition to not containing physical contaminants, flavors or foreign aromas [33].

Table II. Results of physicochemical and microbiological analysis of instant powdered beverage established by NTE INEN 2471 [33].

Requirement	Value obtained	Maximum
Moisture (%) m/m	0.94	5.00
pH in reconstituted product	3.73	4.20
Total coliforms (CFU/g)	<10	10
E. coli (CFU/g)	<10	10
Molds (MFU/g)	<10	10
Yeast (YFU/g)	<10	10

The results obtained from the physicochemical analyses do not exceed the maximum limits according to INEN 2471 [33], since the measurements performed comply with the requirements mentioned in Table II. Determining the moisture content is to achieve product stability, which allows better preservation of the product by avoiding the development of microorganisms, which require the presence of water. Similarly, pH is an intrinsic factor of the product, which is a measure of acidity or alkalinity of the product, indicating the concentration of hydrogen ions [52]. In reference to the value obtained, the beverage maintains a stable color between pH 3.5 and 5.5 [53].

The analysis of molds and yeasts was carried out using PetrifilmTM plates, in which a value of <10 MFU g^{-1} and <10 YFU g^{-1} was obtained, evidencing the absence of propagating units, complying with the microbiological quality indicated according to Ecuadorian regulations. In this context, it is important to emphasize the importance of carrying out microbiological analyses to ensure the safety of each process in the food chain, in order to avoid intoxication or infection in consumers.

K. Sensory Analysis

Sensory quality is linked to the acceptance of the product by the consumer, which depends on the individual, time and space. There is a close relationship between the person and the food, which has physical and chemical characteristics that are perceived by the taster [54].

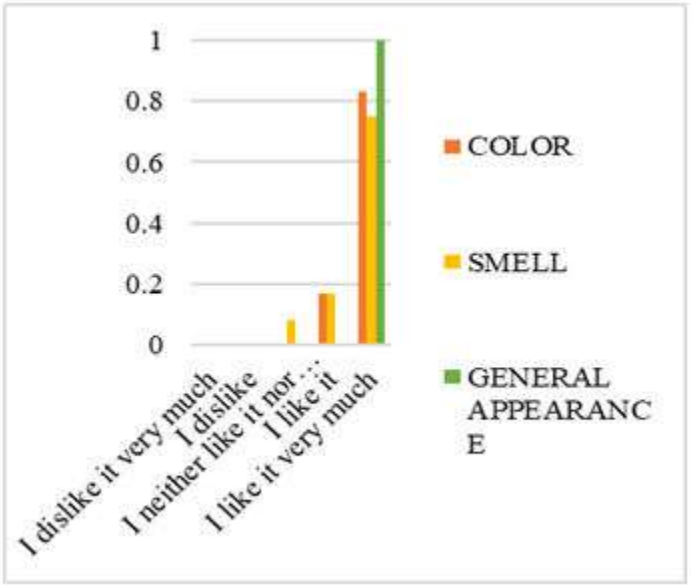

Figure 2: Results of the hedonic scale performed on the powder mixture

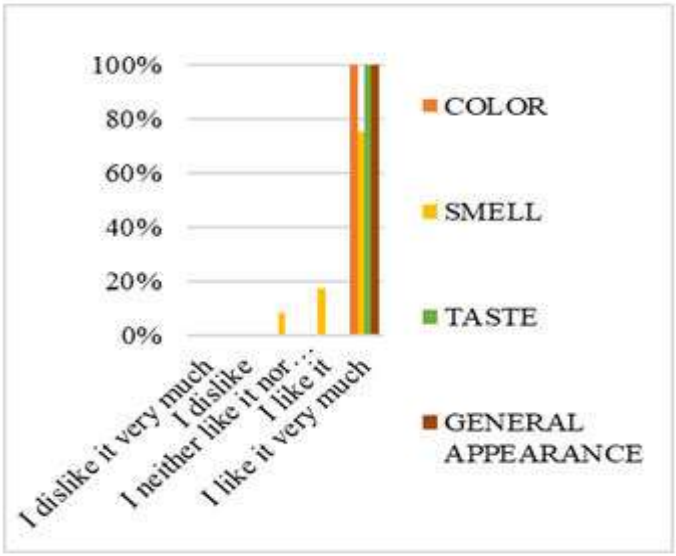

Figure 3: Hedonic scale results for the product reconstituted in water

Figure 2 shows 96% acceptability in the powdered product, while in Figure 3, the appearance, color and flavor resulted in 100%, obtaining a percentage of acceptability of about 97% in the reconstituted product.

These results show that the processed products (powder and reconstituted beverage) not only provide anthocyanins that help the consumer's health, but also because of their sensory characteristics, which make them attractive to the customer's eye.

CONCLUSION

The fruits of *Macelania rupestris* are an important source of antioxidants, especially anthocyanins, thanks to the geographical location and climate in which they grow, becoming a new alternative in the food industry, as it was chosen to develop an instant powdered drink that met the technical requirements of the Ecuadorian Standard and also with a content of 50 mg cyanidin-3-glucoside L^{-1}, daily amount necessary for humans.

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