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Nanoencapsulation and identification of phenolic compounds by UPLC-Q/TOF-MS^2 of an antioxidant extract from *Opuntia atropes*

Running title: Nanoencapsulation of phenolics from *Opuntia*

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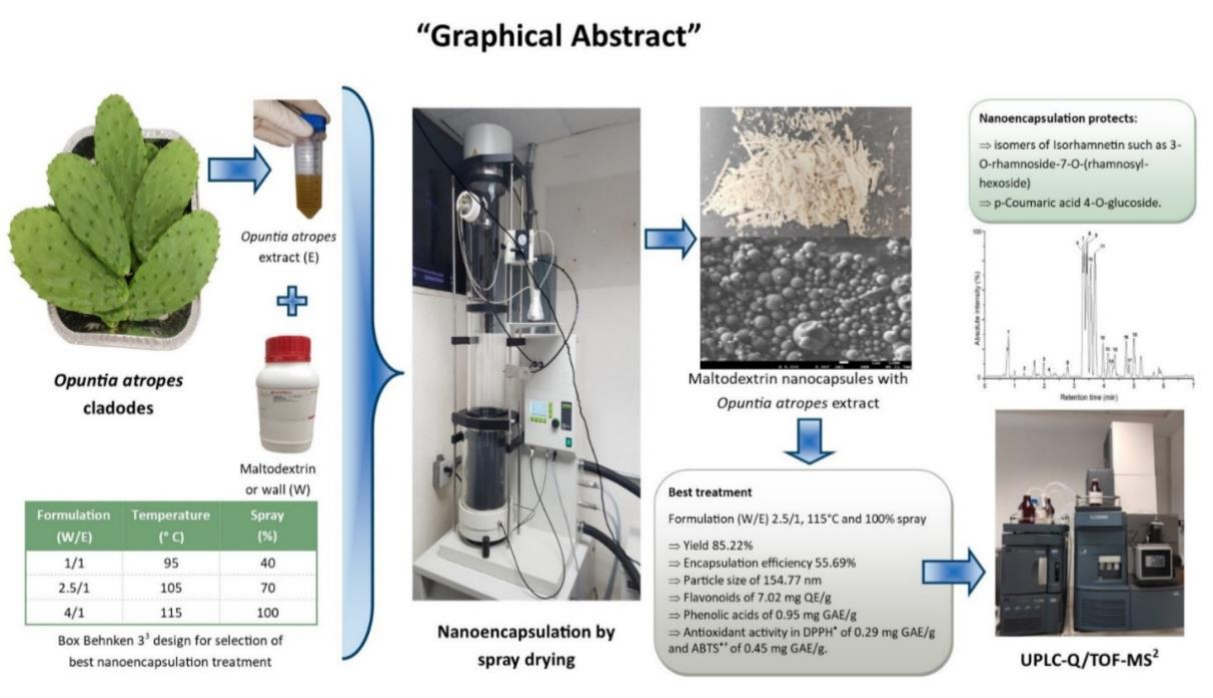
ABSTRACT

Background: Nanoencapsulation is a technique that protects bioactive compounds such as polyphenolic compounds from environmental factors, through a biopolymer that acts as a wall system. Cladodes of *O. atropes* are an important source of polyphenolic compounds, flavonoids being the most abundant, these are mainly in the form of glycosides and their consumption has been related to a decrease in glucose and lipid profile through the elimination of intracellular radicals.

Purpose of the study: To study the formulation and process parameters that allow for obtaining the best experimental conditions for nanoencapsulation and protect the polyphenolic compounds of the *O. atropes* extract.



Methods: Wall materials applied for nanoencapsulation include soy protein isolate, calcium caseinate , and maltodextrin. A 3^3 Box Behnken design was used: wall material extract of *O. atropes* (1/1, 2.5 / 1, 4/1), temperature (95, 105 and 115 ° C) and spray (40, 70 and 100%).Yield, feeding speed, particle size, encapsulation efficiency, phenolic acids, flavonoids, DPPH• (1,1-dipheny l-2-picrilhydrazyl) and ABTS•+(2,2'-azino-bis-3-ethylbenzothia-zolin acid-6-sulfonic) were evaluated. Polyphenolic compounds were identified by UPLC-Q / TOF-MS^2.

Results: Maltodextrin presented better properties in the encapsulation of the extract of *O. atropes*. The 3^3 Box Behnken design made it possible to identify the treatment with the appropriate quality characteristics for the nanoencapsulation process: Formulation 2.5 / 1 (maltodextrin / *O. atropes* extract), 115 ° C and 100% spray resulted in 85.22% yield, feeding speed of 9 mL / h, the particle size of 154.77 nm, 55.69 % encapsulation efficiency, phenolic acids of 0.95 mg GAE / g, flavonoids of 7.02 mg QE / g, and antioxidant activity in DPPH• of 0.29 mg GAE / g and ABTS•+ of 0.45 mg GAE / g. The polyphenolic profile was characterized, and it was confirmed that nanoencapsulation protects some isomers of isorhamnetin 3-*O*-rhamnosido-7-*O*- (rhamnosyl-hexoside) and p-coumaric acid 4-*O*-glucoside.

Conclusion: Maltodextrin is an excellent encapsulating material of the *O. atropes* extract. The formulation and process conditions that favored all the response variables were identified, and it was shown that nanoencapsulation protects the polyphenolic compounds.

Keywords: *O. atropes* extract, nanoencapsulation, polyphenolic profile, UPLC-Q / TOF-MS^2.

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INTRODUCTION

Opuntia spp. is a source of bioactive compounds that are of interest to human health. Alkaloids, indicaxanthin, neobetanin, polysaccharides, and phenolic compounds [1,2] have been isolated from extracts of *Opuntia* spp. PC has antioxidant properties and exhibit a powerful, free radical scavenging capacity and confer protection against transition metal oxidation and lipid peroxidation, a major risk factor in atherosclerosis [3]. Previous research showed that the consumption of a purified isoramnetin extract from *Opuntia ficus-indica* produced a decrease in body weight and total cholesterol in mice with diet-induced obesity [4]. Most of the natural flavonoids are O-glucosides and C-glucosides. The most abundant flavonoid glycosides in plants of the *Opuntia* genus are flavone and flavanol glycosides [5]. *Opuntia* spp. contains flavonoids such as kampferol or isoramnetin that can suppress the accumulation of lipids or inhibit adipogenesis through the regulation of adipogenic genes [6], in addition, these compounds are capable of delaying the pro-oxidant effects in proteins, DNA and lipids through the generation of stable radicals [7], being useful in the prevention of cardiovascular diseases and cancer.

PC from natural sources are easily degraded when exposed to heat, light, oxygen, pH, and digestive enzymes. They also have an unpleasant taste, so their direct use in the food industry is limited [8.9]. The use of an encapsulation system can improve the stability and bioavailability of PC [10]. Encapsulation by spray-drying is a simple and inexpensive technology [11], which offers flexibility to control particle size and morphology [12]. A coating material known as a wall, which allows a protective barrier that is flexible, does not interact with the material to be encapsulated, and allows high encapsulation efficiency, is required to encapsulate a substance. Carbohydrates such as maltodextrin have shown protection against oxidation and high-water solubility [13], and proteins such as soy protein isolate and caseinate allow the formation of strong films [14].

The Büchi Nano B-90 spray dryer has shown that wall materials such as gum arabic, whey protein, polyvinyl alcohol, modified starch and maltodextrin, can produce submicron particles below the 1 µm scale, with yields from 43 % for maltodextrin, up to 94.5% for modified starch [15]. Another study showed that maltrodextrin presented a high encapsulation efficiency of 86.3% for polyphenols from black currant (*Ribes nigrum L.*) [16]. It has been reported that, under optimal conditions, 71% encapsulation of pomegranate polyphenols is obtained using maltodextrin and 82.9% using soy protein isolate, and the stability of polyphenolic compounds is maintained up to 85% and 95% % respectively, in storage conditions of 60 ° C for 60 days [17], which has shown the protective effect that encapsulation provides to polyphenolic compounds.

The evaluation of multiple factors in the formulation and processing of the spray drying encapsulation of bioactive compounds such as PC is of great interest in both the scientific and industrial sectors, to manufacture innovative high-value products with health benefits [18]. The response surface methodology can be used to define the effect of independent variables, alone or in combination, to optimize and find the appropriate process conditions to obtain nanocapsules with suitable characteristics [19]. The Box Behnken design is an RMS design, slightly more efficient than the central composite design, but more efficient than the three-level factorial design, since it allows a reduction in the number of experiments to be performed, which translates into less consumption of reagents and less laboratory work, development of mathematical models that allow evaluating the relevance and statistical significance of the effects of the factors

under study, as well as evaluating the interaction effects between the factors [20].

Nanoencapsulation of bioactive compounds has provided satisfactory results for the formulation of submicron particles, with yields ranging from 70% to 90% for small amounts of the sample [15,21]. This research aims to study, using a Box Behnken response surface design, different factors that directly influence the production obtaining of nanocapsules with *O. atropes* extract. Therefore, it is established that the nanoencapsulation process will protect the antioxidant activity of the phenolic compounds extracted from *O. atropes*.

METHODS

Materials: Young cladodes of *O. atropes* of 60 ± 30 g were collected in Ziracuaretiro, Michoacán, México. The identification of the *Opuntia* species was made in the herbarium of the Faculty of Biology of the Michoacana University of San Nicolás de Hidalgo (Morelia, Mexico). The nopal cladodes were washed, disinfected, cut into 1 cm pieces, oven-dried at 50°C/48h, triturated, and sieved until producing flour with a particle size ≤ 260 µm. Maltodextrin, calcium caseinate, folin-ciocalteu, gallic acid, quercetin, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, potassium persulfate, methanol and Amberlite XAD16N, with high purity, were obtained from Sigma Aldrich (Mexico). Sodium carbonate, sodium nitrite, sodium hydroxide, and aluminum chloride of reactive grade were obtained from MEYER. Soy protein isolate was obtained from GNC. Acetonitrile, methanol, water, and formic acid, all grade LC-MS, were purchased from Fisher Scientific Chemicals (Fair Lawn, NJ, USA).

Extraction of phenolic compounds: To extract the PC from *O. atropes*, 10 g of nopal flour was dispersed in 100 mL of 70% ethanol and placed in a shaking water bath SW22 (Julabo, Seelbach, Germany) at 100 rpm, 47.8 °C for 2 h. 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 RE100-Pro (DLAB Scientific Inc., USA) at a temperature of 40 °C. The aqueous extract was filtered again with Whatman No. 5 filter paper. The aqueous extract was used to carry out the wall material selection tests.

Selection of wall material and nanoencapsulation conditions process: Five different formulations were evaluated to be used in the nanoencapsulation process of the aqueous extract of *O. atropes*, which considered the following wall materials: F1, maltodextrin; F2, soy protein isolate; F3, soy protein isolate + maltodextrin (1/1); F4, calcium caseinate; and F5, calcium caseinate + maltodextrin (1/1). A ratio of wall material/extract of *O. atropes* (W/E) of 2.5/1 was used in all treatments. The solids content was adjusted to 1% in formulations F1, F4 and F5, and in F2 and F3, it was adjusted to 0.5% because they had a larger particle size. Solutions were vacuum filtered with Whatman No. 5 filter paper. The nanoencapsulation was carried out in a Nano Spray Dryer Unit B-90 (Büchi Labor Technik AG, Flawil, Switzerland). The parameters used were 7 µm mesh, 105 °C inlet temperature, 110 ± 1 L/min gas flow, 33 ± 1 mbar vacuum and 70% spray. The morphology and size of the nanocapsules obtained were analyzed by Scanning Electron Microscopy. The samples were metalized with copper under vacuum conditions and were observed in a scanning electron microscope JSM-7600F SEG-SEM (JEOL Ltd. UK).

Nanoencapsulation using RSM Based on the results obtained from the tests described in previos section, maltodextrin was the wall material selected to be included in all the treatments used in the Response Surface Methodology (RSM) experiments. The

combination of three levels, with three factors each, was considered in the experimental design used to nanoencapsulate the extracts, as follows: formulation W/E (maltodextrin or wall material /extract of O. atropes) being 1/1, 2.5/1, and 4/1, temperature of 95, 105 and 115 °C and spraying being 40, 70 and 100%, using a Box Behnken response surface design, to obtain 17 experimental runs which included 5 repetitions at the central point. Each treatment was adjusted to 1% solids and vacuum filtered with Whatman No. 5. Subsequently, the solution of each treatment was processed in a Nano Spray Dryer B-90B (Büchi Labortechnik AG, Flawil, Switzerland), the gas flow was maintained at 110 ±1 L/min and the vacuum was 33 ± 1 mbar and a 7 µm mesh was used. The response variables were: yield (%), feeding speed (mL/h), particle size evaluated in an SZ-100 nanoparticle analyzer (HORIBA, Ltd. Headquarters, Japan) encapsulation efficiency, phenolic acids [22.23], flavonoids [24] and antioxidant activity by capture of the radical DPPH• [23,25] and ABTS•+ [26].

Determination of the phenolic profile by UPLC-ESI-Q/TOF-MS²: For identification of PC in the spray-dried crude extract of *O. atropes*, purification was carried out with Amberlite XAD16N. 1 ml of the extract was placed in a glass Pasteur pipette that was used as a column for purification, and this was washed 3 times with 1 ml distilled water and the recovery of the extract was carried out with 3 ml absolute ethanol and dehydrated at 50 °C/24 h. Subsequently, a 500 ppm solution was prepared with HPLC grade water and was filtered on Whatman paper with a 0.2 µm pore and placed in 2 mL glass vials. For the identification of PC present in the nanocapsules, 300 mg nanocapsules were ruptured with 3 ml of methanol: acetic acid: water (50:8:42), stirred for 1 min in a Vortex VTX-5 (Scientific, USA) and 40 min in an ultrasonic bath (CIVEQ 8892) and centrifuged at 3087 g/10 min. The supernatant was washed 3 times with 3 ml of methanol: ethanol (1:1) and centrifuged at 3087 g/10 min after each wash. The supernatant from the 3 washes was placed in an oven at 50 °C to evaporate the solvent, and purification with Amberlite XAD16N was then carried out. The obtained sample was prepared at 500 ppm with HPLC grade water, filtered on paper with a 0.2 µm pore, and placed in 2 mL glass vials. For the evaluation of the polyphenolic profile, an Acquity ultra-high resolution chromatography was used. 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 (Q-TOF, WATERS, 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.

Statistical analysis: The nanoencapsulation study of the extract of *O. atropes* was performed using a Box-Behnken design by RSM. The results were analyzed through the Design Expert 11 statistical package. The chromatograms were produced using OriginPro 2016 software.

RESULTS AND DISCUSSION

Selection of wall material: Scanning electron microscopy (SEM) is commonly used to examine spray-dried powders as it provides valuable information on particle morphology, such as particle size, shape, and surface structure. In Figure 1 are shown the SEM images of the nanocapsules of the different treatments. The F1 presented well defined nanocapsules, with a spherical shape and a smooth surface. The particles obtained did not form aggregates, and the particle size is varied, where most of the particles are smaller than 1 µm, and according to what is described by Fang and Bhandari et al. [27], the capsules its in the nanometric scale, since the sizes of the particles formed through nano-spray encapsulation are classified into macro (> 5 mm), micro (1 µm to 5 mm) and nano (<1 µm). In F1, maltodextrin was used as a wall material. In F3, where soy protein isolate and maltodextrin (1/1) were used, nanocapsules of varied morphology were observed, from spherical to amorphous and some collapsed capsules. In F2, F4 and F5, a limited amount of nanocapsules were obtained. Most of them were irregularly shaped microcapsules, with a widely dented surface, which was attributed to the contraction of the particles during the drying process [17], and the chemical nature of the proteins used as the wall material. According to the results observed by SEM, the most appropriate material to carry out the nanoencapsulation of the *O. atropes* extract was maltodextrin. Maltodextrin minimizes PC contact with oxygen [28], lasts longer, releases encapsulated materials under simulated conditions of the digestive tract [29], provides non-hygroscopic properties [30], and therefore, could avoid the high hygroscopicity of nanocapsules during storage.

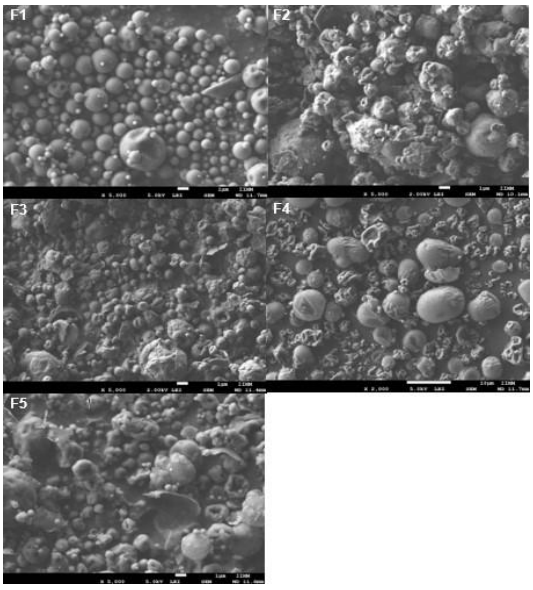

Figure 1. Micrographs of Nanocapsules obtained by SEM.

Fig. 1 shows the SEM images of the nanocapsules of the different treatments. The F1 presented well-defined nanocapsules, with a spherical shape and a smooth surface. The particles obtained did not form aggregates. In F1, maltodextrin was used as a wall material. In F3, where soy protein isolate and maltodextrin (1/1) were used, nanocapsules of varied morphology were observed, from spherical to amorphous and some collapsed capsules. In F2, F4, and F5, a limited amount of nanocapsules were obtained

Nanoencapsulation: To study the optimal conditions for nanoencapsulation of the extract of *O. atropes*, a Box-Behnken design of three factors with three levels each was used, with a total of 17 treatments, where yield, feeding speed, the efficiency of encapsulation, particle size, phenolic acids, flavonoids and antioxidant activity by capture of the radicals DPPH• and ABTS•+ were the response variables. In Table 1, it can be seen that T16 had a higher yield in the recovery of nanocapsules with 94.30%, a higher feeding rate of 18.20 mL/h and an encapsulation efficiency of 89.10%, which was related to a higher spray rate and which was also attributed to the amount of maltodextrin used, which was 4/1 in relation to the extract of *O. atropes*. In previous research, yields ranging from 76 to 96% have been reported for various substances [18], which is consistent with the present study. Gu et al. [31] showed that yield increases with increasing concentration of the wall polymer hypromellose acetate succinate. T2 had the lowest yield, that was 21.43%, and an encapsulation efficiency of 22.87% which was attributed to the fact that the formulation contained a greater amount of extract, which was 1/1 in relation to maltodextrin. The nanocapsules obtained in T2 showed hygroscopicity due to the extract of *O. atropes* which made their recovery difficult, affecting the performance and efficiency of encapsulation. In addition, it is probable that some PC were released during the recovery of the powder due to hygroscopicity, which could favor contact with free radicals thus causing their deterioration [32]. It also presented the lowest feeding rate of 2.80 mL/h, which was related to the low spray rate of the treatment, which was 40%.

The particle size presented by the different treatments ranged between 110-405 nm, with T6 being the smallest nanocapsules with 110.87 nm and T12 with the largest nanocapsules, 405.20 nm. The evaluation of the size of the obtained nanocapsules is important because the smallest particles exhibit a higher release rate of the active compound, compared to the larger ones due to the difference in the exposed surface area [33].

Table 1. Experimental Values for Nanoencapsulation of *O. atropes* extract.

Factors				Response Variables							
T	**F (W/E)**	**TE (°C)**	**SP (%)**	**Y (%)**	**FS (mL/h)**	**PS (nm)**	**EE (%)**	**PA (mg GAE/g)**	**FL (mg QE/g)**	**DPPH• (mg GAE/g)**	**ABTS•+ (mg GAE/g)**
1	1/1	95	70	67.97	7.23	233.80	43.34	**1.72**	8.78	0.65	**0.91**
2	1/1	105	40	**21.43**	**2.80**	163.43	**22.67**	1.66	**11.94**	0.70	0.82
3	1/1	105	100	39.46	12.00	229.80	25.59	1.70	9.85	**0.73**	0.82
4	1/1	115	70	24.62	7.40	127.77	66.35	1.68	8.33	0.40	0.74
5	2.5/1	95	40	50.00	3.00	194.17	31.91	**0.94**	5.80	0.48	0.47
6	2.5/1	95	100	83.13	6.00	**110.87**	53.41	0.96	5.37	0.36	0.48
7	2.5/1	105	70	81.08	11.26	236.63	79.59	1.44	2.84	0.51	0.45
8	2.5/1	105	70	76.10	10.40	268.67	82.42	1.42	1.65	0.51	0.50
9	2.5/1	105	70	70.98	10.00	176.70	81.40	1.41	2.25	0.51	0.47
10	2.5/1	105	70	72.04	9.93	175.30	80.12	1.40	2.20	0.46	0.44
11	2.5/1	105	70	63.24	12.30	200.43	82.37	1.40	2.51	0.48	0.44
12	2.5/1	115	40	58.45	4.00	**405.20**	32.57	1.24	5.83	0.46	0.51
13	2.5/1	115	100	85.22	9.00	154.77	55.69	0.95	7.02	0.29	0.45
14	4/1	95	70	83.24	8.50	225.03	72.23	0.98	**2.04**	**0.24**	**0.32**
15	4/1	105	40	88.89	8.80	243.67	86.77	1.02	2.33	0.35	0.41
16	4/1	105	100	**94.30**	**18.20**	275.00	**89.10**	0.98	1.55	0.34	0.32
17	4/1	115	70	81.27	7.30	146.93	80.72	0.97	1.31	0.25	0.44

T1 had the highest number of phenolic acids with 1.72 mg GAE/g and T2 the highest flavonoid content with 11.94 mg QE/g. The content of PC present in T1 and T2 was due to the higher extract content in their formulation and also indicated that phenolic acids are more thermosensitive than flavonoids, since a higher content of phenolic acids was obtained in a treatment at 95 °C, and a higher flavonoid content at 105 °C. In T5, a lower content of phenolic acids was obtained (0.94 mg GAE/g) and in T14, a lower content of flavonoids (2.94 mg QE/g). Hence, the content of phenolic acids was reduced by almost half in T5 compared to T1 and the flavonoid content was reduced four times in T14 compared to T2.

The highest antioxidant activity in DPPH$^{\bullet}$ was presented by the T3 treatment and by T1 in ABTS$^{\bullet+}$. These treatments presented a greater amount of extract in their formulation that was in a 1/1 ratio (maltodextrin/*O. atropes* extract). On the other hand, the T14 treatment was the one with the lowest antioxidant activity for the capture of the DPPH$^{\bullet}$ radical, which was 0.24 mg GAE/g, and for the ABTS$^{\bullet+}$ it was 0.32 mg GAE/g. T14 is one of the treatments that contain the largest amount of wall material with respect to the nucleus, which was 4/1 (maltodextrin/*O. atropes* extract), so it contains less *O. atropes* extract and therefore, also less antioxidant activity compared to the treatments that presented more of the extract of *O. atropes*.

Pisoschi *et al*. [34] indicated that nanoencapsulation can lead to a decrease in antioxidant activity due to the binding of the encapsulated compound to the biopolymer. In our study, this phenomenon could have contributed to the decrease in antioxidant activity in the treatments that contained more maltodextrin, since for the antioxidant activity in both DPPH• and ABTS•+, there was a decrease of 75% between the treatment with the highest value compared to the treatment with the lowest value for these two response variables.

Table 2 shows the correlations between the dependent variables analyzed, where a strong positive correlation was found between the concentration of phenolic acids and the antioxidant activity due to radical capture by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, being 0.81 in both cases. This indicates that when the phenolic acid content in the nanocapsules increases, the antioxidant activity of both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ also increases. A direct and high correlation was found in flavonoids with antioxidant activity due to capture of the ABTS$^{\bullet+}$ radical, being 0.86, so the greater the number of flavonoids in the nanocapsules, the greater the antioxidant activity based on the ABTS$^{\bullet+}$ radical. A high negative correlation was also obtained between flavonoids and encapsulation efficiency, being -0.86, which indicated that the higher the encapsulation efficiency, the lower the flavonoid content in the nanocapsules.

Four response variables were simultaneously optimized, of greatest interest for this research, which was: yield, encapsulation efficiency, flavonoids, and antioxidant activity by the capture of the ABTS$^{\bullet+}$ radical. The desirability model predicted avalor of 0.56 for these responses, and an optimal treatment (TO) was obtained. The process conditions were as follows: formulation ratio 1/1, temperature 104.7°C, and spray 88.3%

The model predicted a yield of 49.32%, encapsulation efficiency of 53.24%, flavonoids content of 8.43 mg QE/g, and antioxidant activity in ABTS$^{\bullet+}$ of 0.82 mg GAE/g. However, it was observed that T13 presented the best characteristics for nanoencapsulation of the extract of *O. atropes* compared to TO, since it presented a high yield of 85.22%, encapsulation efficiency of 55.69%, flavonoids content of 7.02 mg QE/g, and antioxidant activity in ABTS$^{\bullet+}$ of 0.45 mg GAE/g, being more favorable values than those predicted for the TO. Therefore, T13 was selected as the most suitable to carry out the nanoencapsulation of the extract of *O. atropes*, and both the nanocapsules with extract of *O. atropes,* and the extract of *O. atropes* without encapsulation, were characterized by UPLC-Q/TOF-MS^2 to compare the polyphenolic profile of the *O. atropes* extract in both free and nonencapsulated form.

Table 2. Correlation between the response variables in the nanoencapsulation process

	Feeding speed (mL/h)	Yield (%)	Encapsulation efficiency (%)	Phenolic acids (mg GAE/g)	Flavonoids (mg QE/g)	DPPH• (mg GAE/g)	ABTS •+ (mg GAE/g)
Feeding speed (mL/h)	1.00						
Yield (%)	0.48	1.00					
Encapsulation efficiency (%)	0.63**	0.66**	1.00				
Phenolic acids (mg GAE/g)	-0.01	-0.68**	-0.28	1.00			
Flavonoids (mg QE/g)	-0.48	-0.77**	**-0.86****	0.53*	1.00		
DPPH• (mg GAE/g)	-0.12	-0.64**	-0.57*	**0.81****	0.62**	1.00	
ABTS•+ (mg GAE/g)	-0.33	-0.74**	-0.65**	**0.81****	**0.86****	0.77**	1.00

Significant correlation at the level 0.01** y 0.05*. n=17.

Identification of polyphenolic compounds by UPLC-Q / TOF-MS²: Phenolic compounds in a food matrix are generally free, esterified, and/or linked to other macromolecules, such as polysaccharides, fiber, and proteins [35]. The application of solvents, such as ethanol and water, favors the extraction of these PCs either in their free form (aglycone) or as glycosides.

The chromatographic profiles obtained by UPLC-Q/TOF-MS² of both the nanocapsules with *O. atropes* extract and the *O. atropes* extract (T13) are presented in Table 3A and 3B respectively.

Each PC of both the *O. atropes* extract and nanocapsules with the *O. atropes* extract was identified according to their characteristic aglycone fragment ions by interpreting their fragmentation patterns and by interpreting the spectra of masses that were obtained and it was compared with the information reported in databases such as Phenol-Explorer and MassBank, and with previous research conducted by Guevara et al. [36], Santos et al. [37], Astello et al. [38], Melgar et al. [39] and Mena et al. [40] for the identification of the compounds. The UPLC-Q/TOF-MS2 results for nanocapsules with *O. atropes* extract are presented in Figure 2 (A) and Table 3 (A).

Eighteen peaks were identified, mainly associated with flavonoids of the flavonol type in their glycosidic form, and most of them were tentatively identified as kaempferol glycosides and isorhamnetin glycosides. Kaempferol glycosides were identified at peaks 5, 6, 7, 8, 9, 12, 13, 14, and 16, with isorhamnetin glycosides at peaks 10, 11, 15, 17, and 18. Peak 2 presented the aglycone of isorhamnetin, peak 3 corresponded to *p*-coumaric acid 4-*O*-glucoside and in peak 4, ferulic acid 4-*O*-glucoside was identified. Meanwhile, medioresinol, which is a lignan, was also present in peak 1. The results obtained were similar to those reported because they predominantly found flavonoids in their glycosidic forms in different varieties of cladodes of *O. ficus-indica*, mainly derived from kaempferol and isorhamnetin [36,37,41,42]. The methodology used in this study provides information on the proportions in which the PCs, which are mainly flavonoid glycosides, are present in the crude extract. As can be seen in the chromatogram in Figure 2 (A) and Table 3 (A), the proportion of kaempferol glycosides with respect to isorhamnetin glycosides is higher, and quercetin was not found in its free or glycosylated form as reported by other authors [36,38]. Ferulic acid glycosides have been identified in different varieties of cladodes of the genus *Opuntia*

[36,38] and the presence of glucosides from coumaric acid [36]. In our study, peak 1 corresponded to medioresinol, a lignan, which has not been reported so far in the polyphenolic profile of the *Opuntia* genus, and it was found in a considerable proportion with respect to the flavonoids. Hence, the presence of this lignan increases the number of bioactive compounds in the cladodes of *O. atropes* and, therefore, is of interest to human health. The differences in the polyphenolic profile of the *O.* genus are due to the type of cultivar, stage of maturity, place of origin, harvest season, and environmental conditions [41]. The results obtained for the polyphenolic profile of the extract of *O. atropes* are shown in Figure 2 (B) and Table 3 (B), noting 17 peaks that correspond to PC like those presented in the nanocapsules. The group that predominated were flavonoids of the flavonol type, which were glycosides of kaempferol and isorhamnetin. Peaks 5, 6, 7, 8, 11, 12, 13, and 15 were from kaempferol glycosides, peak 2 indicated the presence of isorhamnetin in the form of aglycone, and peaks 9, 10, 14, 16, and 17 corresponding to isorhamnetin in its glycosylated form. At peak 1, medioresinol was identified, and peaks 3 and 4 corresponded to ferulic acid 4-O-glucoside isomers.

It should be noted that p-coumaric acid 4-O-glucoside was only identified in the nanocapsules, which could have been retained by maltodextrin at the time of being processed in the nano spray dryer. It was also observed that peaks 10 and 11 (Fig. 2 (A)), had greater intensity than peaks 9 and 10 (Fig. 2 (B)). These peaks corresponded to isorhamnetin 3-O-rhamnoside-7-O-(rhamnosyl-hexoside) isomers, and according to the retention time in which they were identified, the same compounds were found in both the extract and the nanocapsules. Hence, it is inferred that the wall material used in the nanocapsules could have contributed to the protection of the isorhamnetin glycosides, and therefore in the decrease in the degradation.

Table 3. Polyphenolic profile of nanocapsules with *O. atropes* extract (treatment 13) obtained by UPLC-Q/TOF-MS^2.

Peak N°	Rt (min)	$[M-H]^-$ (m/z)	Ms^2 Fragment of the molecular ion	Tentative assignment	Polyphenol class	Polyphenol family	Molecular formula
A.	**Nanocapsules whith *O. atropes* extract**						
1	0.80	387.1436	342.1453	Medioresinol	Lignan	Lignans	$C_{21}H_{24}O_7$
2	1.33	315.1125	175.0105	Isorhamnetin	Flavonoid	Flavonols	$C_{16}H_{12}O_7$
3	1.98	325.1315	163.0905	*p*-Coumaric acid 4-*O*-glucoside	Phenolic acid	Hydroxycinnamic acids	$C_{15}H_{18}O_8$
4	2.25	355.1382	175.0108	Ferulic acid 4-*O*-glucoside	Phenolic acid	Hydroxycinnamic acids	$C_{16}H_{20}O_9$
5	2.79	755.1544	375.1250	Kaempferol 3-*O*-glucosyl-rhamnosyl-glucoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{20}$
6	3.28	739.1664	173.1364	Kaempferol 3-*O*-(rhamnosyl-galactoside)-7-*O* rhamnoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
7	3.34	739.1675	175.0103	Kaempferol 3-*O*-(rhamnosyl-galactoside)-7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
8	3.40	739.1659	175.0101	Kaempferol 3-*O* (rhamnosyl-galactoside)-7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
9	3.44	739.1636	431.2135	Kaempferol 3-*O*-(rhamnosyl-galactoside)-7-*O*-rhamnoside (Isomer III)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
10	3.56	769.1654	739.1613	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$

Peak N°	Rt (min)	$[M-H]^-$ (m/z)	Ms2 Fragment of the molecular ion	Tentative assignment	Polyphenol class	Polyphenol family	Molecular formula
11	3.70	769.1671	739.1619	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (Isomer I)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
12	3.97	593.1393	217.1594	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
13	4.12	593.1398	175.0105	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
14	4.30	593.1395	175.0103	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
15	4.36	623.1449	175.0109	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$
16	4.74	593.1408	175.0108	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer III)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
17	4.84	623.1441	175.0107	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$
18	5.00	623.1422	175.0103	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$
B. *O. atropes* extract							
1	0.78	387.1415	342.1432	Medioresinol	Lignan	Lignans	$C_{21}H_{24}O_7$
2	1.33	315.1111	175.0099	Isorhamnetin	Flavonoid	Flavonols	$C_{16}H_{12}O_7$
3	2.29	355.1360	175.0097	Ferulic acid 4-*O*-glucoside	Phenolic acid	Hidroxycinnamic acids	$C_{16}H_{20}O_9$
4	2.36	355.1363	175.0099	Ferulic acid 4-*O*-glucoside (Isomer I)	Phenolic acid	Hidroxycinnamic acids	$C_{16}H_{20}O_9$
5	2.87	755.1499	361.1723	Kaempferol 3-*O*-glucosyl-rhamnosyl-glucoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{20}$
6	3.40	739.1607	643.1956	Kaempferol 3-*O*-(rhamnosyl-galactoside)-7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
7	3.46	739.1620	175.0097	Kaempferol 3-*O*-(rhamnosyl-galactoside)-7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
8	3.54	739.1603	365.2122	Kaempferol 3-*O*-(rhamnosyl galactoside)-7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{33}H_{40}O_{19}$
9	3.68	769.1613	739.1583	Isorhamnetin 3-*O*-rhamnoside-7-*O*- (rhamnosyl-hexoside)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
10	3.82	769.1624	739.1594	Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (Isomer I)	Flavonoid	Flavonols	$C_{34}H_{41}O_{20}$
11	4.11	593.1376	175.0099	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
12	4.28	593.1367	175.0098	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
13	4.45	593.1371	175.0099	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
14	4.52	623.1407	175.0099	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	Flavonoides	Flavonols	$C_{28}H_{32}O_{16}$
15	4.91	593.1359	175.0096	Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside (Isomer III)	Flavonoid	Flavonols	$C_{27}H_{30}O_{15}$
16	5.00	623.1403	175.0099	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside (Isomer I)	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$
17	5.15	623.1392	175.0095	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside (Isomer II)	Flavonoid	Flavonols	$C_{28}H_{32}O_{16}$

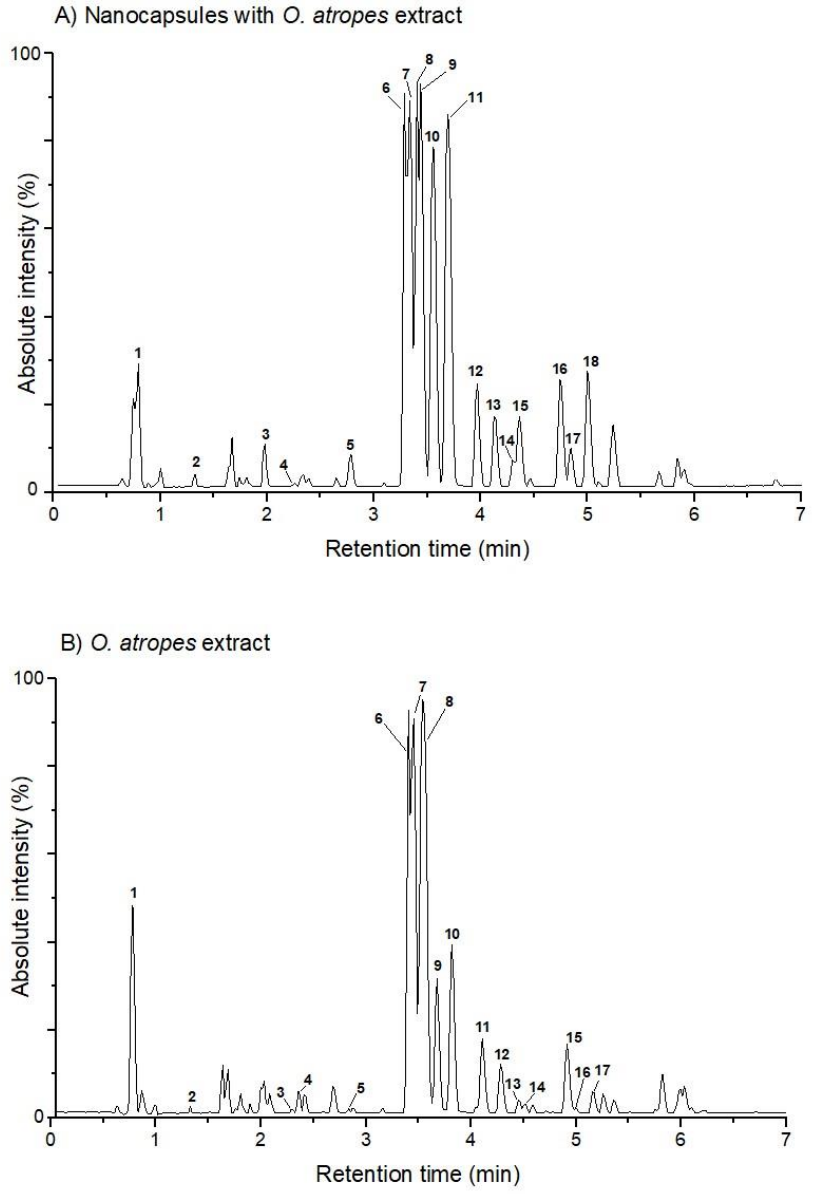

Figure 2. Peak chromatogram of the polyphenolic compounds obtained by UPLC-Q/TOF-MS^2. For the assignment of each peak see Table 3A and 3B.

CONCLUSIONS

Maltodextrin presented better properties in the encapsulation of the extract of *O. atropes* by forming well-defined, spherical, nanometric capsules. A similar polyphenolic profile was identified in the extract of *O. atropes* and nanocapsules with the extract of *O. atropes*, with most of the compounds being glucosides of kaempferol and isorhamnetin. Detection of medioresinol lignan was found for the first time. Nanoencapsulation contributed to the preservation of the polyphenolic compounds of the extract of *O. atropes*, mainly isorhamnetin 3-O-rhamnoside-7-O-(rhamnosyl-hexoside) isomers and p-coumaric acid 4-O-glucoside.

List of Abbreviations: *O. atropes: Opuntia atropes*, EQ / g: quercetin equivalent / gram, EAG / g: gallic acid equivalent / gram, PC: phenolic compounds, RSM: response surface methodology, DPPH$^{\bullet}$: 1,1-dipheny l-2-picrilhydrazyl, ABTS$^{\bullet+}$: 2,2'-azino-bis-3-

ethylbenzothia-zolin acid-6-sulfonic, W/E maltodextrin or wall material /extract of *O. atropes*, SEM: Scanning Electron Microscopy, UPLC: ultra-high-resolution liquid chromatograph, F1: Nanocapsules of maltodextrin with *O. atropes* extract, F2: Nanocapsules of soy protein isolate with *O. atropes* extract, F3: Nano capsules of soy protein and maltodextrin with *O. atropes* extract, F4: Nanocapsules of calcium caseinate with *O. atropes* extract, F5: Nanocapsules of calcium caseinate and maltodextrin with *O. atropes* extract, T: Treatment, F: Formulation, TE: Temperature, SP: Spray, Y: Yield, FS: Feeding speed, PS: Particle size, EE: Encapsulation Efficiency, PA: Phenolic Acids, FL: Flavonoids, UPLC-Q/TOF-MS2: Ultra High Resolution Liquid Chromatograph coupled to an accelerated orthogonal mass spectrometer equipped with an electrospray ionization source, Rt: retention time.

Conflict of interest: The authors have no conflicts of interest to declare.

Author's contributions: *Tranquilino-Rodriguez performed the experimental research, Martinez Flores and **Rodiles-Lopez designed the study, ***Martinez-Avila contributed to the analysis of compounds obtained by UPLC-Q/TOF-MS^2.

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