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Physicochemical, functional, and antioxidant characterization of *Opuntia oligacantha* (xoconostle Ulapa) endocarp flours

Caracterización fisicoquímica, funcional y antioxidante de la harina de endocarpio de *Opuntia oligacantha* (xoconostle Ulapa)

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ABSTRACT

The endocarp of *Opuntia oligacantha*, known as xoconostle Ulapa, is composed of seeds and mucilage, and is considered a residue. This article aims to describe, based on its physicochemical and functional characterization, the endocarp flour of *Opuntia oligacantha* for its applicability in the food industry. The flour was obtained through a dehydration and grinding process, after which its physicochemical, nutritional, technological, and antioxidant capacity properties were determined. The results showed that the flour has an acidic pH (4.1 ± 0.05), humidity (7.51 ± 0.22), and aw (0.31 ± 0.00). L*=53.67 ± 0.71; red, a*=10.00 ± 0.31 and yellow, b*= 14.55 ± 2.27 giving a red-brown color. Low fat amount (6.61 ± 0.04%) and high amount of dietary fiber (76.46 ± 1.25%). High value of water solubility index, activity, and emulsion stability (21.82 ± 2.99%, 65.12 ± 1.03%) and 99.42 ± 0.99% respectively; and a low oil absorption (0.149 ± 0.21 mL/g). This flour has significant antioxidant activity (40.75-53.32%) for FRAP, ABTS, and DPPH tests. *Opuntia oligacantha* endocarp flour could be recommended in foods that require modifying their texture, thanks to its ability to absorb water and fat.

KEY WORDS: Antioxidants, endocarp, flour, *Opuntia oligacantha*.

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RESUMEN

El endocarpio de *Opuntia oligacantha*, conocido como xoconostle Ulapa, se compone de semillas y mucílago, y se considera un residuo. Este artículo tiene como objetivo describir a partir de su caracterización fisicoquímica y funcional la harina del endocarpio de *Opuntia oligacantha* para su aplicabilidad en la industria alimentaria. La harina se obtuvo a través de un proceso de deshidratación y molienda, posteriormente se determinaron sus propiedades fisicoquímicas, nutricionales, tecnológicas y de capacidad antioxidante. Los resultados mostraron que la harina tiene un pH ácido (4.1 ± 0.05), humedad (7.51 ± 0.22) y aw (0.31 ± 0.00) bajas. L*=53.67 ± 0.71; rojo, a*=10.00 ± 0.31y amarillo, b*= 14.55 ± 2.27 que dan un color rojo-marrón. Baja cantidad de grasa (6.61 ± 0.04 %) y alta cantidad de fibra dietética (76.46 ± 1.25 %). Alto valor de índice de solubilidad en agua, actividad y estabilidad de emulsión (21.82 ± 2.99 %, 65.12 ± 1.03 %) y 99.42 ± 0.99 % respectivamente; y una baja absorción de aceite (0.149 ± 0.21 mL/g). Esta harina tiene una actividad antioxidante significativa (40.75-53.32 %) para las pruebas de FRAP, ABTS y DPPH. La harina de endocarpio de *Opuntia oligacantha* podría recomendarse en alimentos que requieran modificar su textura gracias a que es capaz de absorber agua y grasa.

PALABRAS CLAVE: Antioxidantes, endocarpio, harina, *Opuntia oligacantha*.

Introduction

Cacti occupied an economic, social, and religious place in Mesoamerica, being important in the Aztec civilization gastronomy (Sáenz *et al*., 2013). Currently, their production continues to grow and is important to this day; an instance of this is that, in 2014, the Agri-Food and Fisheries Information System (SIAP) recorded a production of 11,048 tons of xoconostle in Mexico (SIAP, 2018). More than 1,500 varieties of cacti have been identified worldwide (Arias-Rico *et al*., 2020), which have stood out for their capacity to develop in arid and semi-arid areas (Kalegowda *et al*., 2017). Among these cacti is the nopal (Opuntia sp.), a family of cactus plants, within which some members produce acidic fruits known as “xoconostles” (Hernández-Fuentes *et al*., 2015), these have been the subject of a limited number of investigations.

The xoconostle is made up of 3 structures (Figure 1). The mesocarp, which is used in the production of sweets, jellies, and drinks, and the epicarp and endocarp (made up of seeds and mucilage), which are commonly removed and considered waste (Morales *et al.,* 2012; 2015).

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Among the studies carried out with the xoconostle is that of Morales *et al.* (2012), where it was determined that the seeds contained in the inner part of the fruit (endocarp) have a greater amount of fiber compared to the fleshy part of the fruit (mesocarp). Continuing with their research, Morales *et al*., (2014) observed the presence of unsaturated fatty acids and antioxidant properties in the endocarp. Later, Kalegowda *et al.* (2017) identified that the mucilage obtained from this fruit exhibits interesting properties, such as the ability to emulsify and bind substances, as well as anti-inflammatory, anti-ulcer properties, and benefits in cholesterol regulation.

In Mexico, there are investigations on native species of xoconostle, such as *Opuntia oligacantha*, known as xoconostle Ulapa, which has a significant socioeconomic relevance (Hernández-Fuentes *et al.,* 2015). Within the studies of this fruit species, researchers have discovered that the endocarp contains phenolic compounds that have the potential to extend the shelf life of products such as yogurt (Cenobio-Galindo *et al.,* 2019), and it reduces the activity of enzymes such as α-amylase and α-amyloglucosidase (Medina-Pérez *et al.,* 2019).

In this context, the purpose of this study was to evaluate the physicochemical, nutritional, technological, and antioxidant properties of the flour derived from the endocarp of *Opuntia oligacantha*, in order to consider its application as an additive in the food industry.

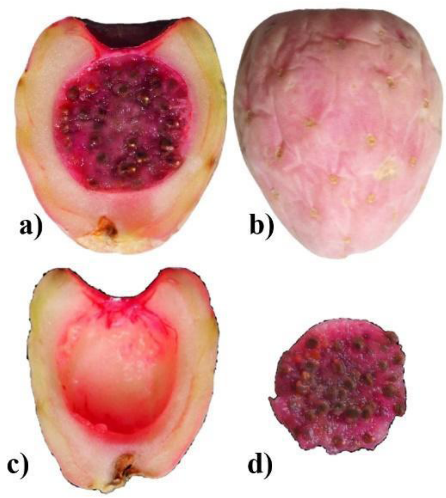

Figure 1. Dissection of the fruit of *Opuntia oligacantha* (xoconostle Ulapa). Anatomy of the complete fruit in longitudinal section (a) and its structures: epicarp, peel (b), mesocarp: pulp (c); and endocarp: seeds and mucilage (d).

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Material and Methods

Obtaining the xoconostles

The xoconostles were collected in the San Agustín Tlaxiaca municipality, Hidalgo, Mexico, during the period from December 2021 to January 2022. This place is located at a of 20°06´52´N, 98°53´12´ W, and an altitude of 2340 meters above sea level. The collected xoconostles were chosen based on their commercial maturity, that is, those with a red color were selected.

Obtaining the flour

In total, 55 kg of xoconostles were collected, which were washed and cut in half lengthwise to separate the seeds with their mucilage from the epicarp and mesocarp. The endocarp samples (seeds with mucilage) underwent a two-stage dehydration process. The first stage was a partial dehydration at room temperature for 24 h, and the second stage was carried out in a drying oven (model 83 L Felisa FE-292D, Mexico) with air at 60 °C for 72 h; subsequently, the dehydrated seed was processed in a cereal mill (model WEG Mod MSL1J, Mexico) and sifted using a 0.6 mm sieve (USA Standard Test Sieve, W.S Tyler). The resulting flour was stored in polyethylene bags at room temperature and protected from light until use.

Physicochemical properties of *Opuntia oligacantha* endocarp flour

Flour yield was determined as described by Maray *et al.* (2018). The weight of the fresh endocarp (raw material) was obtained as well as the weight after drying (dehydrated product) in a drying oven (83 L Felisa FE-292D, Mexico). The percentage yield was calculated using the following equation:

Equation 1.

Yield %=(weight of dehydrated product g/weight of raw material g)x100

Soluble solids

The total soluble solids (°Brix) was determined in the fruit juice with a refractometer (Generic Home 019, Tokyo, Japan). This parameter is associated with the degree of ripeness of the fruit as described by Guzmán-Maldonado *et al*. (2010).

pH

The pH value was determined in a 10 g sample with 100 mL of distilled water using a digital potentiometer (Hanna instruments, HI98107, Mexico) previously calibrated, the determinations were performed in triplicate (Guimarães *et al*., 2020).

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Water activity (aw)

This parameter was determined in triplicate at 25 ± 1.5°C using a water activity meter (Aqualab Series 4TE, Decagon, Pullman, WA, USA), measuring the variation of the water partial pressure (Dick *et al.,* 2020).

Color

Color was measured using the CIELab color scale, using a colorimeter (Minolta CM-508d Tokyo, Japan) setting the illuminant D65 and observation angle of 10°, where L* indicates luminosity (L* = 0 or 100 indicate black or white, respectively), a* is the chromaticity axis between green (-) to red (+), and b* the axis between blue (-) to yellow (+), as described by López-Cervantes *et al*. (2011).

Nutritional composition

The moisture content was determined in a drying oven (83 L Felisa FE-292D, Mexico) with hot air at 105°C until a constant weight was obtained, as established by the AOAC (1975).

Ash determination

The ash content was determined gravimetrically by incineration of the sample at 550°C for 5 h in a muffle furnace (Felisa FE-361, Mexico), as described by the AOAC (1975).

Protein determination

The nitrogen content was determined by the Kjeldahl method (Kjeldahl digester Buchi K-436, Flawil, Switzerland), and the protein content was calculated with the conversion factor 6.25 from this parameter, as described by the AOAC (1975).

Fat determination

Fat content was determined in the sample after moisture extraction. The test was performed by the Soxhlet method (Buchi E816-HE Fat Extraction System, Flawil, Switzerland), using petroleum ether, as described by the AOAC (1975).

Determination of crude fiber

The crude fiber determination was obtained from the defatted sample, digested with sulfuric acid and sodium hydroxide, as described by the AOAC (1975), by the Van Soest method (Labconco LAC300001-00 crude fiber equipment. USA).

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Determination of carbohydrates

The carbohydrate content was calculated by difference. The results of moisture, ash, protein, fat, and crude fiber were added and subtracted at 100 % (Asouzu, *et al.,* 2020).

Equation 2

% Carbohydrates=100% - (%M + %A+ %CP + %EE + %CF)

Where: M is moisture; A, ash; CP, crude protein; EE, ether extract; CF, crude fiber.

Determination of dietary fiber

The content of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) was determined with an enzymatic kit for total dietary fiber analysis (100A-TDF, Switzerland) according to the AOAC (1975).

Technological properties

The water absorption index (WAI) was determined according to the method described by Guimarães *et al*. (2020) and Lopera-Cardona *et al*. (2016). 5 g of the sample was mixed with 30 mL of distilled water in a previously weighed 50 mL Falcon tube. The mixture was gently stirred for 2 min and left to stand for 30 min. The mixture was then centrifuged (Hermle Z326K Centrifuge, Labortechnik Gmbh, Germany) at 3000 g for 30 min, the supernatant was decanted to determine the water soluble solids index (WSI). The WAI was expressed as the weight of the gel obtained after removing the supernatant per g of sample according to Equation 3.

Equation 3.

WAI (g/g) = [(sediment weight g - sample weight g] /sample weight g)

Water soluble solids index (WSI)

The decanted supernatant to obtain WAI was deposited in a previously weighed tray and was evaporated at 105°C in a drying oven (83 L Felisa FE-292D, Mexico) until a constant weight was obtained. The WSI was expressed as an original percentage of the dry solids of the sample weight in g using equation 4 (Lopera-Cardona *et al*., 2016).

Equation 4.

WSI (%) = ([sample weight g - dry sediment weight g]/sample weight g)x 100

Oil absorption index (OAI)

This was determined according to the method of Amaya-Cruz *et al.* (2018) and Guimarães *et al.* (2020) with some modifications. In a previously weighed 50 mL Falcon centrifuge tube, 1 g of sample was mixed with 6 mL of vegetable oil for 60 s. The tubes with the mixture were left to stand for 30 min at room temperature, then they were centrifuged (Hermle Z326K, Labortechnik Gmbh, Germany) at 3000 g for 20 min and the volume of the supernatant was measured. The oil absorption index was expressed as mL of bound oil per gram of sample on a dry basis (equation 5).

Equation 5.

$$OAI\ (mL/g) = ([V1 - V2]/m$$

Where: V1, is the initial volume of oil (mL) that was mixed with the *O. oligacantha* endocarp flour; V2, is the volume of oil (mL) that was not absorbed after centrifugation; and m is the weight of the flour sample (g).

Swelling capacity (SC)

It was determined according to the method described by Dick *et al.* (2020) and Lopera-Cardona *et al*. (2016). 3 g of the sample were added in a 50 mL graduated cylinder. The cylinder was gently tapped on a fixed surface, and the volume was recorded. Subsequently, 30 mL of distilled water was added, and the mixture was left to rest for 60 min. After the time had elapsed, the volume was measured. The swelling capacity was expressed as the multiple of the original volume calculated with the following equation.

Equation 6.

$$SC\ (mL/g) = \text{volume after standing (mL)} / \text{initial volume (mL)}$$

Bulk density (BD)

It was determined according to the method described by Dick *et al.* (2020) and Asouzu *et al*. (2020). A previously treated 10 mL graduated cylinder was filled with flour up to the 10 mL mark and gently tapped on a fixed surface until the fill did not decrease by more than 10 mL. The bulk density was expressed as grams of sample per unit volume using equation 7.

Equation 7.

$$BD\ (g/mL) = \text{sample weight g/volume after tapping (mL)}$$

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Emulsion activity (EA) and emulsion stability (ES)

These parameters were determined by modifying the method described by Kaur & Singh (2005) and Lopera-Cardona *et al.* (2016). In a 20 mL centrifuge tube, a 20% (w/v) aqueous flour suspension was homogenized (IKA-Ultra Turrax T18-S1 digital homogenizer, Wilmington, USA) at 1000 g for 30 s. The speed was increased to 24000 g for 5 min, and simultaneously, 20 mL of vegetable oil was gradually added to obtain the emulsion. The emulsion was left to stand for 30 min at room temperature. It was then centrifuged (Hermle Z326K centrifuge, Labortechnik Gmbh, Germany) at 3500 g for 25 min. The emulsion activity was expressed as a percentage of the height of the emulsified layer relative to the total height, using the following equation.

Equation 8.

EA (%) = ([Height of emulsifying phase (cm) /Total height (cm)] x 100)

The formed emulsion was heated to 85 ± 3°C in a water bath for 15 min. Then, the emulsion was left to stand for 30 min at room temperature; the volume of the emulsifying phase and the total volume were measured. The ES was expressed as a percentage of the emulsifying activity after heating.

Equation 9.

ES (%) = ([A1/A2]x 100)

Where: A1 is the height of the emulsified phase before heating (cm), and A2 is the height of the emulsified phase after heating (cm)

Texture profile analysis (TPA) of *Opuntia oligacantha* endocarp gel

The gel was prepared by modifying the method of Quintero-García *et al*. (2021) with 16, 18, and 20% flour suspensions in distilled water. These were prepared using a thermal shaker (Lab Equipment, model 85-2, USA) at 37.1° for 30 minutes. The samples were then cooled in a cold water bath and poured into 60 x 15 mm Petri dishes and stored at 4 ± 2°C for 24 h. The texture profile analysis (TPA) of *Opuntia oligacantha* endocarp flour gel was measured on a texturometer (Brookfield CT3-4500, Spain) equipped with a TA35 cylindrical probe (diameter 10 mm, height 50 mm), with a 2-cycle compression at 50% of its original height. Petri dishes with 7 mm thick gel samples were placed on the support plate, specifying an activation load of 0.067 N and a constant crosshead speed of 1 mm/s. From the resulting time-force curves, hardness, adhesiveness, cohesiveness, elasticity, and firmness values were analyzed. Three replicates were made for each sample.

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Obtaining the aqueous extract from the endocarp flour of *Opuntia oligacantha*

In 10 mL of distilled water, 1 g of flour was homogenized and centrifuged (Hermle Z326K centrifuge, Labortechnik Gmbh, Germany) at 5000 g for 10 min at 4°C. The supernatant was collected (aqueous extract); from this sample, total phenols and antioxidant activity were quantified in triplicate (Monteiro *et al.,* 2021).

Determination of total phenolic compounds

In 10 mL of water, 1 g of flour was homogenized and centrifuged (Hermle Z326K centrifuge, Labortechnik Gmbh, Germany) at 5000 g for 10 min at 4°C. The supernatant was collected (aqueous extract), and from this sample, total phenols and antioxidant activity were quantified in triplicate (Monteiro *et al.,* 2021).

Total phenolic compounds were determined with the Folin Ciocalteu reagent from Sigma-Aldrich, St. Louis, Missouri, USA, as described by Monteiro *et al.* (2021) with modifications. 1,580 mL of aqueous extract and 100 µL of Folin Ciocalteu were mixed; after 8 minutes, 300 µL of 20% sodium carbonate was added. The samples were placed in a water bath at 50°C for 15 min. The absorbance was determined at 765 nm. The results were calculated using a standard curve ($y=0.0984x+0.057$) and expressed as mg gallic acid equivalents from Sigma-Aldrich, St. Louis, Missouri, USA, per g of extract.

Determination of antioxidant capacity

The antioxidant capacity was determined by spectrophotometric method (UV-1280 Shimadzu UV-vis spectrophotometer, Japan). A 1 mM Trolox standard curve, prepared with 0.00129 g of Trolox in 5 mL of 50% ethanol was used to measure the antioxidant capacity on the DPPH and ABTS+ radicals. The ferric reducing antioxidant power (FRAP) was determined with an iron standard curve, for which a 2 mM iron sulphate stock solution was prepared (0.0028 g in 5 mL of water). Determinations were performed in triplicate.

DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP (Ferric Reduction Antioxidant Power) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

DPPH radical elimination capacity.

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical was prepared at a concentration of 60 µM, in 15 mL of methanol and stored in the dark until use. 0.1 mL of aqueous extract of *O. oligacantha* endocarp flour was mixed with 3.9 mL of the DPPH solution. The capacity of the flour to stabilize the DPPH radical was measured at λ 517 nm. The results were calculated using

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a Trolox standard curve and expressed as mmol equivalents of Trolox/100 g of extract (Brand-Williams *et al.,* 1995; Monteiro *et al.*, 2021).

ABTS radical elimination capacity.+

980 µL of ABTS dissolved in water at a concentration of 7 mM and 20 µL of aqueous extract *of O. oligacantha* endocarp flour were mixed and left to stand for 20 min at room temperature, protected from light. The ABTS (2,2'-azinobis-(3-ethyl benzothiazoline-6-sulfonate ammonium)) radical elimination activity was determined at λ 734 nm. The results were calculated using a Trolox standard curve and expressed as mmol equivalents of Trolox/100 g of extract (Monteiro *et al.,* 2021).

Determination of ferric-reducing antioxidant power (FRAP)

A 30 µL aliquot of *O. oligacantha* endocarp flour extract was taken, plus 970 µL of TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine acid) solution prepared with 25 mL of acetate buffer, 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3$-H_2O solution. FRAP analysis was determined at λ 593 nm. Results were calculated using a ferrous sulfate standard curve and expressed as mmol of Fe(II)/100 g of extract (Benzie & Strain, 1996; Monteiro *et al*., 2021; Thaipong *et al.,* 2006).

Data analysis

Data were analyzed using descriptive statistics, where the characterization parameters *of O. oligacantha* endocarp flour for each test (n=3 or n=10) were expressed as the mean ± standard deviation using Stargraphics Centurion XVI 16.1.03 (32 bits) (2009) Program.

Results and Discussion

Physicochemical properties of *Opuntia oligacantha* endocarp flour

The physicochemical properties of *O. oligacantha* endocarp flour are presented in Table 1. The yield obtained in this work for *O. oligacantha* endocarp flour was 24.24 ± 0.20%, higher than the range of 12-18% reported for *Opuntia matudae* endocarp flour (Guzmán-Maldonado *et al*., 2010). The differences in flour yield may be because *O. oligacantha* was dehydrated with hot air *and O. matudae* with freeze-drying. Morales-Tapia *et al.* (2022) have shown that flour drying by freeze-drying has a lower yield than hot air drying, since in freeze-drying, water sublimation (solid-gas) occurs, causing greater water loss than in hot air drying (Bashir *et al.,* 2020) and finally the amount of water in the flour after drying influences the yield (Guimarães *et al.,* 2020). In addition, as more water is lost in freeze-drying, water-soluble solids may also be lost, which favors low yield (Maray *et al.,* 2018).

The pH of the *O. oligacantha* endocarp flour (4.13 ± 0.05) was lower than that obtained for *Opuntia ficus indica* cactus-fruit (prickly pear) flour (5.58) reported by Batu *et al.* (2018). The acidic pH of the flour prevents the growth of microorganisms; this favors the safety of the products when used as a condiment. The differences in pH are since xoconostles are normally more acidic than prickly pears due to the presence of organic acids, such as malic, citric, and oxalic; however, the place of harvest and the degree of ripeness of the fruit also influence (Ayadi *et al.,* 2009; Fernández-Luqueño *et al.,* 2021).

The Brix degree values are an indication of the degree of ripeness of the fruits. In this sense, the endocarp flour of *O. oligacantha* showed a value of 6.0 ± 0.00 °Brix, which indicates that it was obtained from a ripe fruit. This result is similar to the values of total soluble solids presented by the species of *Opuntia spp.* (xoconostles), which range between 1.10 -1.35 °Brix and 4.28-6.12 °Brix for immature and ripe fruits, respectively (Hernández-Fuentes *et al.,* 2015).

Table 1. Physicochemical properties of *Opuntia oligacantha* endocarp flour

	Opuntia oligacantha	**Other Cacti**
Rendering (%)	24.20 ± 0.20	12-18 ± 1-3[1]
°Brix	6.00 ± 0.00	5.36 ± 0.11[2]
pH	4.13 ± 0.05	5.58[3]
Aw	0.31 ± 0.00	0.37 ± 0.00[4]

[1]*Opuntia matudae* (Guzmán-Maldonado *et al*., 2010), [2]*Opuntia oligacantha* (Hernández-Fuentes *et al*., 2015), [3]*Opuntia ficus indica* (Batu *et al*., 2018), [4]*Opuntia monacantha* (Dick *et al*., 2020). The results are expressed as mean ± standard deviation, except the pH value for *Opuntia ficus indica.*

The endocarp flour of *O. oligacantha* showed an aw of 0.31 ± 0.00, lower than that reported for *Opuntia monacantha* (0.37 ± 0.00) (Dick *et al.,* 2020). Regardless of the species, the differences between the values of our study and those reported for *O. monacantha* may be based on the fact that both botanical sources were dehydrated at different temperature/time conditions (60°C/36 h and 45°C/16 h, respectively) to obtain the flour. The aw is a key parameter for the conservation of flours. Despite the differences, it has been reported that an aw value less than 0.5 decreases the development of pathogenic microorganisms and physicochemical damage in flours, increasing their shelf life and being used as functional additives (Morales-Tapia et al., 2022).

Table 2 shows the color results obtained for *O. oligacantha* endocarp flour, which showed a luminosity (L*= 53.67 ± 0.71) greater than that reported for *Opuntia joconostle* and *O. matudae* flours (L*= 33.5 ± 0.1 and 22.4 ± 0.0, respectively). The red intensity (a*) in *O. oligacantha* endocarp flour (10.00 ± 0.31) was similar to that reported for *O. matudae* flour (a*= 10.4 ± 0.0), but greater than that of *O. joconostle* flour (a*= 5.3 ± 0.0). The yellow intensity (b*) for O. oligacantha endocarp flour was 14.55 ± 1.27; this value was similar to that obtained for *O. joconostle* flour (14.0

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± 0.0) but higher than that of *O. matudae* flour (9.6 ± 0.0) (Arias-Rico *et al.,* 2020). Regarding the value of b*= 14.55 ± 2.27, this was greater than a*= 10.00 ± 0.31, and although both values are positive, they indicate that the yellow color predominates over the red according to the CIELab parameters. It has been reported that the color in *Opuntia* flours is due to the betalain content in the fruits; Specifically, betacyanins confer red-purple colors and betaxanthins yellow-orange colors (Cota-Sánchez *et al*., 2016; Medina-Pérez *et al.* 2020); this indicates that there is a higher content of betaxanthins than betacyanins in the endocarp flour of *O. oligacantha*.

Table 2. Color parameters L, a and b in the endocarp flour of *Opuntia oligacantha.*

Color	OoEF
Luminosity (L*)	53.67 ± 0.71
Red intensity (a*)	10.00 ± 0.31
Yellow intensity (b*)	14.55 ± 2.27

OoEF: *Opuntia oligacantha* endocarp flour. Results are expressed as mean ± standard deviation.

Nutritional composition of *Opuntia oligacantha* endocarp flour

Table 3 shows the nutritional composition values obtained for *O. oligacantha* endocarp flour. The nutritional composition of the flours is very varied, depending on the species (De Andrade *et al.,* 2021), genetic variety (Keyata *et al*., 2020), cultivation location, soil conditions, fruit harvest season (Zepeda *et al*., 2009), particle size, time, and flour drying temperature (Deli *et al.,* 2019).

The endocarp flour of *O. oligacantha* had a moisture content of 7.51 ± 0.22%. Arias-Rico *et al*., (2020) reported moisture values of 7.2 and 8.2% for the flours of *O. joconostle* and *O. matudae*, respectively; meanwhile Dick *et al.* (2020) found that the moisture content for *O. monacantha* flour was 5.13%. The above moisture percentages are below 10%. This ensures the stability of the flour during its storage (Deli *et al.,* 2019), since the reduction of the moisture percentage reduces bacterial growth (Du *et al*., 2019); therefore, if used as an ingredient in food, it can favor shelf life (Guimarães *et al.,* 2020).

On the other hand, the endocarp flour of *O. oligacantha* had an ash content of 2.85 ± 0.12%, protein of 6.29 ± 0.01%, and fat of 6.61 ± 0.04%, which were close to the ash (1.95 ± 0.08%), protein (6.84 ± 0.22%) and fat (7.15 ± 0.23%) contents obtained for an endocarp flour of *O. matudae*. These differences can be attributed to the fact that they are different species collected in different geographic locations, since the fruits of *O. matudae* were obtained from the states of Mexico, Puebla, and Guanajuato, while those of *O. oligacantha* were collected in the Hidalgo state (Guzmán-Maldonado *et al.,* 2010).

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The carbohydrate content in the endocarp flour of *O. oligacantha* was 39.00 ± 1.59%, 57% lower than that reported for an endocarp flour of *O. matudae* (81.77%) (Guzmán-Maldonado *et al.,* 2010). It has been reported that 75% of the endocarp is made up of mucilage (Morales *et al.,* 2014) and that the carbohydrate content depends on the degree of maturity of the fruits, thus immature fruits have a higher mucilage content and, therefore, a higher carbohydrate content necessary for their development (López-Cervantes *et al.,* 2011; Sutton *et al.,* 1981).

Table 3. Nutritional composition of *Opuntia oligacantha* endocarp flour.

Parameter	Result
Humidity (%)	7.51 ± 0.22
Ash (%)	2.85 ± 0.12
Protein (%)	6.29 ± 0.01
Fat (%)	6.61 ± 0.04
Carbohydrates (%)[a]	39.00 ± 1.59
Raw fiber (%)[b]	37.72 ± 1.69

Results are expressed as mean ± standard deviation of three replicates. [a]Carbohydrates = 100% - (%H + %C + %PC + %EE + %FC).

Total dietary fiber is the sum of soluble (gums, pectin, and mucilage) and insoluble (cellulose, hemicellulose, and lignin) fractions. These fractions of dietary fiber may vary according to the season in which the fruit was harvested. In winter (dry season), cacti tend to form more insoluble fiber in their cell wall, which serves as a barrier to prevent water loss (Ventura-Aguilar *et al*., 2017). In this context, we harvested our fruit in December, which is why the endocarp flour of *O. oligacantha* is high in total, insoluble and soluble dietary fiber with values of 76.46 ± 1.25; 73.82 ± 1.19, and 2.64 ± 0.06%, respectively, in comparison with *O. matudae* endocarp flour (1.02 ± 0.04, 0.72 ± 0.10 and 0.30 ± 0.04% respectively) (Guzmán-Maldonado *et al.,* 2010). In general, dietary fiber is important because it promotes beneficial physiological effects such as laxative, anticholesterolemic, antihyperglycemic, and has a prebiotic effect by improving the growth of bacterial flora in the large intestine (Bodner & Sieg, 2009); it also improves the texture and quality of food (Das *et al*., 2020). Similarly, insoluble dietary fiber can prevent fat loss from food thanks to its lipophilic affinity, which is important in palatability (Bodner & Sieg, 2009), since it improves elasticity and softness in food products such as bakery products (Zhu *et al*., 2020). In addition, it affects intestinal regulation and reduces the risk of prostate cancer (Merenkova *et al.,* 2020).

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Technological properties of *Opuntia oligacantha* endocarp flour

The technological properties of *O. oligacantha* endocarp flour are shown in Table 4. *O. oligacantha* endocarp flour had a water absorption index (WAI) of 1.648 ± 0.05 gH2O/g dry matter; this value was lower than that obtained for *O. ficus indica, O. inermis*, and *O. monacantha* cactus (nopal) flour (6.85 ± 0.03, 3.15 ± 0.01, and 7.03 gH2O/g dry matter, respectively) (Ayadi *et al.,* 2009; Dick *et al.,* 2020). These differences in WAI among cacti may be due to the species (Dick *et al.,* 2020) and the soluble fiber content in the flour, since this type of fiber has been described as responsible for water absorption (Asouzu *et al*., 2020); therefore, the low WAI value obtained in this study for the endocarp flour of *O. oligacantha* may be due to the low soluble fiber content (2.64 ± 0.06%), when the other reported species showed values higher than 5.8 ± 0.4% soluble fiber.

Table 4. Technological properties of *Opuntia oligacantha* endocarp flour.

Properties	OoEF
Water absorption Index (WAI) %	1.648 ± 0.06
Water solubility index (WSI) %	21.827 ± 2.99
Oil absorption index (OAI) mL/g	0.149 ± 0.21
Swelling capacity (mL/g)	1.866 ± 0.11
Bulk density (g/mL)	0.688 ± 0.00
Emulsion capacity (%)	65.12 ± 1.03
Emulsion stability (%)	99.42 ± 0.99

Results are expressed as mean ± standard deviation of three replicates. OoEF: *Opuntia oligacantha* endocarp flour.

The water solubility index (WSI) obtained in this work for the endocarp flour of *O. oligacantha* was 21.827 ± 2.99 %; higher than the 5.23 ± 0.02 % described for flour of *O. ficus indica (*Ayadi *et al*., 2009). The high value of WSI in the endocarp flour of *O. oligacantha* is since it has more soluble solids (hydrophilic compounds) such as glucose, fructose, and saccharose than the flour of *O. ficus indica* (Ayadi *et al.,* 2009; Morales *et al.,* 2015).

On the other hand, the endocarp flour of *O. oligacantha* presented an oil absorption index (OAI) value of 0.149 ± 0.21 mL of oil/g dry matter, this value was lower than those reported for flours of *O. ficus indica, O. inermis,* and *O. monacantha* (1.29 ± 0.01, 1.31 ± 0.01, and 1.26 g of oil/g dry matter, respectively) (Arias-Rico *et al.,* 2020; Ayadi *et al*., 2009; *Dick et al.,* 2020). The low OAI content in the endocarp flour of *O. oligacantha* is probably since the endocarp has a low

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content of lipophilic compounds (fatty acids and tocopherols) compared to the peel or the whole fruit (Arias-Rico *et al.,* 2020). A high OAI value in foods is recommended to prevent fat loss during processing and promote palatability, in addition to increasing fat excretion in the intestine (Yang *et al.,* 2021). In this case, *O. oligacantha* endocarp flour, with a low OAI value, can be recommended for the development of healthy low-fat products aimed at the population with low tolerance to fat palatability in foods (Amaya-Cruz *et al.,* 2018).

Other important technological aspects to consider in a flour are its swelling capacity (SC) and bulk density (BD). Regarding SC, the *O. oligacantha* endocarp flour showed a value of 1.866 ± 0.01 mL/g, which was lower than that of the *O. monacantha* flour (17.49 mL/g) (Dick *et al,* 2020). The low SC value in this research is probably due to the low WAI value shown by the *O. oligacantha* endocarp flour since SC is an indicator of WAI, both related to water absorption (Abirami *et al.,* 2014). On the other hand*, O. oligacantha* endocarp flour had a high bulk density (BD) of 0.688 g/mL, similar to that obtained for *Opuntia ficus indica* flour (0.647- 0.703 g/mL) (Ayadi *et al.,* 2009).

The emulsification capacity (EC) and emulsion stability (ES) obtained for *O. oligacantha* endocarp flour were 65.12 ± 1.03 and 99.42 ± 0.99 %, respectively. These values are higher than those observed for *Opuntia dillenii flour* (EC= 55-65 %, ES= 40-50 %) (Kalegowda *et al.,* 2017).

On the other hand, the endocarp flour of *O. oligacantha* has 10 times more protein than *O. dilleniid,* as reported by Medina *et al.* (2007). Protein is indispensable in the emulsifying activity because it acts as a surfactant; that is, it can bind two immiscible liquids (Abirami *et al.,* 2014; Du *et al.,* 2019).

Table 5. Textural Properties of *Opuntia Oligacantha* Endocarp Flour (OoEF)

Suspension (%)	Hardness (g)	Adhesiveness (J)	Cohesiveness	Elasticity Index	Firmness
16	132.00 ± 37.42^{a}	0.0015 ± 0.00^{a}	1.0550 ± 0.04^{c}	1.5100 ± 0.16^{b}	1.0943 ± 0.05^{b}
18	240.17 ± 18.00^{a}	0.0045 ± 0.00^{c}	0.9767 ± 0.01^{b}	1.5067 ± 0.05^{b}	2.2987 ± 0.14^{c}
20	1134.48 ± 101.16^{b}	0.0025 ± 0.00^{b}	0.0400 ± 0.00^{a}	0.1750 ± 0.02^{a}	0.3775 ± 0.07^{a}

The results are expressed as the mean ± standard deviation of 3 replicates. Different superscript letters indicate significant statistical differences between the suspensions.

The TPA results of the OoEF are reported in Table 5. In general, it is observed that the values of hardness, adhesiveness, cohesiveness, elasticity, and firmness increase from 16 to 18%, but decrease to 20% because possibly at this flour concentration, there is no longer an interaction of Van der Waals forces. Quintero-García *et al.* (2021) measured the texture of the mucilage flour of *O. ficus indica* and reported values of hardness, cohesiveness and elasticity lower than those found in this work (11.29 ± 0.04 g, 0.72 ± 0.01 and 0.63 ± 0.01, respectively); while the adhesiveness (3.21 J) was higher than that obtained for *O. oligacantha* flour. Normally, the higher the hardness and firmness, the less sticky the gel is, decreasing the adhesiveness because the force of attraction between the surface of the sample and the surface of the texturometer probe is lower (Talens Oliag, 2017). In addition, Acevedo *et al*. (2013) mention that the texture parameters depend on the composition of the flour and that protein and soluble fiber increase the viscosity (Zarate-Diego *et al*., 2021; Du *et al.,* 2019).

The metabolism of living organisms generates reactive oxygen and nitrogen species that are very unstable and react with the rest of the cellular macromolecules. When the generation of these species exceeds the antioxidant defenses of the organism, a pathological state known as oxidative stress is generated, which leads to the oxidation of lipids, proteins, and even nucleic acids, thus triggering health disorders, such as diabetes mellitus, hypertension, cancer, multiple sclerosis, Alzheimer's, and other neurodegenerative disorders (Apak *et al*., 2016; Dibacto *et al.,* 2021; Lu *et al.,* 2021; Vázquez-Ovando *et al.,* 2022). Antioxidants are molecules capable of stabilizing or eliminating reactive species, either through the donation of an electron, an H atom, or both (Apak *et al.,* 2016). Currently, the consumption of antioxidant-rich food products has been positively linked to a reduction in the risk of developing chronic non-communicable diseases. In addition, the food industry prefers natural antioxidant ingredients from plant foods over those of synthetic origin because the latter have been shown to generate toxicity at high concentrations (Dibacto *et al.,* 2021). The antioxidants present in fruits and vegetables can be mainly categorized into three groups: vitamins, carotenoids, and phenolic compounds, the latter being the most potent in terms of antioxidant activity, as indicated by Thaipong *et al.* (2006). On the other hand, to promote the use of plant residues, the production of flours is a viable option since they can preserve the bioactivity and nutritional properties of the plant in natura (Coimbra *et al.,* 2023).

Table 6 shows the parameters related to the antioxidant capacity of *Opuntia oligacantha* endocarp flour, as well as its phenolic compound content.

There are different techniques to determine the antioxidant capacity of molecules. Some are based on their ability to stabilize radicals such as DPPH and ABTS+; others, such as FRAP, are based on measuring the power to reduce the ferric ion to ferrous (Griffin & Bhagooli, 2004; Vázquez-Ovando *et al.,* 2022). The ABTS test is applied to hydrophilic and lipophilic antioxidant systems, while DPPH applies to hydrophobic or lipophilic systems (Floegel *et al.,* 2011; Thaipong *et al*., 2006; Vázquez-Ovando *et al.,* 2022). In the case of FRAP, this method applies to relatively insoluble food matrices (Vázquez-Ovando *et al.,* 2022); it has been observed that it does not react with the sulfhydryl or thiol group of the amino acids cysteine and methionine responsible for the elimination of free radicals, so FRAP should not be applied to protein-rich antioxidant systems for there may be an alteration in the percentage of FRAP inhibition (Benzie & Devaki, 2018; Çekiç *et*

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al., 2009). On the other hand, Amadi *et al.* (2017) reported the existence of these sulfurous amino acids in cacti such as *O. dillenii*, recommending them as a supplement in malnutrition problems.

Table 6. Total phenolic compounds content and antioxidant capacity of *Opuntia oligacantha* endocarp flour.

Properties	OoEF
Total phenolic compounds	4.93 ± 0.08
DPPH (2,2 Diphenyil picrylhydrazyl)	51.97 ± 0.30
ABTS (2,2'-azinobis- (3-etil benzotiazolin-6 amonium sulfanate)	53.32 ± 2.68
FRAP (Ferric reducing antioxidant power)	40.75 ± 0.63

Values are expressed as the mean ± standard deviation of 3 replicates. Total phenolic compound results are expressed in mg gallic acid equivalents (GAE/100 g dry basis). DPPH and ABTS results are expressed in mmol Trolox equivalents (TE/100 g extract). FRAP results are expressed as mmol Fe(II)/100 g extract. OoEF: *Opuntia oligacantha* endocarp flour.

Phenolic compounds are natural antioxidants that contribute to extending the shelf life of foods, have antibacterial properties due to their ability to affect the permeability of bacterial membranes, and may also help prevent chronic diseases in those who consume these foods (Cenobio-Galindo *et al.* 2019; Yang *et al.* 2018). These compounds are developed in the fruit in defense against insects (Ventura-Aguilar *et al.,* 2017). In contrast, they are associated with antioxidant activity, so it is important to measure them. In this sense, the endocarp flour of *O. oligacantha* has a higher content of phenolic compounds compared to that reported for the endocarp flour of *O. joconostle* and *O. matudae* (0.11 ± 0.01 and 0.05 ± 0.01 mg of gallic acid equivalents (GAE)/g of extract, respectively) (Morales *et al*., 2015). Regarding the antioxidant capacity, the endocarp flour of *O. oligacantha* presented inhibition values of the DPPH and ABTS radicals 4 to 8 times higher than those obtained for endocarp extracts of *O. joconostle* (DPPH = 6.20 ± 0.32 mmol TE/100 g of extract, ABTS = 8.51 ± 0.82 mmol TE/100 g) (Dávila-Hernández *et al.,* 2019); that is, the endocarp of *O. oligacantha* has 4-8 times more antioxidant power than the endocarp of *O. joconostle*, and this effect could be associated with a higher content of phenolic compounds.

Differences in the amount of both total phenols and antioxidant capacity can be attributed to the genotype, species, crop, and even the growth conditions of the xoconostle (Hernández-Fuentes *et al.*, 2015). Furthermore, the presence of these compounds can also vary depending on the part of the fruit being examined (Fernández-Luqueño *et al.,* 2021).

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Conclusions

Opuntia oligacantha endocarp flour has important physicochemical, nutritional, technological, and antioxidant properties for the food sector. The endocarp is mainly considered a residue of the fruit, so it has hardly been studied. The results of this research show that the flour has low aw and moisture values that can increase its shelf life, and it also has an acidic pH that can prevent the growth of microorganisms. Due to the low amount of fat and high amount of dietary fiber, *O. oligacantha* endocarp flour can be used in the preparation of dietary foods. Additionally, *O. oligacantha* endocarp flour has high values of water solubility, activity, and emulsion stability; these properties have the potential to modify the texture of foods due to its hydration properties as well as a low percentage of oil absorption recommended in low-fat foods. Finally, this flour has important antioxidant activity that can improve the shelf life of products and favor consumer health.

Authors' contributions

Conceptualization of the work PMA, ORD, GDFR; development of the methodology GDFR, GTR, PMJ; software management, DLRAJL, RRNE, GDFR, PMA; experimental validation, FME, PMA, ORD, GDFR, analysis of results, PMA, GDFR, PMJ, DLRAJL; Data management, PMA, ORD, GDFR, FME; writing and preparation of the manuscript, PMA, ORD, GDFR, RRNE, DLRAJL; writing, review and editing, PMA, DLRAJL, ORD, GDFR, GTR; project manager GDFR.

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