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Effect of two dehydration processes on extracts from *Opuntia atropes* and characterization of polyphenolic compounds by ultra high-resolution liquid chromatograph coupled with mass spectrometry

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ABSTRACT

The objective of this research was to evaluate the polyphenolic compounds, the antioxidant activity, and the individual identification of the different types of phenolic compounds in dried extracts of *Opuntia atropes*. *O. atropes* extract was dehydrated in a drying oven at 50°C/24 h and by nano-spray drying at 105°C. Drying by nano-spray did not affect the content of phenolic compounds or the antioxidant activity of the *O. atropes* extract, presenting values of 11.80 ± 0.61 mg of gallic acid equivalent/g of phenolic acids, 22.26 ± 1.02 mg quercetin equivalent/g of flavonoids and inhibition percentage of the radical DPPH$^{\bullet}$ (2,2-. diphenil-1-picrilhidrazil) and ABTS$^{\bullet+}$ (2,2′Azinobis(3-ethylbenzothiazoline-6 sulfonic acid)) of 17.92 ± 0.31 and 54.08 ± 0.48, respectively. In the powdered extracts of *O. atropes*, glycosides of kaempferol and isorhamnetin were mainly identified by ultra high-performance liquid chromatography coupled to mass spectrometry. Mediorresinol lignan was identified for the first time in this *Opuntia* species.

RESUMEN

El objetivo de esta investigación fue evaluar los compuestos polifenólicos, la actividad antioxidante y la identificación individual de los diferentes tipos de compuestos fenólicos en extractos secos de *Opuntia atropes*. El extracto de *O. atropes* se deshidrató en un horno de secado a 50°C/24 h y mediante secado por nano-pulverización a 105°C. El secado por nano-pulverización no afectó el contenido de compuestos fenólicos ni la actividad antioxidante del extracto de *O. atropes*, presentando valores de 11.80 ± 0.61 mg EAG (equivalentes de ácido gálico)/g de ácidos fenólicos, 22.26 ± 1.02 mg EQ (equivalentes de quercetina)/g de flavonoides y porcentaje de inhibición del radical DPPH$^{\bullet}$ (2,2-difenil-1-picrilhidrazilo) y ABTS$^{\bullet+}$ (ácido 2, 2′ Azinobis-3-etil- benzotiazolin-6 sulfónico) de 17.92 ± 0.31 y 54.08 ± 0.48, respectivamente. En los extractos en polvo de *O. atropes*, se identificaron principalmente los glucósidos de kaempferol e isoramnetina mediante cromatografía líquida de ultra alta resolución acoplada a espectrometría de masas. El lignano de mediorresinol se identificó por primera vez en esta especie de *Opuntia*.

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1. Introduction

The consumption of the different species of *Opuntia* spp. (Cactaceae family) is highly related to a lower risk of chronic diseases associated with oxidative stress, such as cancer, diabetes, and cardiovascular diseases (Antunes-Ricardo et al., 2015; Keller et al., 2015; Rodríguez-Rodríguez et al., 2015;). These biological effects have related to the anti-inflammatory and antioxidant properties of the polyphenols present in *Opuntia* spp. (Luca et al., 2020; Myint et al., 2020). *Opuntia* Mill., is the most important genus of these Cactaceae and includes many species and varieties. *Opuntia ficus-indica* (L.) Mill. is the specie most studied worldwide. However, there are other species that are consumed, such as *Opuntia atropes* Rose, and of which there are few reports (López-Gutiérrez et al., 2015; Tranquilino-Rodríguez et al., 2020; Valadares et al., 2020). Recent studies have shown that cladodes of *Opuntia* spp. are good candidates for the development of new healthy foods, due to their high content of phenolic-type compounds, such as flavonoids (Serra et al., 2013). These physiologically active compounds are present mainly as glycosides and it has been shown that the sugar residues in flavonoids, their number and type, affect their biological activity and are related to differences in bioavailability (Antunes-Ricardo et al., 2015). In general, polyphenolic compounds and other antioxidant compounds have become more important economically and its increasingly used in the nutraceutical and cosmetic industries (Marqués et al., 2013). In the food sector, a wide range of products enriched with polyphenolic compounds have begun to be developed for the prevention of chronic diseases, which have been very well received by the consumer (Gómez & Martinez, 2018; Spina et al., 2019). To achieve separation processes, as well as the identification and characterization of bioactive compounds, plant materials must first undergo an extraction process (Gligor et al., 2019). Various extraction techniques such as maceration have been used to obtain antioxidants and other bioactive

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compounds from different parts of the plant (Benattia & Arrar, 2018). After extraction, it is recommended to carry out a drying process to increase the stability of the compounds and preserve their shelf life in storage for a longer time (Akbarian et al., 2013). The traditional drying process can damage quality by causing oxidative damage, browning, change in taste and solubility. During hot air drying, polyphenols can have enzymatic and non-enzymatic degradation (Abhay et al., 2016; Salazar et al., 2018). This is favored when the drying temperature of polyphenolic compounds exceeds 50°C (Aruwa et al., 2019; De Torres et al., 2010). Therefore, to maintain the quality of the polyphenols, the drying process must be carried out under a controlled temperature environment, preferably low, or applying vacuum (Wang et al., 2009). Spray drying is a simple, fast, reproducible and scalable drying technology that allows mild temperature conditions, suitable for heat-sensitive bioactive compounds and maintains the quality of nutrients, colors and flavors (Fazaeli et al., 2012). The powders produced are of high quality and generally have low moisture content and water activity, so they are resistant to microbiological and oxidative degradation, resulting in high storage stability (Islam et al., 2016; Shishir et al., 2018). The objective of this research was to evaluate two methods of drying in an extract of *O. atropes*, which allow the preservation of polyphenolic compounds with antioxidant activity, as well as the individual identification of the different types of phenolic compounds present in the extract.

2. Materials and methods

The cladodes of *O. atropes* were obtained from Ziracuaretiro, Michoacán, Mexico, and were identified by the Herbarium of the Faculty of Biology of the Universidad Michoacana de San Nicolás de Hidalgo.

2.1. Dehydration and characterization of the extract of *O. atropes*

The young cladodes of *O.* atropes (3 months) of 80 ± 40 g were disinfected with 1% sodium hypochlorite solution/15 min, then washed with distilled water, spines removed, cut into 1 × 1 cm pieces, and dehydrated at 50°C for 48 h in a oven (Felisa® FE-292, Mexico). The dehydrated samples were triturated in a blender (Oster®, 450,010,000 model, 400-watt power) and sieved to obtain nopal flour with a particle size of less than 260 µm. The nopal flours were stored in frozen at −5°C until their analysis.

The extracts of polyphenolic compounds of *O. atropes* were obtained according to the conditions described in a previous study by this same team (Tranquilino-Rodríguez et al., 2020), with minor modifications. Briefly, the extraction method was carried out as follows, 10 g of nopal flour were placed in 100 mL of 70% ethanol in a 250 ml Erlenmeyer flask, which was placed at 50°C in a shaking water bath SW22 (Julabo®, Seelbach, Germany) at 100 rpm for 120 min. The extract obtained was centrifuged at 3,087 g/10 min and vacuum filtered with Whatman filter paper No. 2, 4 and 5 until obtaining a particle size ≤2.5 µm. The ethanol was then removed in a rotary evaporator (Science Med® RE 100-Pro) at a temperature of 40°C, coupled to a vacuum system, obtaining an aqueous extract, that was vacuum filtered with Whatman paper No. 5 to obtain a particle size ≤ 2.5 microns. The extract of *O. atropes* adjusted to 1% of total solids, was dehydrated by two methods, drying in an oven at 50°C and drying by nano-spray at 105°C. For drying in the oven (Felisa® FE-292, Mexico), 20 mL of the *O. atropes* extract were placed in a glass Petri dish 100 × 20 mm, and placed in the oven at 50°C for 24 h and the dehydrated extract was collected with a stainless-steel spatula. Nano-spray drying was carried out in a Nano Spray Dryer Unit B-90 (Büchi® Labortechnik AG, Flawil, Switzerland). The process parameters were, inlet temperature of 105°C, outlet temperature of 49°C, air flow of 110 L/min, vacuum pressure of 33 mbar, aspersion of 70%, spray mesh size of 7 µm and feeding speed of 36 mL/h (5 h of process). The sample was collected with a silicone spatula. The powdered extracts (EOSE and EOSNA) were stored in an amber bottle and in a desiccator at 4°C until their analysis. The quantification of phenolic acids, flavonoids and antioxidant activity by capture of the radical DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ this was carried out both in the *O. atropes* extract before drying (EO), in the *O. atropes* extract dried in an oven at 50°C (EOSE) and, in the *O. atropes* extract dried by nano-spray (EOSNA). In the extracts obtained in powder (EOSE and EOSNA), it was determined; yield (%), humidity (%), particle size (nm), colour (luminosity, chroma, °hue) and polyphenolic compounds by UPLC-Q/TOF-MS^2.

2.1.1. Determination of total phenolic acids or phenols

The phenolic acids were assayed using the Folin–Ciocalteu method, as described by Makkar et al. (1993), with minor modifications suggested by Treviño-Gómez et al. (2017). An aliquot of 250 µL of the extract was added to 250 µL of Folin–Ciocalteu reagent (2 N) and 250 µL of 20% Na_2CO_3, stirred and incubated at 40°C for 30 mins. Distilled water (2 mL) was added, and the mixture was stirred. Absorbance was read at 750 nm in a VELAB® (Germany) spectrophotometer. Gallic acid was used as the standard. The results were expressed as gallic acid equivalents per gram of dry sample (mg GAE/g).

2.1.2. Determination of total flavonoids

The total flavonoids were assessed following the method proposed by Liu et al. (2002). A 150-µL aliquot of each sample and 150 µL of 5% $NaNO_2$ were mixed. Then, 150 µL of 10% $AlCl_3$ and 1 mL NaOH 0.1 M were added and stirred. The absorbance was measured at 510 nm (VELAB® spectrophotometer). Quercetin was used as the standard. The results were expressed as quercetin equivalents per gram of dry sample (mg QE/g).

2.1.3. DPPH$^{\bullet}$ antioxidant assay

The scavenging of DPPH$^{\bullet}$ radicals was determined, as detailed by Randhir and Shetty (2007), with the modifications proposed by Treviño-Gómez et al. (2017). A 50-µL aliquot of each sample was mixed with 2.95 mL of 60 µM methanol–DPPH$^{\bullet}$ solution, to obtain a volume of 3 mL. The samples were stirred for 10 s and maintained for 30 min in darkness. Gallic acid was used as the standard. Absorbance was read at 517 nm (VELAB® spectrophotometer), and the results were expressed as gallic acid equivalents per gram of sample according to the calibration curve prepared with the

same standard and the % inhibition of the DPPH$^{\bullet}$ radical according to the following formula:

$$\%\ inhibition = \frac{A_R - A_s}{A_s} * 100$$

A_R = Absorbance of the reference standard. A_S = Absorbance of the sample.

2.1.4. ABTS$^{\bullet+}$ antioxidant assay

The ABTS$^{\bullet+}$ scavenging activity was evaluated according to Re et al. (1999) method. A stock solution containing 2:1 v/v of 7 mM ABTS$^{\bullet+}$ and 2.45 mM potassium persulfate solution was left to stand at room temperature for 12 to 16 h, and then adjusted with ethanol until an absorbance of 0.70 ± 0.02 nm at 734 nm was reached. Afterward, 15 µL of each sample was mixed with 1,485 µL of the prepared ABTS$^{\bullet+}$ solution. After 15 mins, the absorbance was measured at 734 nm (VELAB® spectrophotometer), and the results were expressed as gallic acid equivalents per gram of sample according to the calibration curve prepared with the same standard and the % inhibition of the ABTS$^{\bullet+}$ radical according to the following formula:

$$\%\ inhibition = \frac{A_R - A_s}{A_s} * 100$$

A_R = Absorbance of the reference standard. A_S = Absorbance of the sample.

2.1.5. Determination of yield

The percentage yield of the *O. atropes* dehydrated extracts were determined gravimetrically (Gereniu et al., 2017).

$$Yield\ (\%) = \frac{Weight\ of\ dry\ extract\ collected}{Inicial\ weight\ of\ extract^1} * 100$$

[1]Total solids content of the extract was determined gravimetrically by oven-drying (105°C) until a constant weight (4 h) was reached.

2.1.6. Determination of moisture

A porcelain capsule at constant weight was used for each sample, 3 g of extract were weighed into each capsule and placed in a drying oven (Felisa®) 105°C/4 h. Subsequently, they were placed in a desiccator for 40 min and weighed to constant weight (AACC, 2000). The moisture percentage was calculated based on the following formula:

$$Moisture\ (\%) = \frac{W_w - W_d}{S} * 100$$

W_W = weight of the capsule and the wet sample in grams. W_d = weight of the capsule and dry sample in grams. S = weight of the wet sample in grams.

2.1.7. Determination of particle size

A solution of 1 mg of extract in 1 mL of distilled water was prepared for each treatment. The measuring was evaluated in a nanoparticle analyzer (Horiba® SZ-100, Ltd., Japan). The particle size was expressed in nm.

2.1.8. Determination of colour

Determination of color. A manual colorimeter (BYK® Gardner, USA) was used, with an illuminant and standard observer D65/10 ° at T = 24°C. The samples (EOSE and EOSNA) were placed in a 4 cm container of diameter. The thickness of each sample was 1 cm and the parameters of luminosity, chroma or saturation and the °hue were determined.

2.1.9. Identification of polyphenolic compounds by UPLC-Q/TOF-MS^2

For the identification of polyphenolic compounds of the *O. atropes* extract obtained both by drying in an oven (EOSE) and by drying by nano-spray (EOSNA), a solution was prepared at 500 ppm with HPLC grade water for each sample and filtered on paper Whatman with 0.2 µm pore and was placed in the 2 mL glass chromatography vials.

For the evaluation of the polyphenolic profile, an ultra-high-resolution liquid chromatograph was used (ACQUITY® UPLC I-Class, Singapore). The qualitative identification of the polyphenols was performed with a BEH PHENYL analytical column (2.1 mm x 100 mm, 1.7 µm, WATERS, UK) operated at 40°C. Gradient separation was performed for each sample using a mobile phase of solvent A: water with 0.1% (v/v) formic acid and solvent B: 100% acetonitrile, with a constant flow rate of 0.3 mL per min. The samples were injected (3 µL) with an automatic sampler at a scan time of 10 min, starting with the 90% A and 10% B gradient program, followed by 87% of A and 13% of B at 0.5 min, 85% of A and 15% of B at 2.0 min, 83% of A and 17% of B at 3.50 min, 80% of A and 20% of B at 5.0 min, and finally 10% of A and 90% of B at 8.50 min. The UPLC system was coupled to a Q-TOF orthogonal accelerated Q-TOF mass spectrometer (XEVO® G2-XS Q-TOF 4 K, UK) equipped with an electrospray ionization source. The PDA detector was used to record the chromatograms. The detection of the mass spectra was carried out in the negative ion mode in an m/z mass range of 50–1200 Da, using a capillary voltage of −3.5 and +4.0 kV, dry gas temperature of 210°C, 8.0 L gas flow per min, 2.0 bar nebulizer pressure, and 1 Hz spectrum speed. Automatic MS/MS experiments were performed using a 15–35 V ramp collision energy with argon as the collision gas and adjusting the scan time every second. Each polyphenolic compound was identified according to its characteristic aglycone fragment ions, through the interpretation of its fragmentation patterns and with the interpretation of the mass spectra that were obtained, these data were compared with databases such as Phenol-Explorer and MassBank and with the information reported by other research (Astello et al., 2015; Guevara-Figueroa et al., 2010; Melgar et al., 2017; Mena et al., 2018; Santos-Zea et al., 2011).

2.2. Statistic analysis

Results obtained from the triplicates were reported as a mean ± standard deviation (SD). Data were subjected to Student's t tests or one-way analysis of variance (ANOVA) followed by Tukey's test using JMP6 software (SAS, Institute, Cary, NC, USA). P values < .05 were regarded as significant. Chromatograms obtained by UPLC -ESI-Q/TOF-MS^2 were plotted using OriginPro 2016 software.

3. Results and discussion

3.1. Dehydration and characterization of the extract of O. atropes

No significant difference was found in the content of phenolic acids (P > .05) in the extracts, being 11.13 mg GAE/g for the *O. atropes* extract before drying and 10.62 mg GAE/g for

Table 1. Dehydration of *Opuntia atropes* extract.

Tabla 1. Deshidratación del extracto de *Opuntia atropes*.

Parameter	*O. atropes* extract before drying (EO)	*O. atropes* extract nano-spray drying at 105°C (EOSNA)	*O. atropes* extract oven drying at 50°C/24 h (EOSE)
Phenolic acids (mg GAE/g)	11.13 ± 0.38 [a,b]	11.80 ± 0.61 [a]	10.62 ± 0.27 [b]
Flavonoids (mg QE/g)	24.05 ± 1.44 [a]	22.26 ± 1.02 [a]	19.22 ± 0.09 [b]
DPPH$^{\bullet}$ (mg GAE/g)	1.71 ± 0.04 [a]	1.65 ± 0.03 [a]	1.63 ± 0.04 [a]
% Inhibition (10 mg)	18.32 ± 0.42 [a]	17.92 ± 0.31 [a]	17.76 ± 0.46 [a]
ABTS$^{\bullet+}$ (mg GAE/g)	5.66 ± 0.00 [a]	5.70 ± 0.05 [a]	5.19 ± 0.11 [b]
% Inhibition (10 mg)	53.49 ± 0.07 [a]	54.08 ± 0.48 [a]	49.30 ± 1.08 [b]

Results are expressed as mean ± standard deviations (SD) of three determinations. [a, b, c] Different letters in the same row are significantly different, as shown by Tukey' test $p<0.05$.

EOSE and of 11.80 mg GAE/g for EOSNA (Table 1). The flavonoid content of the *O. atropes* extract before drying was 24.05 QE/g and there was no significant difference ($P > .05$) with EOSNA, which was 22.26 QE/g; however, in EOSE there was a difference significant ($P < .05$) and flavonoids of 19.22 QE/g were quantified, so there was a decrease of 20.08% with respect to the *O. atropes* extract before drying. For the antioxidant activity by capturing the radical DPPH$^{\bullet}$, no significant difference was found in the % inhibition of the *O. atropes* extract before drying, which was 18.32%, compared to the two drying treatments, which was around 17% (Table 1). For the antioxidant activity as a function of the ABTS$^{\bullet+}$ radical, there was no statistically significant difference between the *O. atropes* extract before drying (53.49%) compared with the EOSNA (54.08%), however, was found less inhibition in EOSE (49.30%) (Table 1), which indicated that the oven drying treatment decreased 7.8% of the antioxidant activity for this radical.

The *O. atropes* extract dehydrated in an oven (EOSE), was obtained in the form of dark amber crystals, and its recovery was difficult, due to the presence of high hygroscopicity and adherence to the glass petri dish, and therefore, presented a low yield, 49.70% (Table 2). The extract of *O. atropes* dried by nano-spray (EOSNA), was obtained in the form of a fine beige color powder, and presented low hygroscopicity, so that its recovery was higher, obtaining a yield of 74.29%, which is classified as a high yield for the use of this technology (Arpagaus et al., 2017).

The moisture in the obtained extracts was determined. The EOSE extract presented 9.67 ± 0.86% and the EOSNA extract presented 4.38 ± 0.42% (Table 2). Moisture is a critical factor in dehydrated products since it determines their shelf life based on the deterioration reactions that take place in the product. As mentioned, the EOSE extract presented high hygroscopicity, which increased the moisture content of the extract, this moisture could favor contact with free radicals, thus causing their deterioration (Silva et al., 2013). On the other hand, in the EOSNA extract, the stability of the bioactive compounds and antioxidant activity could be related to the lower moisture and hygroscopicity.

The color parameters of the extracts are shown in Table 2. The parameters were evaluated; luminosity, this parameter was evaluated in a range from 0 to 100, where 100 was white and 0 was black. The saturation or chroma was also determined and the purity or how vivid the color is indicated, so that dull colors can be differentiated near center and become more vivid as they move away from center and finally the °hue was quantified, which is the visual appreciation of color, the angle found and the tone this represented was: 90 ° = yellow (HunterLab, 2012). For EOSE, luminosity values of 26.83 ± 1.99, chroma of 16.37 ± 0.53 and °hue of 75.30 ± 0.92 were obtained, so this extract presented a bright dark yellow color, whereas EOSNA presented luminosity values of 66.54 ± 1.24, chroma of 11.33 ± 1.14 and °hue of 88.35 ± 2.42, so this extract presented a light and opaque yellow color (Figure 1). The color in antioxidant extracts is an important quality parameter. Brown colors are associated with oxidation processes. This characteristic could be verified, because the EOSE extract presented a darker color, and therefore, lower polyphenolic and antioxidant content compared to EOSNA.

The size of the particles was evaluated in the two dehydrated samples (Table 2). It was obtained that the extract of *O. atropes* obtained by EOSE was in the micrometric scale >1 µm (1307.8 ± 81.18 nm), while the particles obtained by EOSNA were of nanometric size <1 µm (105.4 ± 25.40 nm) as reported by Fang and Bhandari (2012), Ezhilarasi et al. (2013), and Wui (2015). With nano-spray drying technology (Buchi B-90), it is possible to obtain ultrafine powders with a size smaller than 500 nm, compared to the aggregation in the form of flakes obtained by other methods such as oven drying or lyophilization (Chopde et al., 2020). The main advantage of nano-spray drying is the production of nano-scale particles, which improves the bioavailability of bioactive components, due to a higher surface–volume ratio,

Table 2. Characterization of dehydrated *Opuntia atropes* extracts.

Tabla 2. Caracterización de extractos de *Opuntia atropes* deshidratados.

Parameter		*O. atropes* extract nano-spray drying at 105°C (EOSNA)	*O. atropes* extract oven drying at 50°C/24 h (EOSE)
Yield (%)		74.29 ± 3.09 [a]	49.70 ± 7.38 [b]
Moisture (%)		4.38 ± 0.42 [b]	9.67 ± 0.86 [a]
Colour	Luminosity	66.54 ± 1.24 [a]	26.82 ± 1.99 [b]
	Chroma	11.33 ± 1.14 [b]	16.37 ± 0.53 [a]
	°hue	88.35 ± 2.42 [a]	75.30 ± 0.92 [b]
Particle size (nm)		105.4 ± 25.40 [b]	1307.8 ± 81.18 [a]

Results are expressed as mean ± standard deviations (SD) of three determinations. [a, b,] Different letters in the same row are significantly different, as shown by Student' test $p<0.05$.

Table 3. Polyphenolic profile from EOSE obtained by UPLC-Q/TOF-MS^2.

Tabla 3. Perfil polifenólico de EOSE obtenido por UPLC-Q/TOF-MS^2.

Peak N°	Rt(min)	[M-H]$^-$ (m/z)	Ms^2 Fragment of the molecular ion	Tentative assigment	Polyphenol class	Polyphenol family	Molecular formula
1	0.78	387.1490	195.1070	Medioresinol	Lignan	Lignans	$C_{21}H_{24}O_7$
2	1.98	325.1316	175.0132	*p*-Coumaric acid 4-*O*-glucoside	Phenolic acid	Hydroxycinnamic acids	$C_{15}H_{18}O_8$
3	3.39	739.1702	175.0130	Kaempferol 3-*O*-(rhamnosyl-galactoside) -7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
4	3.49	739.1711	175.0131	Kaempferol 3-*O* (rhamnosyl-galactoside) -7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
5	3.63	769.1743	175.0131	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
6	3.78	769.1764	175.0135	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (Isomer I)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
7	4.84	593.1451	175.0128	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
8	5.10	623.1503	175.0129	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$

Rt = retention time. [M-H]$^-$ = molecular ion. Ms^2 = mass analyzer.

Table 4. Polyphenolic profile from EOSNA obtained by UPLC-Q/TOF-MS^2.

Tabla 4. Perfil polifenólico de EOSNA obtenido por UPLC-Q/TOF-MS^2.

Peak N°	Rt (min)	[M-H]$^-$ (m/z)	Ms^2 Fragment of the molecular ion	Tentative assigment	Polyphenol class	Polyphenol family	Molecular formula
1	0.78	387.1469	195.1059	Medioresinol	Lignan	Lignans	$C_{21}H_{24}O_7$
2	1.98	325.1367	175.0133	*p*-Coumaric acid 4-*O*-glucoside	Phenolic acid	Hydroxycinnamic acids	$C_{15}H_{18}O_8$
3	3.39	739.1693	175.0129	Kaempferol 3-*O*-(rhamnosyl-galactoside) -7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
4	3.49	739.1671	175.0122	Kaempferol 3-*O*-(rhamnosyl-galactoside) -7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
5	3.63	769.1717	175.0126	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
6	3.78	769.1743	329.2764	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (Isomer I)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
7	4.84	593.1470	329.2766	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
8	5.10	623.1507	329.2765	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$

Rt = retention time. [M-H]$^-$ = molecular ion. Ms^2 = mass analyzer.

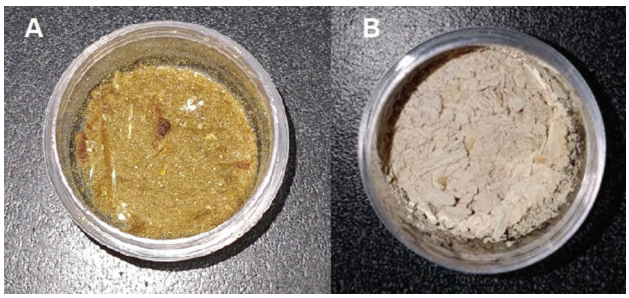

Figure 1. Dehydrated extracts (a) EOSE and (b) EOSNA.

Figura 1. Extractos deshidratados (a) EOSE y (b) EOSNA.

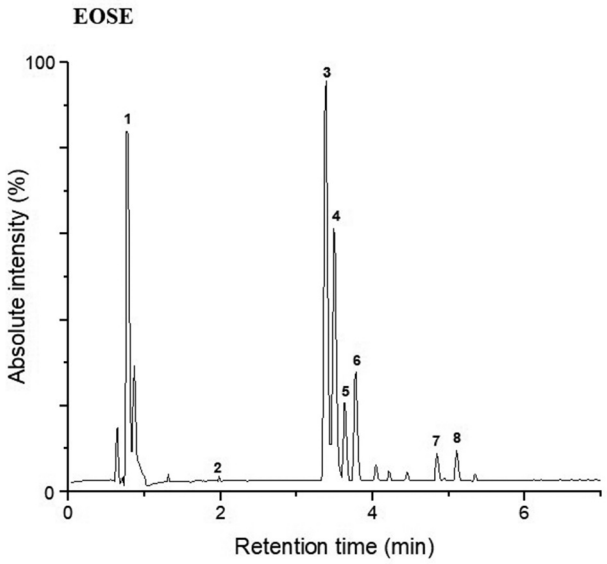

Figure 2. Peak chromatogram of the polyphenolic compounds obtained by UPLC-Q/TOF-MS^2 for EOSE. For the assignment of each peak see Table 3.

Figura 2. Picos del cromatograma de compuestos polifenólicos obtenidos por UPLC-Q/TOF-MS^2 para EOSE. Para la asignación de cada pico ver la Tabla 3.

stability, and a higher rate of penetration into cells (Arpagaus et al., 2018).

Spray dryers can dry a product very quickly compared to other drying methods and are suitable for heat-sensitive products such as polyphenols, as the exposure time to high temperatures is short (Sandoval-Peraza et al., 2016). Spray drying has been shown to have higher retention of phenolic compounds and flavonoids compared to lyophilization in papaya pulp (Gomes et al., 2018). A previous study showed that spray drying did not affect the polyphenolic and antioxidant content of a black rice (*Oryza sativa* L., var. Artemide) extract (Papillo et al., 2018). It has also been shown that freeze-drying and oven drying at 45°C do not affect the polyphenolic and antioxidant content of the pulp and peel of the fruit of *O. ficus-indica* (Aruwa et al., 2019). And that lyophilization is a more efficient method to preserve polyphenolic compounds with antioxidant activity, in *Opuntia* spp. cladode flour, compared to spray drying or tunnel drying (Martínez-Soto et al., 2016). However, until now the impact of nano-spray drying or oven on the polyphenolic extract of cladodes of *O. atropes* had not been reported.

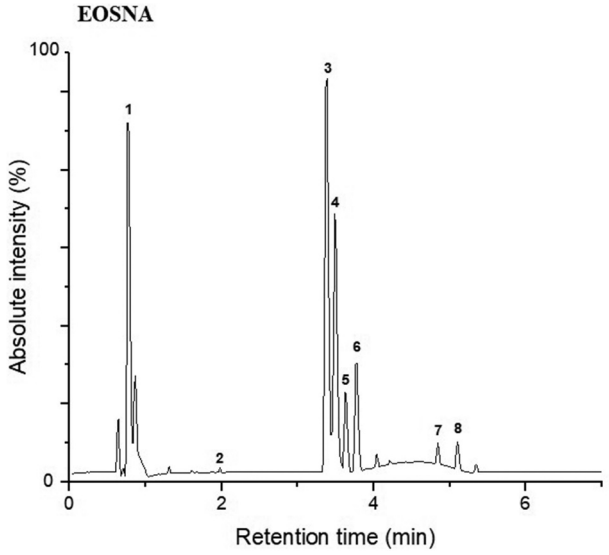

Figure 3. Peak chromatogram of the polyphenolic compounds obtained by UPLC-Q/TOF-MS^2 for EOSNA. For the assignment of each peak see Table 4.

Figura 3. Picos del cromatograma de compuestos polifenólicos obtenidos por UPLC-Q/TOF-MS^2 para EOSNA. Para la asignación de cada pico ver la Tabla 4.

Based on these results, it was established that drying by nano-spray at 105°C did not affect the content of polyphenolic compounds (phenolic acids and flavonoids) or the antioxidant activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) of the *O. atropes* extract, in addition the nano-spray drying at 105°C presented some advantages over drying in an oven at 50°C, which are related to the yield, moisture, color, particle size and the content of flavonoids and antioxidant activity in ABTS$^{\bullet+}$, however, if nano-spray drying is not available, drying in an oven at 50°C/24 h could be a feasible method for the dehydration of *O. atropes* extract. The decrease in the amount of some polyphenolic compounds such as flavonoids and the antioxidant activity of the extract should be considered, lower quality characteristics and that the drying time is longer.

Lemos et al. (2016) reported 1.2 mg GAE/g of phenolic acids or phenols and 2.26 mg QE/g of flavonoids in flour from *O. atropes* and Guevara-Figueroa et al. (2010) reported concentrations of up to 5.2 mg GAE/g for phenolic acids and 9.7 mg QE/g for flavonoids also in *O. atropes* flour. The content of both phenolic acids and flavonoids in this study were higher than those reported by Lemos et al. (2016) and Guevara-Figueroa et al. (2010). These variations in the polyphenol content are related to the agroecological conditions of the region where the cladodes were collected, variety, cladode maturity, extraction method and compound quantification (Koolen et al., 2013).

The tests with radical's DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ are used because they show an idea of the structure and its relationship in the antioxidant activity of the compounds contained in the samples (Bernini et al., 2018), this antioxidant characterization provided us valuable information on the chemical reactivity of the compounds present in the samples and that effectively present the ability to eliminate free radicals by electron transfer. It has been reported that the values of the total antioxidant activity in extracts of *Opuntia* spp. its correlated with the content of flavonoids mainly (Fernández-López et al., 2010). The extracts obtained in this study presented a significant number of polyphenolic compounds, and their antioxidant activity was also shown, so cladodes of *O. atropes* can be attractive foods for the food industry as functional ingredients, to prevent or reduce the risk of chronic degenerative diseases.

3.2. Identification of polyphenolic compounds by UPLC-Q/TOF-MS^2

Tables 3 and 4 show the polyphenolic profiles of the oven-dried *O. atropes* extract (EOSE) and the extract dried by nano-spray (EOSNA) respectively, obtained by UPLC-Q/TOF-MS^2. It is important to mention that this is a tentative chemical characterization of the polyphenolic compounds present in the *O. atropes* extracts. Figures 2 and 3 show the retention times and the 8 peaks found for both EOSE and EOSNA respectively, in both cases they correspond mainly to polyphenolic compounds of the flavonoid type, from the group of flavonols, which are kaempferol glycosides and isorhamnetin, see Tables 3 and 4, as can be seen, the two extracts presented very similar chromatograms, and the peaks corresponded to the same polyphenolic compounds. Therefore, the dehydrated extracts of *O. tropes* (EOSE and EOSNA) conserved similar proportions of these bioactive compounds.

Tables 3 and 4 show that for the two dehydration treatments (EOSE and EOSNA), peaks 3 and 4 belong to kaempferol 3-*O*-(rhamnosyl-galactoside) −7-*O*-rhamnoside; however, these 2 compounds had different retention times; therefore, its designated as isomers, and in peak 7, kaempferol 3-*O*-galactoside 7-*O*-rhamnoside was detected. In peaks 5 and 6, two isomers of isoramnetin 3-*O*-rhamnoside-7-*O*- (rhamnosyl-hexoside) were present, and in peak 8 another isoramnetin glucoside was found, which was isoramnetin 3-*O*-glucoside 7 -*O*-rhamnoside. Peak 1 corresponded to medioresinol, which is a lignan, which has not been reported so far in the polyphenolic profile of the genus *Opuntia*, this compound was found in a similar proportion with respect to kaempferol glycosides (kaempferol 3-*O*- (rhamnosyl-galactoside) −7-*O*-rhamnoside), for this reason its identification in *O. atropes* is important. Lignans are attributed many physiological properties that positively influence human health (Durazzo, 2018). Its intake has been mainly related to its possible chemopreventive actions against cancer, due to its phytoestrogenic properties and in the prevention of cardiovascular diseases (Anandhi et al., 2018; Xiao et al., 2018). The presence of this lignan increases the number of bioactive compounds in the cladodes of *O. atropes* and, therefore, its interest in human health, either as an individual bioactive molecule or in conjunction with the other molecules present in the extract. Finally, a phenolic acid was detected in peak 2, which was *p*-coumaric acid 4-*O*-glucoside in a low proportion with respect to the other polyphenolic compounds. It is noteworthy to mention that the methodology used in this study (UPLC-Q/TOF-MS^2), does not indicate the concentration of each polyphenolic compound present in the crude extract; however, it does provide us information about the proportion of the polyphenolic compounds, which are mainly flavonoid glycosides, and as can be seen in the chromatograms presented in Figures 2 and 3, the proportion of kaempferol glycosides with respect to isorhamnetin glycosides is greater, and the lignan (mediorresinol) is detected in a very similar proportion to kaempferol glycosides.

The results found in this study coincide with what was previously reported by Park et al. (2007) who indicated that

both kaempferol and isorhamnetin are largely found as glycosides in flowers, fruits and cladodes of *Opuntia* spp. De Santiago et al. (2018), reported flavonoids in cladodes of the species of *O. ficus-indica* as the predominant polyphenolic compounds, of which approximately 80% were derived from isorhamnetin, while those derived from quercetin and kaempferol were in lower concentration. In the research carried out by Santos-Zea et al. (2011) identified the flavonoids present in different varieties of *Opuntia* cladodes such as isorhamnetin and kaempferol, mainly in their glycosidic forms in crude extracts. Flavanols such as kaempferol and isorhamnetin 3-*O*-glucosides, identified in this study have been associated with the antioxidant effect, an anti-inflammatory effect assigned to intestinal inflammation and cardioprotective effects (Kuti, 2004; Matias et al., 2014); therefore, the consumption of these extracts can provide important health benefits.

4. Conclusions

Nano-spray drying did not affect the polyphenol content or antioxidant activity of the *O. atropes* extract. Polyphenolic screening for *O. atropes* showed the majority presence of flavonols kaempferol and isorhamnetin in the form of glycosides and medioresinol, which is a lignan in this nopal species, was identified for the first time. The contribution in the identification of new bioactive molecules is relevant since it contributes to the existing relationship between the consumption of *O. atropes* with the potential benefits for human health. The extracts of *O. atropes* obtained in this work could be used as nutraceuticals and, therefore, have potential for application in the food and health industries.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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