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Characterization of functional components flour epicarp papaya (*Carica papaya* L) as a source of natural pigments

Caracterización de los componentes funcionales de la harina de epicarpio de papaya (*Carica papaya* L) como fuente de pigmentos naturales

Viviana Andrea Velasco-Arango[1], John Edwin Sotelo-Barbosa[2], Luis Eduardo Ordoñez-Santos[3], José Igor Hleap-Zapata[4*]

[1]*Agroindustrial engineer, vavelasco@unal.edu.co, ORCID: 0000-0003-2514-1267, Universidad Nacional de Colombia - Palmira, Palmira, Colombia.*
[2]*Agroindustrial Engineer, jesotelob@unal.edu.co, ORCID: 0000-0001-5582-4099, Universidad Nacional de Colombia - Palmira, Palmira, Colombia.*
[3]*Doctor of Biological Sciences, leordonezs@unal.edu.co, ORCID: 0000-0002-8958-027X, Universidad Nacional de Colombia - Palmira, Palmira, Colombia.*
[4]**Doctor in Food Engineering, jihleapz@unal.edu.co, ORCID: 0000-0001-9692-5443, Universidad Nacional de Colombia - Palmira, Palmira, Colombia.*

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ABSTRACT

Keywords:

Antioxidant, Peel Papaya, Natural Dyes, Phenolics, Tropical Fruit.

The papaya (*Carica papaya* L) is a fruit rich in antioxidant substances and an important source for obtaining bioactive compounds. Its production, worldwide, for the year 2017, was 13.3 million tons. In its industrial processing is obtained approximately between 15 and 20%, in relation to the weight of the fruit, peels or epicarp, which are susceptible to be used to obtain organic compounds such as carotenoids and polyphenols, among others, also contributing to mitigate the effects on the environment, as generally, these peels are thrown into solid waste dumps, generating serious environmental pollution problems. The objective of this research was to characterize physicochemically the carotenoid pigments obtained from the papaya epicarp. A papaya epicarp flour (HEP) was processed and determined, both in it and in the fresh epicarp, pH, titratable acidity, soluble solids, color parameters according to the CIELab method, water activity, moisture content and dry matter. Carotenoid content, antioxidant activity and phenolic compounds content were also determined. The results showed high values for physicochemical parameters. The content of carotenoid compounds for the fractions of β-carotene, α-carotene, β-criptoxantine, zeaxanthine and lycopene ranged between 8.587 and 4.070 mg/100g of epicarp, with the highest value corresponding to β-criptoxantine and the lowest value the lycopene fraction. The antioxidant activity, expressed as inhibition of the DPPH radical, gave a value of 58.77 ± 3.038 IC50 mg/ml. The content of phenolic compounds measured in mg gallic acid equivalents/g gave a result of 24,948 ± 0,728. The data obtained allow us to conclude that this flour can be used as a source of bioactive compounds and natural pigments both in the food industry and in technical and pharmaceutical industries

RESUMEN

Palabras clave:

Antioxidantes, Cáscara de papaya, Colorantes naturales, Compuestos fenólicos, Frutas tropicales.

La papaya (*Carica papaya* L) es una fruta rica en sustancias antioxidantes y fuente importante para la obtención de compuestos bioactivos. Su producción, a nivel mundial, para el año 2017, fue de 13,3 millones de toneladas. En su procesamiento industrial se obtiene aproximadamente entre el 15 y el 20%, con relación al peso de la fruta, de cáscaras o epicarpio, las cuales son susceptibles de ser aprovechadas con el fin de obtener compuestos orgánicos tales como carotenoides y polifenoles, entre otros, contribuyendo, además, a mitigar los efectos sobre el medio ambiente, ya que generalmente, estas cáscaras son arrojadas a los vertederos de residuos sólidos, generando serios problemas de contaminación ambiental. El objetivo de esta investigación fue caracterizar fisicoquímicamente los pigmentos carotenoides obtenidos del epicarpio de papaya. Se procesó una harina de epicarpio de papaya (HEP) y se determinó, tanto en ella como en el epicarpio en fresco, pH, acidez titulable, sólidos solubles, parámetros de color según el método CIELab, actividad de agua, contenido de humedad y materia seca. Igualmente, se determinó el contenido de carotenoides, la actividad antioxidante y el contenido de compuestos fenólicos.

***Corresponding author.**
E-mail address: jihleapz@unal.edu.co (José Igor Hleap Zapata)

Los resultados mostraron valores altos para los parámetros fisicoquímicos. El contenido de compuestos carotenoides para las fracciones de β-caroteno, α-caroteno, β-criptoxantina, zeaxantina y licopeno osciló entre 8,587 y 4,070 mg/100g de epicarpio, siendo la de mayor valor la correspondiente a β-criptoxantina y la de menor valor la fracción de licopeno. La actividad antioxidante, expresada como inhibición del radical DPPH, arrojó un valor de 58,77 ± 3,038 IC50 mg/ml. El contenido de compuestos fenólicos medido en mg de ácido gálico equivalentes/g dio un resultado de 24,948 ± 0,728. Los datos arrojados permiten concluir que dicha harina puede ser utilizada como fuente de compuestos bioactivos y pigmentos naturales tanto en la industria alimenticia como en industrias técnicas y farmacéuticas.

Introduction

Developments that have reached the health sciences, especially nutrition, show that the catastrophic and increased risk and mortality disease can be lessened by adequate intake of fruits and vegetables because of its excellent nutritional and functional properties, which they are directly linked to the physiological and metabolic processes in humans [1], [2]. estimated, globally, 59% of the total annual deaths are due to noncommunicable diseases such as cardiovascular disease, obesity, diabetes, cancer and respiratory diseases, and it is assumed that the sixth leading cause of this is the low intake Fruits and vegetables.

Papaya (*Carica papaya* L) shows a worldwide economic importance due to its high antioxidant activity and pleasant aroma [3]. This fruit belongs to the family Caricaceae originated in tropical and subtropical regions of America and Africa [4] and 2017 world production of 13.3 million tonnes [2], which has been in production was estimated steady growth in recent years due to its excellent sensory, nutritional and digestive properties [5]. Production in Colombia for the same year was 191,000 tonnes [6], ranking among the top ten producers in the world [7]. In processing the fruit is obtained as waste, a significant amount of shells (exocarp), seeds and fibers; shells represent between 15 and 20% of the total weight of the fruit [8]. The final destination of these shells, in most cases, is available at inappropriate sites, or dumping them in water sources, generating processes of environmental pollution and disease development [9]. In the literature there are no much research related to the industrial use of the epicarp papaya, which is rich in organic compounds such as starch, dietary fiber, proteolytic enzymes, pectin, lycopene, carotenoids, polyphenols, vitamins A, B and C, among others [7], [8], [10].

Of the listed substances, strong interest has carotenoid pigments, which are important source of natural dyes that can be used in the food, pharmaceutical or cosmetological as prevent and combat some degenerative diseases by acting as antioxidants bioactive [11]. Ordoñez and Ledesma [12] determined the concentration of lycopene in some tropical fruits like chonto tomato (*Solanum lycopersicum*), papaya (*Carica papaya*) and guava (*Psidium guajava*) and compared their physicochemical properties and found that this pigment is in significant concentrations and therefore these fruits can be a benefit to counteract degenerative diseases such as cancer and diabetes. To obtain these carotenoids are available various techniques [13], [14], highlighting the annotated in the work done by Finch et al. [15]. The aim of this investigation was to study the extraction of carotenoid pigments and characterization from epicarp papaya, in order to give added value to said residue and thereby help mitigate the negative impact that they generate in the environment.

Materials and methods

Location

The research was conducted at the Laboratory of Technology of Fruits and Vegetables of the Universidad Nacional de Colombia - Palmira, which is located in the city of Palmira, Valle de Cauca, Colombia located at 1001 meters above sea level and an average temperature of 24 ° C.

Obtaining the raw material

Shells papaya were obtained directly from fruits, which were purchased fresh in a supermarket in the city of Palmira, Valle de Cauca, Colombia to a degree of maturity 6 (yellow) optimal to be consumed fresh. With the help of a sharp knife in stainless steel shells they were removed by seeking free epicarpio pulp. The shells obtained were weighed and packed in sealed polyethylene bags for the vacuum using a sealing machine EGARVAC SCP BASIC B. (Vacarisses, Barcelona, Spain) to be preserved under refrigeration (8 ± 1 ° C) for 24 hours prior to performing the respective analyzes and measurements. For measurements, the shells were dried using a process of direct drying in an oven at 60 °C to constant weight (12.5% w/w) and were subsequently ground using an IKA M-20s3, USA mill obtaining a flour in powder form, with an average particle diameter of 0.25 mm, which was weighed in order to establish the yield of the flour in relation to the initial raw material (epicarp).

Physico-chemical parameters

Both the epicarp papaya fresh (EPF) to flour epicarp papaya (HEP), made from the same, the following physicochemical parameters were determined: weight (g), pH, titratable acidity (%) solids soluble (° Brix), color coordinates (L *, a *, b *, C, h), water activity and moisture content (%). Similarly carotenoid content, antioxidant activity and phenolic content for processed flour was determined. All tests were performed in triplicate.

Determination of pH

A portion of the sample was placed for analysis in a beaker and water was added distilled in a ratio of 1:10, with continuous agitation. a sample solution 30 ml, to which was introduced the electrode pH meter (MP 230, Metter Toledo, Switzerland) calibrated with a buffer solution at 10% (w / w) distilled water (Standard took technical Colombian NTC-4592) [16].

Determination of titratable acidity

One gram of the sample was weighed and analyzed was placed in a beaker with 10 ml of distilled water. Continuous stirring a homogeneous solution, to which were added three drops of phenolphthalein and titrated with a solution of sodium hydroxide at 0.1 N until obtaining a pH of 8.1 to 8.2 as obtained Colombian Technical standard 4623 [16]. Acidity percentage was calculated by the following equation:

$$\% Acidez = (ml\ NaOH \times 0{,}1 \times F.Eqvt)/(ml\ muestra) \times 100 \quad (1)$$

Determination of soluble solids

10 g of the sample were taken to analyze and distilled water was added 1:10. The sample was mixed, with continuous agitation in a beaker of 250 ml and then an aliquot of said sample was taken and placed on the lower prism of the refractometer (ATAGO POCKET PAL - 1, Japan). The measurement is expressed in ° Brix according to Colombian Technical Standard NTC - 4624 [16].

Determination of the color

Determining the color in the samples was performed on a colorimeter (CHROMA MICOLTA METTER CR-400, Japan), through which the CIELab parameters International Commission on Illumination evaluated. D65 illuminant and 2 ° observer (equipment calibrated with a white ceramic plate with reference values Y = 89.5, x = 0.3176 and y = 0.3340) were used. Additionally, the pitch (h) was calculated and chromaticity (C) using the following equations:

$$C = (a^{*2} + b^{*2})^{(1/2)} \quad (2)$$

$$h° = \arctan(b^*/a^*) \quad (3)$$

Determination of water activity

Following AOAC 978.19 [17] standard and using the AquaLab, 4te equipment, USA this parameter was determined for each of the samples to be analyzed.

Determination of moisture content

According to the thermogravimetric principle the moisture content was determined in each of the samples. To this 0.5 g sample was weighed and placed at 105 ° C in a moisture analyzer HB43

halogen-S Metter Toledo, Switzerland, in the AOAC 925.09 method. the moisture content of the sample in percent wet weight was obtained.

Determining the carotenoid content

Determining the total carotenoid content in the HEP it was carried out using the protocol described by Ordonez et al. [11]. 0.1 g of sample were placed in a test tube previously covered with foil and were added 7 ml of a mixture ethanol-hexane (4: 3 v / v). The test tube was placed in a heat bath at 16 ° C for one hour with continuous stirring, was subsequently added 1 ml of distilled water and stirring was continued for one hour. A the end of this process, 3 ml of the organic phase was taken and absorbance readings of the extract were performed in a spectrophotometer (Jenway, 6320D, England) versus hexane as white at wavelengths 450, 441, 451 and 472 nm. concentration (mg / g sample) using the extinction coefficients E% in hexane was calculated: 2560, 2800, 2460.

$$C.T. = \frac{A*V*10^3}{E^{1\%}*W} \quad (4)$$

A - Absorbance at specific wavelength (450 nm for β-carotene, 444 nm for α-carotene, β-451 nm for cryptoxanthin, zeaxanthin 451 nm and 472 nm for lycopene,
V - volume of the extract in ml,
W - weight of sample in grams
E1% - extinction coefficient for a 1% solution in hexane.

Determination of antioxidant activity. The antioxidant activity, expressed as percent inhibition of DPPH radical (2,2-diphenyl-1-picrylhydrazilin radical, Sigma-Aldrich Chemical) was performed by the method described by Martinez-Giron et al. [18]. To this, 0.25 g of sample were weighed flour papaya and deposited in an Erlenmeyer lined with aluminum foil to prevent light action. They were added 25 ml of methanol and the mixture was stirred for one minute. Subsequently, the obtained solution was filtered to obtain the extract. The sample was prepared from a ml of the extract and two ml of 0.1 mM DPPH I cooked. Both the sample and control were agitated and allowed to stand for one hour in the dark at room temperature. Finally a Jenway computer 632D Spectrophotometer was used, England for absorbance readings of both the sample and control at a wavelength of 517 nm. The percentage of antioxidant activity (% AA), in terms of inhibition of DPPH radical was calculated using the following equation:

$$\%\,AA = \frac{A_{517\ Control} - A_{517\ Muestra}}{A_{517\ Control}}\ x\ 100 \quad (5)$$

The results obtained, expressed in IC50, which indicates the concentration required to inhibit sample to 50% of the free radical DPPH. The IC50 parameter was obtained from setting the linear equation obtained from the ratio between the concentrations of the sample and the percent inhibition.

Determination of phenolic compounds

It was performed according to the method described by Folin-Ciocalteu Singleton et to the. [19] and Teow et to the. [20]. 0.5 g of HEP were weighed and brought to a 50 ml Erlenmeyer lined with foil. They were added 10 ml of a mixture of ethanol and water in 80:20 (v / v). The mixture was kept under constant stirring for three minutes at room temperature. Then the mixture was subjected to the action of ultrasound, using a Ultrasonic Cleaner-DCPowerFull-Hagavish, Israel, for 20 minutes at 25 ° C. After this process, the sample was filtered, and 0.5 ml of the filtrate was mixed with distilled water and 0.5 ml of the Folin-Ciocalteu reagent. The obtained mixture was allowed to react for three minutes at room temperature and was subsequently added one mL of anhydrous sodium carbonate to 20% and brought to a temperature bath set at 45 ° C for 15 minutes. Finally, the sample was allowed to cool to subsequently perform absorbance readings at a wavelength of 765 nm on a Spectrophotometer Jenway 632D, USA. A calibration curve with gallic acid (pattern) was performed. The results were expressed in mg of gallic acid equivalents per gram of extract HEP.

Statistic analysis

The data obtained were subjected to an analysis of variance (ANOVA), and to compare the differences between means of different parameters was analyzed Tukey test was used with significance level of 5%. All analyzes were performed in triplicate and are reported as mean ± standard deviation.

Results and discussion

In Table I physicochemical results relate to the EPF and the EPF.

Table I. Physicochemical Properties for Fresh Papaya Epicarp (epf) and Flour Epicarpio Papaya (hep)

	Fresh papaya epicarp (EPF)	Flour papaya epicarp (HEP)
pH	5,560 ± 0,074	5.800 ± 0.186
Soluble solids (Brix)	0.700 ± 0,012[a]	5,600 ± 0,115b
Titratable acidity (% citric acid)	0.250 ± 0,004[a]	4,180 ± 0,161b
Water activity (Aw)	0.986 ± 0,050[a]	0.419 ± 0,054b
Moisture content	82.62 ± 1.140	10.84 ± 0.270
Color coordinates		
L *	55.060 ± 0,970[a]	67.310 ± 0,120b
to*	8,360 ± 0,700[a]	7,260 ± 0,050a
b *	33,630 ± 0,920[a]	39,810 ± 0,150a
C	34.654 ± 0,880[a]	40.466 ± 0,150a
H	76.039 ± 0,060[a]	79.664 ± 0,080a

Values with different letters in the same row are significantly different (p <0.5) among themselves. L * 0 = black and 100 = white; a *: -60 +60 = = green and red; b *: -60 and +60 = blue = yellow; Hue angle (h): 90 ° = yellow, 180 ° = green and red = 0 °; Chromaticity (C): distance from the coordinates in the source to point of certain color. Results reported as mean ± standard deviation for n = 3.

During the drying process, in the preparation of HEP, it was a decrease in the weight of 86.88% corresponding to the elimination of moisture content. Regarding pH, this was slightly higher for HEP compared to EPF was observed, although no statistically significant differences between the two values (p <0.05) were observed. These data are consistent with those proposed by Chaiwut et al. [21] who worked with papaya shells to obtain proteases and presented by the Rinaldi et al. [22] who showed physical and chemical characterization of papaya shells. The pH variation not presented both work as noted in the results of the present investigation may be because the decrease in the amount of hydrogen ions,

Regarding the titratable acidity and soluble solids, statistically significant differences (p <0.05) they were noted between the epicarp papaya and flour obtained from it. Similar results for the acidity, obtained Mendy et al. [23] who worked with papaya coated with aloe vera and Ali et al. [24] who developed a job with fresh papaya coated with chitosan stored under refrigeration. Similarly, the work presented by Ordoñez-Santos et al. [11] showed results consistent with those obtained in this research, working with waste papaya. For the soluble solids content (° Brix) Albertini et al. [25] in a study developed with freshly chopped papaya they reported variation in this parameter comparing the pulp with epicarpio.

But nevertheless, data reported by Rinaldi et al. [22] for shells papaya variety Formosa presented higher values possibly due to the variety and state of maturity of the papaya used. The water activity for the two samples showed statistically significant differences (p <0.05). These results may be due to decreased free water contained in the epicarp, which during the drying process is removed almost completely, remaining trapped in the cells only water monolayer cell associated and bound water these. The results of water activity in the HEP were similar to those shown by Serna-Cock et al. [26] who developed a job shell three varieties of mango. [22] for shells papaya variety Formosa presented higher values possibly due to the variety and state of maturity of the papaya used. The water activity for the two samples showed statistically significant differences (p <0.05). These results may be due to decreased free water contained in the epicarp, which during the drying process is removed almost completely, remaining trapped in the cells only water monolayer cell associated and bound water these. The results of water activity in the HEP were similar to those shown by Serna-Cock et al. [26] who developed a job shell three varieties of mango. [22] for shells papaya variety Formosa presented higher values possibly

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Both the water activity as pH and moisture content are critical parameters in alteration processes and food spoilage. Consequently, the data obtained in this investigation for these variables show a very low possibility of deterioration caused by microbial enzymatic reactions or action since, as discussed by Fernandez-Lopez et al. [27], they become slower in foods with low water activity (range 0.11 to 0.40).

The color of a particular agroindustrial raw material is a quality tool for acceptance or rejection of a particular end product, being one of the most important attributes in the appearance of the product [28]. Table I shows the values of the color coordinates for both the EPF as for HEP. The coordinate of lightness (L *) showed the greatest variation with statistically significant difference ($p < 0.05$), which means that the final flour was darker. This possibly due to isomerization processes of some components, mainly carotenoids [29] and to the browning process caused by the high temperatures during the drying process. Corresponding to the coordinates (a * red-green) and (b * yellow-blue) data showed no significant differences comparing the two samples analyzed. Values for skin papaya were similar to those obtained by Santamaria et al. [30] for papaya variety Maradol and presented by Ordoñez-Santos et al. [11] who analyzed a variety of residues tropical fruits, including papaya. The drying process of the epicarp papaya led to an increase of the values of b * coordinate, the pitch (h) and chroma (C), while the coordinate value a * decreased. This can be explained by the characteristics acquired flour during the drying process, where the main components like carbohydrates, are subjected to high temperatures. Values for skin papaya were similar to those obtained by Santamaria et al. [30] for papaya variety Maradol and presented by Ordoñez-Santos et al. [11] who analyzed a variety of residues tropical fruits, including papaya. The drying process of the epicarp papaya led to an increase of the values of b * coordinate, the pitch (h) and chroma (C), while the coordinate value a * decreased. This can be explained by the characteristics acquired flour during the drying process, where the main components like carbohydrates, are subjected to high temperatures. Values for skin papaya were similar to those obtained by Santamaria et al. [30] for papaya variety Maradol and presented by Ordoñez-Santos et al. [11] who analyzed a variety of residues tropical fruits, including papaya. The drying process of the epicarp papaya led to an increase of the values of b * coordinate, the pitch (h) and chroma (C), while the coordinate value a * decreased. This can be explained by the characteristics acquired flour during

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Table II shows the data obtained for the carotenoid content in HEP. The results showed that the major fraction of carotenoids was obtained for β-cryptoxanthin, while the minor fraction was presented for lycopene. The data obtained are consistent with those shown by Ordoñez-Santos et al. [11] (5.88 mg β-carotene / 100g) who worked with papaya residues. On the contrary, they exceed ose shown by Molina et al. [31], who worked with th epicarp passionfruit (*Passiflora edulis*) (3.85 mg β-carotene / 100g) and those obtained by Repo and Encina [32] who developed a job with aguaymanto fruit (*Physalis peruviana*) (2 64 mg β-carotene / 100g). However, the data displayed by Noronha et al. [33] for epicarpios tucumã (*Astrocaryum vulgare*) (average of 10, 88 mg β-carotene / 100g) and (*Bactris gasipaes*) (9.97 mg average β-carotene / 100 g) obtained under various extraction conditions (ratio of ethyl acetate in acetone, and solid-liquid ratio time agitation). The variation between the data of this investigation and those found by other authors with respect to the carotenoid content, may be due to factors pre postharvest handling and, as well as genetic factors own fruit used [34].

The antioxidant activity of HEP showed a value of 58.77 ± 3.038 IC50 mg / ml (mean ± standard deviation of three replicates). The value obtained presented a figure lower than that shown by Eleojo et to the. [35] (IC50 87.08 mg / ml) who worked in an extract from epicarp (Opuntia ficus indica) drying the sun, as well as so provided by FATEN and Rehab [36] also working with dry extract nopal skin (IC50 88.47 mg / ml). Conversely, slightly exceeds the data presented by Calvache et al. [10] who worked with papaya epicarp (IC50 54.86 mg / ml) and presented by Flores et al. [37] who obtained a value of 31.67 ± 4.82 IC50 mg / ml working with shells bioprocessed papaya. Likewise, the work presented by Reyes-Munguia et al. [38] presented antioxidant activity values below this research dried papaya extracts peel measures at different extraction times (5, 8 and 12 minutes respectively 39, 43 and 54 IC50 mg / ml). Regarding the content of phenolics, a value of 24.948 ± 0.728 mg EAG / g was obtained. this value is less than that found by Raja et al. [39] (378 mg EAG / g) who worked with flour papaya leaves. However, it exceeds the data provided by Morais et al. [40] (3.15 mg GSD / g) who worked with shells papaya dried in a conventional oven and obtained by Molina-Quijada et al. [41] who found values between 7.18 and 10.60 for shells of different grape varieties. Data obtained show that a higher or lower concentration of phenolic compounds or degree of hydroxylation, uptake activity radical DPPH varies, and thus the antioxidant activity, which depends primarily on the properties of reduction and oxidation , which can play an important role in absorbing and neutralizing free radicals. Moreover, the variation in the content of phenols in the HEP

Table II. Concentration Carotenoid Flour Epicarpio Papaya (hep)

Sample	HEP Carotenoid (mg / 100g)
β-carotene	5.628 ± 0.1046
α-carotene	5.145 ± 0.0957
β-cryptoxanthin	5,857 ± 0.1046
Zeaxanthin	5,809 ± 0.1080
Lycopene	4.070 ± 0.0757

can be due to genetic characteristics, to the degree of maturity, to weather conditions, temperature, and fertilizer use and storage conditions fruit which was obtained said flour [42]. which can play an important role in absorbing and neutralizing free radicals. Moreover, the variation in the content of phenols in the HEP can be due to genetic characteristics, to the degree of maturity, to weather conditions, temperature, and fertilizer use and storage conditions fruit which was obtained said flour [42]. which can play an important role in absorbing and neutralizing free radicals. Moreover, the variation in the content of phenols in the HEP can be due to genetic characteristics, to the degree of maturity, to weather conditions, temperature, and fertilizer use and storage conditions fruit which was obtained said flour [42].

Conclusions

Flour papaya epicarp shown as an important ingredient for the development of food products with functional character thanks to its high antioxidant activity associated with the content of carotenoids and phenolic compounds, as well as its capacity as a source of natural pigments. The physicochemical properties analyzed show values within the ranges established for product development not only for the food industry, but for the pharmaceutical industry.

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