Biotecnia
Revista de Ciencias Biológicas y de la Salud
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Bioactive compounds identification and physicochemical characterization from *Nopalea cochenillifera* (L.) Salm-Dyck cladodes flour

Identificación de compuestos bioactivos y características fisicoquímicas de la harina de cladodios de *Nopalea cochenillifera* (L.) Salm-Dyck

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ABSTRACT

Nopalea cochenillifera (L.) Salm-Dyck is a scarcely studied cactus; its characterization contributes to identify the bioactive compounds it contains and its functional properties, which will allow the generation of information on potential uses and applications. The aim of this work was to physicochemically characterize *N. cochenillifera* cladodes flour and identify the phenolic compounds it contains. In general, *N. cochenillifera* flour is low in calories (337 kcal/100 g) with high total dietary fiber content (18.41%). In addition, it exhibits good water (11.04%) and oil (2.05%) absorption capacity, while swelling capacity was 25 mL/g DW. The soluble and hydrolyzable polyphenols content were 207.92 and 647.99 mg EAG/100 g DW, respectively. In addition, they showed antioxidant activity by DPPH$^{\cdot}$ (15.28 mmol TE/g DW), FRAP (20.97 mmol TE/g DW), and ABTS$^{\cdot+}$ (51.31 mmol TE/g DW) methods. Furthermore, six phenolic acids (gallic, ferulic, chlorogenic, p-coumaric, syringic, and neochlorogenic) were identified by HPLC. According to the results, *N. cochenillifera* cladodes flour is an important source of fiber and bioactive compounds with interesting functional properties. In this context, *N. cochenillifera* flour could be used as an ingredient in the formulation of functional foods. However, further, studies are needed on the shelf life and optimizing its preservation process, transformation, and functional potential.

Keywords: *Nopalea cochenillifera* (L.) Salm-Dyck, antioxidant capacity, dietary fiber, bioactive compounds, physicochemical characterization.

RESUMEN

Nopalea cochenillifera (L.) Salm-Dyck es un nopal poco estudiado, su caracterización contribuye a identificar los compuestos bioactivos y las propiedades funcionales que posee, esto permitirá, generar información sobre potenciales usos y aplicaciones. El objetivo del trabajo fue caracterizar fisicoquímicamente la harina de cladodios de *N. cochenillifera* e identificar los compuestos bioactivos que contiene. En general, la harina de *N. cochenillifera* tiene bajas calorías (337 kcal/100 g) con alto contenido de fibra dietética (18.41%). Además, exhibe buena capacidad de absorción de agua (11.04%) y aceite (2.05%), mientras que la capacidad de hinchamiento fue de 25 mL/g base seca (bs). El contenido de fenoles solubles y polifenoles hidrolizables fueron 207.92 y 647.99 mg EAG/100 g bs respectivamente. Además, de presentar actividad antioxidante por DPPH$^{\cdot}$ (15.28 mmol TE/g bs), FRAP (20.97 mmol TE/g bs) y ABTS$^{\cdot+}$ (51.31 mmol TE/g bs), se identificaron cinco ácidos fenólicos. De acuerdo con los resultados, la harina de *N. cochenillifera* es una fuente importante de fibra y compuestos bioactivos con propiedades funcionales. En este contexto, podría ser utilizada como ingrediente funcional en la formulación de otros alimentos. Sin embargo, son necesarios futuros estudios sobre la vida útil de la harina, así como la optimización de su proceso de conservación, transformación y potencial funcional.

Palabras clave*: Nopalea cochenillifera* (L.) Salm-Dyck, Capacidad antioxidante, fibra dietética, Compuestos bioactivos, Caracterización fisicoquímica

INTRODUCTION

Currently, there is a trend to consume foods that are low in carbohydrates but high in dietary fiber, as well as those containing functional ingredients. Fiber consumption has been associated with health benefits for modulating the intestinal microbiota. In addition, it regulates blood glucose and lipid (cholesterol and triglycerides) levels, contributing to the control and prevention of non-transmissible chronic diseases such as diabetes, dyslipidemias, and obesity (Holscher, 2017; **Ríos-**Hoyo *et al.*, 2017; Weickert and Pfeiffer, 2018).

In the literature, fresh nopal cactus is described as a vegetable with high water (88–95%) and carbohydrate (3–7

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Recibido: 4 de junio de 2021
Aceptado: 16 de octubre de 2021

g) content, low protein (0.5–1 g), lipid (0.2 g), and calorie (27 kcal/100 g) content (Sánchez-Tapia *et al.*, 2017; Stintzing and Carle, 2005; Inglese *et al.*, 2018). In addition, it contains bioactive compounds such as polyphenols, which have antioxidant activity. It has been reported that these compounds present in nopal decrease metabolic endotoxemia, glucose intolerance, lipogenesis and metabolic inflexibility (Sánchez-Tapia *et al.*, 2017). According to several authors, the nutritional composition and concentration of bioactive compounds in nopal cactus depends on environmental conditions, species, maturity stage, harvesting season, and/or post-harvest treatments (El-Mostafa *et al.*, 2014; Guevara-Figueroa *et al.*, 2010; Nuñez-López *et al.*, 2013). In Mexico, nopal cactus is consumed fresh (in salads) and the nopal flour is used with other ingredients in a variety of traditional culinary dishes including juices, snacks, and tortillas (Rodríguez-García *et al.*, 2007; Saenz, 2000). Furthermore, it has been used in traditional medicine due to its anti-inflammatory and antimicrobial properties, as well as hypoglycemic activity attributed to the presence of bioactive compounds and dietary fiber (Díaz *et al.*, 2017; NECCHI *et al.*, 2012; Young *et al.*, 2005). Furthermore, over 125 nopal species have been identified in Mexico, where the most commercialized variety is *Opuntia*, while *N. cochenillifera* (L. Salm-Dyck) is one of the least exploited (Inglese *et al.*, 2018).

Native of Mexico, *N. cochenillifera* is endemically distributed across Central America. It can reach a height of 3–6 m and is widely adapted to extreme weather conditions, although its maturation takes longer in cold climates (Scheinvar, 2004). It is considered a wild species, mostly grown in gardens and backyards and used in landscaping, as forage, and/or hedgerows (Lim, 2012). Cladodes are edible, narrow (4–7 cm), and long (20–30 cm); they show a few spines on the bark as compared to other species (Beccaro *et al.*, 2015; Scheinvar, 2004). *N. cochenillifera*, grown all year round and commonly sold locally, and despite having commercial potential, it is undervalued since it is unknown in other markets, especially those far from the cultivation areas (Reyes-Agüero and Aguirre-Rivera, 2016). There is scarce information on the physicochemical and nutritional characterization of the species as well as that of its bioactive compounds and their potential uses. In addition, it has been reported that nopal flours have been used as a food supplement in the production of doughs and bakery products, in order to increase their functional properties (Ayadi *et al.*, 2009; Nabil *et al.*, 2020). Therefore, the objective of this work was to physicochemically characterize *N. cochenillifera* cladodes flour and identify the phenolic compounds that it contains.

MATERIALS AND METHODS

Raw material

The cladodes from *N. cochenillifera* were manually and randomly harvested in the Valley region of Tulancingo, Hidalgo, Mexico. The cladodes were used at commercial maturity, considering the following morphometric variables: 25 cm length, 6 cm width, and 1 cm thickness.

Sample preparation

Three batch cladodes with spines were selected according to the provisions in the Codex Alimentarius (Codex, 2017). First, spines were removed manually, and cladodes were washed with distilled water and disinfected with a NaClO solution (4.5 mg/L). Next, cladodes were cut into strips (10 cm long, 1 cm wide), then dried at 45 ºC for 48 h in a convection oven (3489M-1, Barnstead International, Dubuque, Iowa, US), ground in a mill (UDY Cyclone Sample Mill), and sieved through a 100-mesh screen (US). Finally, powder samples stored in airtight polyethylene bags at room temperature and darkness until analysis.

Proximal analysis

Moisture (method 934.06), total soluble solids (method 932.12), protein (method 978.04), ashes (method 940.26), and lipids (method 950.54) were quantified using the official methods in AOAC (2012). Total carbohydrates were calculated by difference (equation 1), while energetic value was estimated using the equation (2) proposed by Nabil *et al.* (2020).

$$\text{Total carbohydrates (g/100 g)} = 100 - (\text{lipids} + \text{ash} + \text{proteins}) \quad (1)$$

$$\text{Energy (Kcal/100 g)} = 4^{*} (\text{proteins} + \text{carbohydrates}) + 9^{*} (\text{lipids}) \quad (2)$$

Dietary fiber

Dietary fiber (DF) was assessed by the enzymatic-gravimetric method with modifications by Mañas and Saura-Calixto (1995). First, samples (0.5 g) mixed with 25 mL of phosphate buffer (0.08 M, pH 6) was heated at 100 °C for 5 min, and then treated with 25 µL of thermostable α-amylase (A-3306, Sigma Chemical Co., St Louis MO, US) at 100 °C/35 min, after that, samples were cooled at ambient temperature. Furthermore, the pH of the solution was adjusted at 7.5 with NaOH (1N); next, 50 µL of protease (Sigma Chemical Co., St Louis MO, US Sigma, P-5380 at 50 mg/mL of phosphate buffer) were added, and samples incubated in a water bath at 60 °C/35 min, and cooled at ambient temperature. The pH was adjusted at 4.5 with HCl (1N), and 150 µL of β-amyloglucosidase (Sigma Chemical Co., St Louis MO, US Sigma, A-9913) were added, and the solution was heated again at 60 °C/35 min. Finally, the solution was centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 3000 *x g*, 25 ºC, for 15 min, to separate the soluble and insoluble fractions. The supernatants collected from the centrifugation were dialyzed (D-9652, 33 mm, 12400 Da, Sigma Aldrich) against deionized water for 24 h. The supernatants were dialyzed against water to prevent the loss of soluble dietary fiber (SDF). The dialysates containing SDF (17 mL) were acid hydrolyzed (12 M H_2SO_4) and incubated in water bath at 100 °C for 90 min. The SDF content was calculated using the method proposed by Englyst and Cummings (1988), using 3,5 dinitrosalicylic acid and glucose as standard at 530 nm. On the other hand, residues (insoluble dietary fiber, IDF) previously oven-dried were subject to acid hydrolysis (3 mL of 12 M H_2SO_4 in a water bath at 37 °C for 1

h), followed by the addition of 33 mL of distilled water and kept in a water bath (100 °C for 90 min). Finally, the solution was centrifuged (6000 *x g*, 25 ºC, 15 min) and supernatant was recovered. The IDF content was quantified using DNS reagent at 530 nm, as previously described. The remaining residues of IDF were quantified as Klason lignin (KL) by gravimetric method. IDF was calculated as NSP plus KL, while the total dietary fiber content was the sum of SDF and IDF.

pH

The pH was assessed with a digital potentiometer (HI 2211 PH/MV, HANNA) calibrated before the measurement with reference buffer solutions (pH 4.0 and 7.0), according to AOAC (2012), based on the immersion of an electrode in the solution. The sample (1 g) was mixed in 10 mL distilled water for analysis, and the reading was recorded.

Color attributes

The color was quantified using a colorimeter (CR-410, Konica Minolta, Sensing Inc., Osaka, Japan) previously calibrated. Results were expressed according to the rectangular coordinate system L*a*b*.

Apparent density

The apparent density was determined using the method reported by Kaur and Singh (2005). Density was calculated in a graduated cylinder previously tared with 10 mL of the sample, and the sample weight was calculated as grams per volume unit (g/mL).

Water solubility index and water absorption capacity

The water solubility index (WSI) and the water absorption capacity (WAC) analysis were performed following the methodology proposed by Anderson *et al.* (1969), with minor modifications. The sample (2.5 g) was homogenized with 30 mL distilled water, kept at 30 ºC in a water bath for 30 min, and then centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 3000 *x g*/30 min. The supernatant was decanted and oven-dried (3489M-1, Barnstead International, Dubuque, Iowa, US) at 90 ºC for 24 h. Additionally, the pellet weight was recorded, and WSI and WAC were calculated with equation 3 and 4, respectively. The results of WSI are expressed in percentage, and WAC as g of retained water per g dry weight.

$$WSI\ (\%) = \frac{\text{Weight of dry solids in supernatant}}{\text{Weight of dry sample}} * 100 \quad (3)$$

And

$$WAC = \frac{\text{Residue weight, which contained the water (g)}}{\text{Original weight of cladodes flour (g)}} \quad (4)$$

Oil absorption capacity

Oil absorption capacity (OAC) was assessed following the methodology by Anderson *et al.* (1969) with minor modifications. The sample (2.5 g) was mixed with 30 mL common corn oil in a 50-mL conical tube, shaken in a vortex for 1 min and then centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 3000 *x g*/30 min, and the supernatant was decanted. The pellet weight was recorded, the OAC was calculated using the equation (5), and results expressed as g of oil retained per g of sample.

$$OAC = \frac{\text{residue weight, which contained the oil (g)}}{\text{Original weight of cladodes flour (g)}} \quad (5)$$

Swelling capacity

The swelling capacity (SC) was determined using the method described by Robertson *et al.* (2000). The sample (100 mg) was hydrated with 10 mL distilled water in a calibrated cylinder (1.5 cm diameter) at room temperature. Then, after equilibration (18 h), the volume occupied by sample was recorded and expressed as volume per g dry weight.

Total soluble polyphenols, hydrolyzable polyphenols, and condensed tannins quantification

According to the methodology by Pérez-Jiménez *et al.* (2008), an organic-aqueous extraction was performed to quantify the different compounds. The sample (0.5 g) was mixed with 20 mL methanol-acidified water solution (0.8 % HCl 72.8 g/L). The mixture was stirred at room temperature at a moderate speed (10 × ***g*** for 1 h) in a shaker (Heidolph Rex 2, Heidoplh Instruments, Schuwabach, Germany) and then centrifuged at 8000 × *g* for 10 min at 4 °C. Next, the recovered supernatants and the residues were mix with acetone-water solution (20 mL, 80:20 v/v), stirred for 1 h at room temperature and centrifuged under described conditions. Both supernatants were combined to measure soluble phenols (at 750 nm) by the Montreau (1972) procedure, using the Folin-Ciocalteu reagent, and the results expressed as milligrams of gallic acid equivalent per 100 g dry weight (mg EAG/100 g DW), with a standard curve of gallic acid. The residues of the extraction were used to quantify non-extractable polyphenols (NEP) made up of hydrolyzable polyphenols (HP) and condensed tannins (CT). The determination of HP was according to the method proposed by Hartzfeld *et al.* (2002). The residues obtained from the organic-aqueous extraction were hydrolyzed with 20 mL methanol/H_2SO_4, 90:10 (v/v) at 85 ºC for 20 h, and then centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 3000 *x g* at 25 ºC for 15 min, to assess HP in the supernatants using the Folin-Ciocalteu reagent as previously described. The CT were analyzed in the extraction residues, which were hydrolyzed with 10 mL butanol-HCl-$FeCl_3$ (100 ºC, 3 h) and centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 8000 *x g*, for 10 min, to recover supernatants, and absorbance was measure at 555 nm, according to Reed *et al.* (1982). CT were calculated using a standard curve of proanthocyanidins from Mediterranean carob pods (*Ceratonia siliqua* L.), and the CT content expressed as mg/g DW. The quantification of total phenolic compounds was determined with the sum of soluble polyphenols (TSP) and hydrolysable phenols. A multi-mode microplate reader (Synergy HT, Biotek Instruments Inc., Winooski, Vermont, US) was used to quantify soluble and hydrolysable phenols.

Antioxidant activity

The antioxidant capacity of the organic aqueous extracts was quantifying 2,2'-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical cation scavenging activity, analyzed using a modified version of the methodology by Re *et al.* (1999). The radical cation was prepared by mixing 19.3 mg of $ABTS^{\cdot+}$ (7 mM) in 5 mL of potassium persulfate (2.45 mM) and stored (12 - 16 h) in the dark at room temperature under magnetic stirring. The $ABTS^{\cdot+}$ solution was adjusted with phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.7 (± 0.02) at 734 nm. The Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid) was used as a standard and methanol as a blank. Sample (30 ***µL***) was mixed with 255 µL $ABTS^{\cdot+}$ (30 °C for 7 min). The reduction in absorbance was measured at 734 nm. The scavenging of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radicals was performed according to Prior *et al.* (2005) with some modifications. The Trolox sample or standard (30 ***µL***) reacted with 200 µL $DPPH^{\cdot}$ solution (190 µM, using methanol as dissolvent) while absorbance was measured at 517 nm after 10 min. The ferric reducing antioxidant power assay (FRAP) was carried out following the methods by Benzie and Strain (1996), with modifications. The FRAP solution, 10:1:1 (v/v/v) of sodium acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ (2,4,6-tripiridyls-triazine), and 20 mM iron chloride hexahydrate, was tempered to 37 °C before mixing with the samples. The extract of the sample (24 µL) or Trolox standard was added to a 96-well microplate, then mixed with 180 µL FRAP solution using a multichannel dispenser, and the absorbance measured at 595 nm after 30 min. A multi-mode microplate reader (Synergy HT, Bio-Tek, Instrument Inc., Winooski, Vermont, US) was used to quantify the antioxidant activity of described methods. All the methods of antioxidant capacity and results were expressed as mmol Trolox equivalent per g dry weight (mmol TE/g DW).

Carotenoid content

The sample carotenoids were determined according to Ortega *et al.* (2013). Two g of sample were mixed with 7 mL of acetone:petroleum ether 80:20 (v/v) and 0.5 g of $MgCO_3$, then homogenized for 1 min before centrifuged using a high-speed centrifuge (BKC-TH21RL, Biobase, Shandong China) at 15000 rpm for 30 minutes at 4 °C. The supernatant was placed in a separator funnel adding 15 mL of NaCl (20%), the aqueous phase drained, and the organic layer diluted to 10 mL with petroleum ether. Absorbance was measured at 448 nm using a UV/Vis spectrophotometer (Jenway model 6705). The quantification was performed using a ***β-carotene*** calibration curve, and the results expressed as mg β-carotene per 100 g dry weight.

Quantification of phenolic acids by HPLC

The identification of phenolic acids was performed in an HPLC system (1260 Infinity, Agilent Technologies, Waldbron, Germany) equipped with photodiode-array detection and a C18 reversed phase column (particle size 5 µm, diameter 4.6 mm, and length 250 mm, Thermo Scientific®, Sunnyvale, California, US). The mobile phases were 2% acidified water with acetic acid (eluent A) and acidified water (0.5% acetic acid): methanol 10:90 (eluent B). The standards and extracts of the sample were analyzed using a 0% B gradient; 0–35 min, 35% B; 35–55 min, 75% B; 55–60 min, 100% B; and 60–70 min, 0% B, at a flow rate of 0.4 mL/min. The peak areas were detected at 280 and 320 nm for the sample and calibration curve (0.5–300 µg/mL) of gallic, ferulic, chlorogenic, p-coumaric, syringic, and neochlorogenic acids (Sigma-Aldrich, Inc., St. Louis, Missouri, US), were used to identify and quantify the polyphenolic compounds (Jiménez *et al.*, 2014).

Statistical analysis

All results were expressed as mean values ± standard deviation. Data were analyzed using Sigma-Stat version 11. All the analytical measurements were done in triplicate on a same batch of nopal flour, nine determinations were made.

RESULTS AND DISCUSSION

Proximal analysis

Table 1 shows the nutritional composition of the *N. cochenillifera* flour. The moisture content (4.6%) is adequate for storage, which reduces the enzymatic reactions and/or presence of microorganisms (Rodríguez-García *et al.*, 2007). Moreover, the lipid (1.20%) and protein (6.44%) contents were lower than those reported in *Opuntia ficus-indica* flour (lipid 2.3% and protein 8.76%), while the total carbohydrates (75.16%) and ashes (17.2%) in *N. cochenillifera* were higher than those reported (Nabil *et al.*, 2020) in *Opuntia ficus-indica* (74.27% carbohydrates and 11.90% ashes respectively). Additionally, the caloric contribution of cladodes flour was of 337 kcal/100 g, while higher values has been reported for commercial flours from corn and wheat (397.68 kcal/100 g and 384.2 kcal/100 g, respectively) (Kavitha and Parimalavalli, 2014).

The consumption of dietary fiber associates to beneficial effects on consumers' health (Holscher, 2017).

Table 1. Nutritional composition of *N. cochenillifera* cladodes flour.
Tabla 1. Composición nutricional de la harina de cladodios de *N. cochenillifera*.

Parameter	Cladodes flour
Moisture (g/100 g dry weight)	4.6 ± 0.06
Protein (g/100 g dry weight)	6.44 ± 0.82
Lipid (g/100 g dry weight)	1.20 ± 0.04
Carbohydrate (g/100 g dry weight)	75.16 ± 0.97
Ash (g/100 g dry weight)	17.2 ± 0.11
Energy (Kcal/100 g dry weight)	337.2 ± 1.83
Total dietary fiber (g/100 g dry weight)	18.41 ± 0.05
Insoluble dietary fiber (g/100 g dry weight)	16.92 ± 0.19
Soluble dietary fiber (g/100 g dry weight)	1.49 ± 0.21

All values are means ± standard deviation of three determinations per batch.

The total dietary fiber content in *N. cochenillifera* flour was 18.41% (16.92% insoluble and 1.49% soluble), similar to that reported by Stintzing and Carle (2005), who found the average content of fiber in dehydrated *Opuntia* was 18%. Additionally, reports show that the amount of soluble fiber decreases as cladode ages, while insoluble fiber increases (Nabil *et al.*, 2020; Rodríguez-García *et al.*, 2007). The low soluble fiber content in *N. cochenillifera* may associate to the drying temperature (45 ºC) during cladodes flour processing, which is the same as that of the glass transition of mucilage, promoting soft-flexible to rigid state and solubility changes, leading to possible hydrolysis (Ventura-Aguilar *et al.*, 2017). According to the World Health Organization, the recommended fiber dietary intake (RDI) is an average of 25 g/day for both men and women; therefore, adding 15 g of *N. cochenillifera* flour to food products could contribute to at least 10% of the RDI. Subsequently, *N. cochenillifera* flour is a potential source of dietary fiber and its consumption could prevent and control non-transmissible chronic diseases (Bchir *et al.*, 2014). Furthermore, previous reports show that both soluble and insoluble nopal fiber has therapeutic effect on irritable bowel syndrome when nopal when consumed at a 20 g/day dose (Remes-Troche *et al.*, 2021). In this context, it is feasible the use *N. cochenillifera* flour as a functional ingredient in products with low caloric and high dietary fiber contents.

Physicochemical properties

Table 2 shows pH, total soluble solids, and color attributes of cladodes flour that showed a pH of 5.1, consistent with pH reported by Sáenz and Berger (2006), who found pH values from 4.0 to 7.0 in *Opuntia* flours. The total soluble solids in the flour were 6.9 ºBrix, possibly associated with *N. cochenillifera* soluble sugar (glucose) content (Nabil *et al.*, 2020). On the other hand, the color parameters if luminosity (*L), reddening (a), and yellowing (b) of cladodes flour were 67.62, −3.47, and 18.2, respectively, indicating that the flour exhibited a yellow, pale green color, similar to that reported in *O. ficus-indica* powder (Sáenz *et al.*, 2010). The *N. cochenillifera* cladodes flour apparent density was 0.6 g/mL, similar to the reports on spineless *O. ficus indica f. inermis* cladodes powders (Ayadi *et al.*, 2009). This parameter relates to dietary fiber content; insoluble fiber content (mostly insoluble cellulose and hemicellulose) or soluble fiber fraction (mainly mucilages and pectins), which could modify powder density (Nuñez-López *et al.*, 2013).

Functional properties

The WSI relates to the presence of soluble molecules in the flour. The cladodes flour WSI was 5.4; powdered spineless cladodes have shown a higher WSI as compared against spine-covered cladodes (Ayadi *et al.*, 2009). The WAC is a property linked to water retention within a matrix, which largely depends on the physicochemical nature of the flour fiber fraction; it directly affects the technological and functional properties of the flour. The cladodes flour WAC value was 11.4 g of water/g DW, which relates to the water retention capacity due to hydrophilic components in its matrix. *N. cochenillifera* showed a higher WAC value (11.4 g of water/g DW) than that reported in *Opuntia* (8 g of water/g DW) (Ayadi *et al.*, 2009; Nuñez-López *et al.*, 2013). Furthermore, its WAC was higher than those reported for flours made from apples, pears, and carrots; which showed WAC ranging from 1 to 8 g of water/g DW (Ahmad *et al.*, 2016).

Table 2. Physicochemical parameters and functional properties of *N. cochenillifera* cladodes flour.
Tabla 2. Parámetros fisicoquímicos y propiedades funcionales de la harina de cladodios de *N. cochenillifera*.

Parameter	Cladodes flour
pH value	5.1 ± 0.06
Total soluble solids (°Bx)	6.9 ± 0.02
L*	67.62 ± 0.01
a*	-3.47 ± 0.02
b*	18.20 ± 0.01
Bulk density (g/ml)	0.6 ± 0.0
Water solubility index (%)	5.4 ± 0.85
Water absorption capacity (g water/g dry weight)	11.4 ± 0.03
Oil absorption capacity (g of oil/g dry weight)	2.05 ± 0.05
Swelling capacity (mL/g dry weight)	25.0 ± 0.02

All values are means ± standard deviation of three determinations per batch.

On the other hand, the OAC is a relevant parameter to evaluate the hydrophobic nature of the particles that constitute the fiber fraction in the flour (Ventura-Aguilar *et al.*, 2017). *N. cochenillifera* powder showed an OAC of 2.05 g of oil/g DW, comparable to the same OAC value in flours obtained from vegetable sources such as apple and pear (Bchir *et al.*, 2014).

In addition, the SC relates to the dietary fiber content since carbohydrates interact with free polar groups through hydrophilic bonds, retaining them in a matrix. Cladodes flour showed an SC of 25 mL/g DW, which suggests that its components can absorb water due to its capacity to form matrices similar to those of a gel. In addition, these properties promote a low diffusion and a higher absorption of lipid compounds or sugars (Nuñez-López *et al.*, 2013).

These characteristics directly affect the cladodes flour technological and functional properties. They could induce beneficial effects in health by significantly increase chyme viscosity in a dose- or amount-dependent manner. This fact leads to a beneficial regularity by altering fecal viscosity since this property determines the speed at which a substrate transits through the large intestine (McRorie Jr and McKeown, 2017).

Biocompound contents and antioxidant capacity

Table 3 shows the soluble and hydrolyzable polyphenols, tannins, carotenes, and antioxidant activity. The *N. cochenillifera* flour total phenol content was 207.95 mg GAE/100 g DW, slightly higher than the 180 mg GAE/100 g

Table 3. Bioactive compounds content and antioxidant activity of *N. cochenillifera* cladodes flour
Tabla 3. Contenido de compuestos bioactivos y actividad antioxidante de la harina de cladodios de *N. cochenillifera.*

Parameter	Cladodes flour
Total soluble phenols (mg GAE/100 g DW)	207.92 ± 0.74
Hydrolysable polyphenols (mg GAE/100 g DW)	647.99 ± 3.18
Condensed tannins (mg/g DW)	3.55 ± 0.4
Carotenes (mg β-carotene/100 g DW)	4.36 ± 0.2
Antioxidant activity	
$DPPH^{\cdot}$ (mmol TE/g DW)	15.28 ± 0.4
FRAP (mmol TE/g DW)	20.97 ± 0.5
$ABTS^{\cdot+}$ (mmol TE/g DW)	51.31 ± 4.71

All values are means ± standard deviation of three determinations per batch. TE = Trolox equivalent, FRAP = Ferric-reducing antioxidant power assay, $DPPH^{\cdot}$ = 2,2- diphenyl-1-picrylhydrazyl assay, $ABTS^{\cdot+}$ = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay, DW = Dry weight, GAE = Gallic acid equivalent.

DW reported by Gallegos-Infante *et al.* (2009) in *O. ficus-indica* cladodes flour. The difference between these values may relate to the drying process, since previous work showed that different airflow rates and temperatures, modify total phenols (Medina-Torres *et al.*, 2011). The content of hydrolyzable polyphenols in *N. cochenillifera* flour was 647.99 mg GAE/100 g DW. The hydrolyzable forms include a larger proportion of the polyphenols present in *N. cochenillifera* flour than the soluble polyphenols, as previously shown.

On the other hand, tannins are high-molecular-weight polyphenols beneficial to health. Their monomer structure classifies them into two groups: hydrolysable tannins formed by phenolic acids and condensed tannins (Chung *et al.*, 1998). The latter showed values of 3.55 mg/g DW in *N. cochenillifera*, which depends on the maturity of the cladodes (Figueroa-Pérez *et al.*, 2018). Another activity evaluated in Carotenes, is their antioxidant activity, since they are very efficient physical quenchers of singlet oxygen and scavengers of other reactive oxygen species (Fiedor and Burda, 2014). The *N. cochenillifera* cladodes flour carotene content (4.36 mg β-carotene/100 g DW) was higher than that reported for Opuntia cladodes (3.79 mg/100 g DW) subjected to heat treatments. There are reports showing that the extraction of nopal carotenes depends on the presence of mucilage, since these compounds can form complexes with pectin, thus the use of high temperatures for their extraction are necessary (Sáenz *et al.*, 2010, Saenz, 2006; Jaramillo-Flores *et al.*, 2003).

The *N. cochenillifera* cladodes flour antioxidant activity, assessed with $DPPH^{\cdot}$, FRAP, and $ABTS^{\cdot+}$, was 15.28, 20.97, and 51.31 mmol TE/g DW, respectively. The highest antioxidant activity value may relate to the *N. cochenillifera* cladodes flour high polyphenols content. In contrast, the low tannins and carotenes content may influence the lowest value, reported previously, showing that polyphenols significantly correlate with $ABTS^{\cdot+}$ assays, while tannins with $DPPH^{\cdot}$ assays (Figueroa-Pérez *et al.*, 2018). Additionally, the antioxidant capacity shown with FRAP could be mainly due to the tannins and carotenes content, since these compounds are very efficient at controlling singlet oxygen, and phenolic compounds can sequester free radicals and electron transfer ($ABTS^{\cdot+}$) better (Koracevic *et al.*, 2001; Pertuzatti *et al.*, 2014).

Polyphenol profile

The *N. cochenillifera* cladodes flour phenolic compounds (gallic, ferulic, chlorogenic, p-coumaric, syringic, and neochlorogenic) content detected by HPLC, are shown in Table 4 and Figure 1. According to soluble polyphenols chromatographic profile, multiple unidentified peaks are shown, which could correspond to phenolic compounds that have been reported by Figueroa-Perez *et al*. (2018), who identified 15 phenolic acids compounds in nopal cladodes at different maturity stages, from which only 11 were previously identified in commercial and wild *Opuntia* spp. (Guevara-Figueroa *et al*., 2010). Considering the diversity of phenolic compounds reported in other nopal varieties, the unidentified peaks in the *N. cochenillifera* phenolic profile, may include salicylic, protocatechuic, 4-Hydroxybenzoic, vanillic, caffeic, synaptic, eucomic and chlorogenic acids (Astello-García *et al.*, 2015; Figueroa-Pérez *et al.*, 2018; Guevara-Figueroa *et al.*, 2010; Kolniak-Ostek *et al.*, 2020; Mata *et al.*, 2016; Missaoui *et al.*, 2020). Therefore, further studies are necessary to identify *N. cochenillifera* phenolic compounds.

On the other hand, the most abundant phytochemicals identified were gallic, ferulic and chlorogenic acids. The gallic acid content (2708.49 µg/g) indicated in this study was higher than that found by Jun *et al.* (2013). However, the same author reported that ferulic, chlorogenic and p-coumaric acid contents were higher than the data obtained in this study (796.73, 179.70 and 80.58 µg / g, respectively). This difference in the phenolic acid content is likely due to factors such as the cladodes maturity stage, species, geographic conditions, and other environmental conditions (Moussa-Ayoub *et al.*, 2014).

Figueroa-Pérez *et al.* (2018) observed different polyphenol profiles in nopal (*O. fiscus-indica*) cladodes collected at different maturity stages, with association to health beneficial effects. For example, chlorogenic acid relates with

Table 4. Soluble polyphenol content of *N. cochenillifera* cladodes flour.
Tabla 4. Contenido de polifenoles solubles de la harina de cladodios de *N. cochenillifera*.

No.	Compound	Retention time. (min)	Cladodes flour Content (µg/g)
1	Gallic accid	15.65	2708.49 ± 106.59
2	Ferulic acid	53.71	796.73 ± 1.43
3	Chlorogenic acid	36.85	179.70 ± 12.51
4	Coumaric acid	50.05	80.58 ± 12.13
5	Syringic acid	41.52	11.12 ± 0.56
6	Neochlorogenic acid	21.21	9.52 ± 0.28

All values are means ± standard deviation of three determinations per batch.

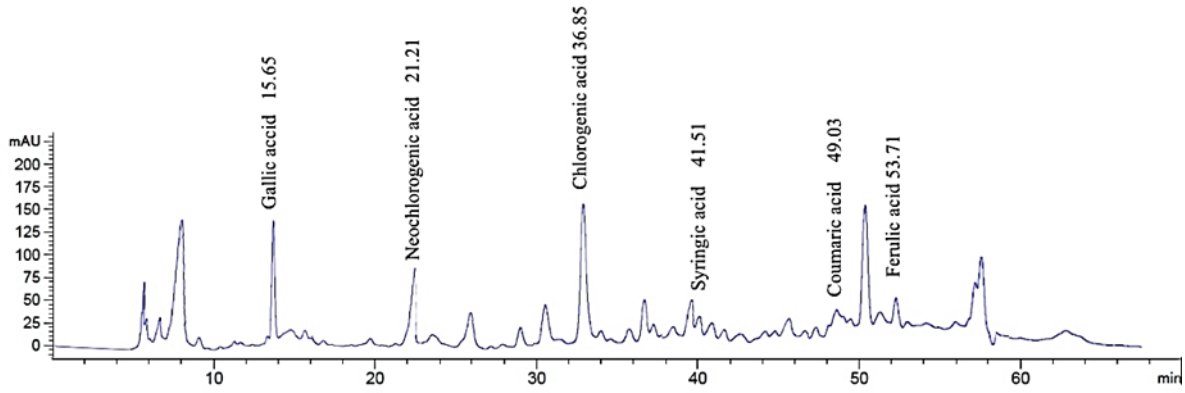

Figure 1. Soluble polyphenols chromatographic profile of *N. cochenillifera* cladodes flour.
Figura 1. Perfil cromatográfico de polifenoles solubles de la harina de cladodios de *N. cochenillifera*.

antidiabetic effects by delaying intestinal glucose absorption and inhibiting hepatic gluconeogenesis, while gallic acid has shown beneficial effects in the prevention and/or management of various disorders, attributed to its antioxidant potential (Kahkeshani *et al.*, 2019; Ong *et al.*, 2012). These results suggest that *N. cochenillifera* cladodes flour may provide antioxidant properties and offer effective protection from free radicals, and support that *N. cochenillifera* could be a promising source of natural antioxidants.

CONCLUSION

The results demonstrated that *N. cochenillifera* has similar properties to the *Opuntia ficus-indica* nopal, and bioactive compounds with antioxidant activity beneficial to the consumers' health. This study is the first step to give an added value to and promote the consumption of this nopal cactus species, which is undervalued in the markets. Furthermore, the cladodes flour phytochemical content and antioxidant activity, makes it a candidate as a functional ingredient in the elaboration of widely used products, such as tortillas and snacks. On the other hand, future studies on the shelf life, optimization, and conservation of cladodes flour are necessary.

ACKNOWLEDGMENTS

One of the authors (HEFI) acknowledges CONACYT-Mexico for the scholarship awarded to study the Master in Food Science at the "Instituto de Ciencias de Agropecuarias" (ICAP-UAEH).

CONFLICTS OF INTEREST.

The authors declare that no conflict of interest exists related with this publication.

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