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Research Article

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Antioxidant Capacity of a Nanoencapsulated Phenolic Extract from *Opuntia atropes* during Corn Tortilla Processing and *In Vitro* Digestion

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Abstract

Corn tortillas are the staple food of central American countries, and their consumption has spread to various parts of the world. However, consumers currently seek more nutritious foods that contain health-promoting compounds. The genus *Opuntia* spp. is a source of bioactive compounds that protect against chronic diseases in humans. The objective of this study was to evaluate the stability of phenolic compounds and the antioxidant capacity of a nanoencapsulated extract of *Opuntia atropes* added to corn tortillas. Phenolic compounds extracted from *O. atropes* cladodes were dehydrated and nanoencapsulated using maltodextrin as the wall material. The encapsulated phenolic extract was incorporated into corn flour, and tortillas were prepared. The total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and $ABTS^{+}$ (2,2'-azino-bis(3-ethylbenzothiazoline 6-sulfonic acid)) radical scavenging in the tortillas were evaluated. *In vitro* digestion was performed to evaluate the stability of the above compounds. Incorporating nanocapsules containing *O. atropes* extract significantly increased the phenolic compound content and antioxidant capacity of the tortillas, yielding values of 0.3885 mg gallic acid equivalents (GAE)/g for total phenols, 0.2421 mg QE/g for total flavonoids and 0.1597 GAE/g for antioxidant capacity by DPPH and 0.2292 mg GAE/g for $ABTS^{+}$ radical scavenging. Likewise, it was shown that, after *in vitro* digestion, the tortillas with nanocapsules (TN) showed a higher content of total phenols and flavonoids that were 0.7109 mg GAE/g and 0.1769 mg QE/g respectively, and higher antioxidant activity by capture of the DPPH radical of 0.2028 GAE/g and $ABTS^{+}$ of 0.3307 mg GAE/g with respect to the control tortillas (CT). It was feasible to incorporate nanocapsules containing *O. atropes* extract, thereby increasing the content of bioactive compounds and preserving the antioxidant capacity of corn tortillas.

Keywords

Corn tortilla, *Opuntia atropes*, Phenolic compounds, Nanoencapsulation, Antioxidant capacity, *In vitro* digestion

Introduction

Mexico is the center of origin of corn (*Zea mays* L.) [1], which is an essential crop in the diet of the Mexican and central American people. After its domestication, maize began to spread from southern Mexico into north and south America [2]. Nixtamalization, the process of cooking maize in alkaline water, is the central technique for the culinary use of maize in Mexico and central America [4]. In this thermal-alkaline process, maize kernels are treated with calcium hydroxide, rested, and ground to obtain a dough that is used to make corn tortillas, which are the most important staple food of the Mexican population [3]. According to the

National Council for the Evaluation of Social Development Policies (CONEVAL), the Mexican population consumes an average of 6 tortillas per person per day in urban areas and 9 in rural areas. Garnica-Romo et al. [5] reported a content of 162 mg of calcium/100 g of tortillas, 7.27% protein, 4.98% fiber, 80.22% carbohydrates, and 4.03% fat, which indicates that tortillas provide an average of 26.4% of daily calcium in urban areas and 39.6% in rural areas (based on 1000 mg of calcium/day), 16.8% of daily protein in urban areas and 25.2% in rural areas (based on a 70 g intake of protein/day), 32.2% of daily fiber in urban areas and 48.4% in rural areas (based on 25 g of fiber intake/day), and 28.4% of total calories in urban areas and 42.7% in rural areas (considering the caloric contribution of proteins, fats, and carbohydrates, based on a 2,200 kcal/day).

Bioactive compounds in corn tortillas have also been studied. These include antioxidants, such as carotenoids, ascorbic acid, and phenolic compounds or polyphenols. According to Astorga-Gaxiola et al. [6], phenolic compounds contribute to the daily intake when tortillas are consumed. White corn tortillas contain 173.61 mg GAE/100 g of phenolic compounds. Six tortillas per day contribute 323.44 mg of phenolic compounds. The intake of phenolic compounds depends on dietary habits and preferences and varies between countries [7]. For example, the daily intake of polyphenols in European countries is approximately 900 mg, while in North and South American countries it is around 900 and 800 mg, respectively [8]. Poland and France had an intake of total polyphenols >1000 mg/day, and Spain was reported at 300 mg/day [8]. In Mexico, Zamora-Ros et al. [9] reported the daily median intake of total polyphenols, flavonoids, phenolic acids, and other polyphenols in adult Mexican women at 694, 235, 361, and 15.1 mg/d, respectively. Marín-Canul et al. [10] reported an intake of polyphenols and flavonoids of 1216 and 121 mg/day in students from Southeastern Mexico.

The tortilla contributes to the daily intake of phenolic compounds in Mexico. However, due to its high consumption in the country, the tortilla could be improved by adding different sources of phenolic compounds capable of preventing chronic degenerative diseases associated with oxidative stress. Cellular oxidation generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) in living organisms, which, when in excess, cause oxidative stress and damage to biological systems [11]. An excess of ROS induces lipid peroxidation, destabilizes and disintegrates cell membranes, and oxidizes DNA and proteins [12], generating several diseases which are associated with oxidative stress. The hydroxyl groups of phenolic compounds can donate a hydrogen atom or an electron to a free radical and therefore act as antioxidants, preventing oxidative stress.

It is essential to measure the antioxidant capacity of chemical compounds that can neutralize the oxidizing action of ROS. *In vitro* antioxidant capacity tests have been developed in which radicals are generated and placed in contact with an antioxidant agent. Antioxidant agents can be compounds such as phenolics and vitamins such as A, C, E and their derivatives, among others. In the literature, several tests are reported to evaluate the antioxidant capacity in which the transfer of a hydrogen atom is involved (for example, the oxygen radical absorption capacity (ORAC test), the hydroxyl radical antioxidant capacity (HORAC test), the total peroxyl radical trapping antioxidant parameter (TRAP test)), or the tests based on the transfer of an electron include the cupric reducing antioxidant power (CUPRAC test) and the ferric reducing antioxidant power (FRAP test). However, there are also tests that involve the transfer of both a hydrogen atom and an electron (for example, ABTS and ABTS tests) [13]. Therefore, the selection of the above-mentioned tests to measure the antioxidant capacity of phenolic compounds such as ABTS and DPPH are reliable.

In the literature, it had been mentioned that dietary sources of phenolic compounds protect against chronic diseases such as cardiovascular and neurodegenerative diseases, diabetes, and cancer [14]. Worldwide, throughout history, numerous plants have been used as a source of phenolic compounds to prevent diseases related to oxidative stress. Among such plants, extracts of *Opuntia* spp., contain phenolic acids (gallic acid, p-coumaric acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, ferulic acid, and salicylic acid) and flavonoids (isoquercetin, isorhamnetin-3-O-glucoside, nicotiflorin, rutin, and narcissin) [15]. Phenolic compounds have beneficial effects on cardiovascular and Alzheimer's disease as well as anti-inflammatory, antidiabetic, anticancer, and hepatoprotective properties [16].

Cladodes of various *Opuntia* species contain more than 40 phenolic compounds [17]. In a study using an ethanol solution at 80% to extract polyphenols in cladodes of *Opuntia ficus-indica*, values of 250.86 mg GAE/100 g and 18.22 mg rutin/100 g were reported for TPC and TFC [18]. Similarly, *Opuntia stricta* was documented to have values for TPC and TFC of 18.014 mg eq gallic acid/100 g and 18.75 mg eq rutin/100 g, respectively [19].

However, phenolic compounds are susceptible to degradation and loss of antioxidant capacity when exposed to factors such as light, oxygen, and temperature. Therefore, they must be protected from such environmental factors. Micro- and nano-encapsulation are techniques in which bioactive compounds are coated with a wall material such as maltodextrin, chitosan, alginate and other polymers that have been studied extensively for their ability to protect phenolic compounds from environmental factors and preserve their antioxidant capacity. In a previous study conducted by our working group, it was demonstrated that the use of maltodextrin as a nanoencapsulating agent for *O. atropes* extract allowed for more efficient preservation of phenolic compounds, mainly flavonoids such as isorhamnetin and kaempferol glucosides [20]. The objective of this study was to obtain a nanoencapsulated phenolic extract from *O. atropes* cladodes and evaluate its phenolic and antioxidant stability when incorporated into corn tortillas and following an *in vitro* digestion process.

Materials and Method

O. atropes

Cladodes of the nopal plant were collected in Ziracuaretiro, Michoacán, Mexico, from a farm specializing in the production of this plant. Cladodes, flowers, and immature fruits were collected and transferred to the Herbarium of the Uni-

versidad Michoacana de San Nicolás de Hidalgo in Morelia, Michoacán, Mexico. The specimen was registered with the herbarium folio number EBUM a27586, and the specified name is *O. atropes* rose. The cladodes of *O. atropes* rose identified and used in the study comply with local or national guidelines with no need for further affirmation.

Extraction of phenolic compounds

The cladodes of *O. atropes* were disinfected, cut into 1 × 1 cm pieces, and dehydrated at 50 °C for 48 h in a convection oven (Ecoshel 9053A, Pharr, TX, USA). The samples were ground and passed through a #60 sieve (U.S.A. Standard Testing Sieve A.S.T.M.E-11 Specification, Daigger, USA) to obtain flour with a particle size <250 µm.

Phenolic extract of *O. atropes* (EOA) was obtained using a 1:10 w/v ratio of cladodes flour:ethanol (70%). The sample was shaken in a water bath (SW22, Julabo, Seelbach, Germany) at 100 rpm and centrifuged at 3.087 g/10 min. The solution was filtered under vacuum through Whatman filter paper to obtain a particle size <2.5 µm and placed in Falcon tubes to determine the phenolic content and antioxidant activity by DPPH and $ABTS^+$ radical scavenging.

TPC and antioxidant capacity

TPC were analyzed using the Folin-Ciocalteu method [21], with some modifications [22]. A 250 µL aliquot of the extract was added to 250 µL of 2 N Folin-Ciocalteu reagent and 250 µL of 20% Na_2CO_3, shaken, and incubated at 40 °C for 30 min. Distilled water (2 ml) was added, and the mixture was vortexed. The absorbance of the samples was read in a spectrophotometer (VELAB, VE-5600UV, TX, USA) at a wavelength of 750 nm. Gallic acid was used as the standard. The results were expressed as mg GAE/g.

TFC were evaluated [23], a 150-µL aliquot of each sample was mixed with 150 µL 5% $NaNO_2$, 150 µL 10% $AlCl_3$, and 1 ml 1 M NaOH. The absorbance of the samples was read on a spectrophotometer at a wavelength of 510 nm. Quercetin was used as a standard, and the results are expressed as quercetin equivalents (mg QE/g).

DPPH radical scavenging was determined [24], with modifications [22]. Briefly, 50 µL of each sample was mixed with 2.95 ml of 60 µM DPPH solution. The samples were vortexed for 10 s, left in the dark for 30 min, and the absorbance was read at 517 nm in a VELAB spectrophotometer. The results were expressed according to the calibration curve prepared with the same standard.

$ABTS^+$ radical scavenging was evaluated [25]. A stock solution containing 2:1 v/v of 7 mM $ABTS^+$ and 2.45 mM potassium persulfate solution was mixed and allowed to stand at room temperature for 16 h. It was then adjusted with ethanol to an absorbance of 0.70 nm at 734 nm. Then, 15 µL of each sample was mixed with 1485 µL of the prepared $ABTS^+$ solution. After 15 min, the absorbance was measured at 734 nm in a spectrophotometer. The results were expressed as mg GAE/g.

Dehydration and nanoencapsulation of *O. atropes* extract

The phenolic extracted was dried with 70% ethanol. Previously, ethanol was removed from the *O. atropes* extract in a vacuum rotary evaporator at 40 °C. The extract obtained was filtered and adjusted to 1% total solids. Dehydration was performed in a nano spray dryer unit B-90 (Büchi Labortechnik AG, Flawil, Switzerland). The process parameters were, inlet temperature of 105 °C, gas flow of 110 L/min, vacuum of 33 mbar, spraying of 70%, and mesh size of 7 µm. The yield percentage of the extracts obtained in powder form was determined.

For nanoencapsulation of the dehydrated extract, an aqueous solution of maltodextrin was prepared with the dehydrated EOA (2.5/1 ratio). The suspension was adjusted to 1% solids, filtered, and processed with a nano spray dryer B-90 Büchi, the gas flow was maintained at 110 L/min, and the vacuum was 33 mbar, using a 7 µm mesh. The nanocapsules obtained were subjected to scanning electron microscopy (SEM) to determine their size and morphology. The samples were metallized with copper under vacuum conditions and observed under a SEM (JSM-7600F SEG-SEM, JEOL Ltd., Tokyo, Japan). In addition, the TPC, TFC, DPPH, and ABTS radical-scavenging activities were measured in the nanocapsules.

Nixtamalization of maize and production and characterization of nixtamalized maize flours control (NMFC)

Sinaloa white corn purchased from PURP, SA de CV, Sinaloa, Mexico was used to prepare the tortillas. The procedure consisted of cooking 1 kg of maize with 3 L of water and 10 g of calcium hydroxide in a gas oven at 92 °C for 35 min. The cooked corn grains were left in a steeping mode at room temperature for 18 h. The cooking liquid was decanted. The cooked maize was washed with 3 L of water and ground in a stone mill to form the dough. The dough was dried in an oven at 50 °C/24 h, ground, and passed through a 60 mesh sieve (Daigger, USA) to obtain the NMFC with a particle size of <250 µm. Then, NMFC was added to 3% nanocapsules containing the *O. atropes* extract (NEOA) and was called NMFN. Then, TPC, TFC, DPPH, and $ABTS^+$ radical-scavenging activities were determined in in NMFC and NMFN.

Production and characterization of tortillas

To prepare the tortillas, the NMFC and NMFN were rehydrated with water until they reached 60% moisture to form the doughs. Then, dough pieces of 30 g were compressed with a tortilla machine to form disks of 13.5 cm diameter and 1.2 mm thickness, and cooked on an iron griddle heated to 250 °C. Each tortilla was cooked on the first side for 17 s, on the second side for 55 s, and on the first side again for 17 s. Two products were obtained as follows: CT (made from NMFC), and tortilla prepared with NEOA TN (prepared from NMFN).

Tortillas were analyzed for moisture, fat, ash, and protein using methods 934.01, 942.05, 3010, and 32.1.22 of the Association of Official Analytical Chemists (AOAC) [26]. Carbohydrates were determined by difference as follows:

% carbohydrates = 100 - (% protein + % fat + ash).

Some quality parameters of tortillas were measured as follows: 1) degree of puffing: three tortillas from each treatment (CT and TN) were cooked, and the puffing of tortillas was recorded observing the percentage of total surface that

puffed, on a scale of 1 to 3, where 1 = puffing between 75 and 100%; 2 = puffing between 25 and 75% and 3 = puffing between 0 and 25% [27]; 2) Tensile strength: tensile tests was performed on three tortillas from each treatment (CT and TN). For the tensile test, a strip 32 mm long, 16 mm wide, and 1.2 mm thick was cut from the central part of the tortilla. The tensile test was performed using a Brookfield CT3 Texture Analyzer (Brookfield Engineering Lab. Inc, Middleboro, MA, USA) with the TA-26 probe. This accessory consisted of a set of gripping pliers in which the tortilla strip was placed and subjected to tension until the rupture. The maximum force required (N) was obtained from the resulting curve. The test conditions were speed 2 mm/s and distance 15 mm; 3) Cutting force: for the cutting test, the same equipment was used applying a flat blade on the central part of the tortilla. The test conditions were speed 2 mm/s, compression distance 10 mm. The N required to cut the tortilla was recorded.

Digestion *in vitro*

The *in vitro* digestion test was performed in dehydrated *O. atropes* extract, NEOA, CT, and TN. The method used was adapted from [28]. The tortillas were previously subjected to a grinding process in a blender for 1 min to simulate chewing. Then, the samples were separately placed in Erlenmeyer flasks, mixed with 0.9% saline, and adjusted to a volume of 20 ml. The samples were acidified to pH 2.0 with 0.5 N HCl, and 1 ml of a porcine pepsin preparation (0.04 g pepsin in 1 ml 0.1 M HCl) was added and incubated at 37 °C in a shaking water bath (SW22, Julabo, Seelbach, Germany) at 95 rpm for 1 h. Then, 5 ml of each sample was centrifuged at 3.087 g/10 min for subsequent analysis of TPC, TFC, and antioxidant activity by DPPH and $ABTS^+$ radical scavenging. The pH was then adjusted to 5.3 with 0.9 M sodium bicarbonate, followed by the addition of 150 µL bile salts: glycolate (0.04 g in 1 ml saline), taurodeoxycholate (0.025 g in 1 ml saline), taurocholate (0.04 g in 1 ml saline), and 75 µL pancreatin (0.04 g in 500 µL saline). The pH was adjusted to 7.4 with 1 M NaOH. The samples were incubated in a shaking water bath (95 rpm) at 37 °C for 2 h to complete the *in vitro* duodenal digestion. Then, the content was centrifuged (CRM GLOBE, Centrificient TD5M-WS, USA) at 3.087 g/10 min and analyzed for TPC, TFC, and antioxidant activity by DPPH and $ABTS^+$ radical scavenging.

Statistical analysis

The results of the present investigation were expressed as mean ± standard deviation. The data were subjected to Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. The analysis was performed using JMP6 software (SAS Institute, Cary, NC, USA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Phenolic extract from *O. atropes* and its characterization

Dehydrated and milled *O. atropes* cladodes were placed in 70% ethanol solution at 48 °C for 2 h to obtain *O. atropes* extract. The EOA obtained by nano spray drying was a fine, cream-colored powder. The values of TPC, TFC, and antioxidant capacity by DPPH and $ABTS^+$ radical scavenging (Table 1) was 11.80 ± 0.61 mg GAE /g, 22.26 ± 1.02 QE/g, 1.65 ± 0.03 mg GAE/g, and 5.70 ± 0.05 mg GAE /g, respectively.

Table 1: Yield, encapsulation efficiency, TPC, TFC and antioxidant capacity of the dried *O atropes* extract.

Parameter	EOA dehydrated	NEOA
Yield (%)	-	85.22 ± 2.93
Encapsulation efficiency	-	55.69 ± 0.82
Total phenolic acids (mg GAE/g)	11.80 ± 0.61[a]	3.32 ± 0.35[b]
Total flavonoids compounds (mg QE/g)	22.26 ± 1.0[a]	24.57 ± 0.66[a]
DPPH (mg GAE/g)	1.65 ± 0.03[a]	1.015 ± 0.10[b]
$ABTS^+$ (mg GAE /g)	5.70 ± 0.05[a]	1.57 ± 0.02[b]

Note: Results are expressed as the mean ± standard deviation of three determinations. Means in the same row with different superscripts are significantly different ($p < 0.05$).

Nanoencapsulation of *O. atropes* extract

The recovery of nanocapsules during nanoencapsulation of the phenolic EOA was 85.22%. The encapsulation efficiency of the phenolic EOA was 55.69%. The TPC, TFC, $ABTS^+$, and DPPH contents of the nanocapsules were 3.32 ± 0.35 mg GAE/g, 24.57 ± 0.66 mg QE/g, 1.015 ± 0.010 mg GAE/g, and 1.57 ± 0.02 mg GAE/g, respectively. The nanocapsules had a smooth surface, little agglomeration between particles, and a spherical and well-defined morphology (Figure 1). Particles of varying size, mostly smaller than 1 µm, were observed, thus falling on the nanometric scale [29]. It has been reported that maltodextrin prolongs the shelf life of encapsulated phenolic compounds and releases the encapsulated materials under simulated digestive tract conditions [30].

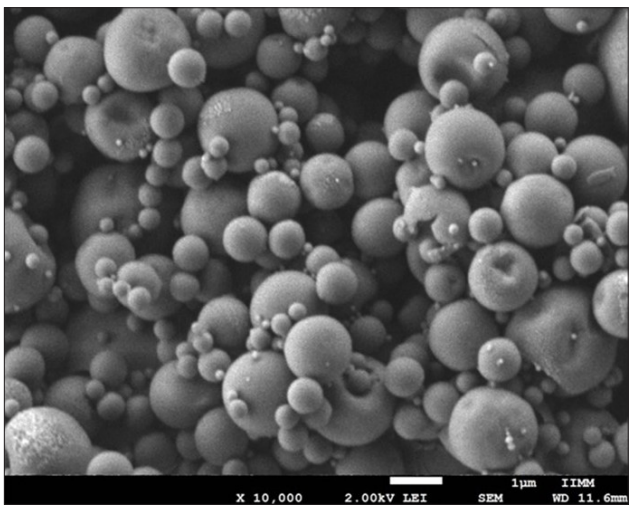


Figure 1: Micrograph of nanocapsules obtained by SEM using maltodextrin as the encapsulation material for *O. atropes* extract.

Chemical composition, phenolic compounds, and antioxidant capacity in flours and tortillas

The chemical composition of flours and tortillas is shown in table 2. The protein content of flours and tortillas was in the range of 8.46% to 8.86%. Heredia-Sandoval et al. [31] obtained a value of 8.6% in nixtamalized corn tortillas, and

Table 2: Chemical composition, TPC, TFC, and antioxidant capacity of nixtamalized flours and tortillas.

Parameter (dry basis)	NMFC	NMFN	CT	TN
Proteins (%)	8.65 ± 0.03^{a,b}	8.46 ± 0.10^{b}	8.86 ± 0.15^{a}	8.53 ± 0.18^{a,b}
Fat (%)	3.91 ± 0.05^{a,b}	4.04 ± 0.21^{a}	3.73 ± 0.28^{a,b}	3.34 ± 0.25^{b}
Ash (%)	1.47 ± 0.01^{b}	1.61 ± 0.06^{a}	1.48 ± 0.00^{b}	1.53 ± 0.01^{a,b}
Carbohydrates (%)	85.96 ± 0.02^{b}	85.57 ± 0.11^{b}	85.92 ± 0.24^{b}	86.58 ± 0.20^{a}
TPC (mg GAE/100 g)	42.00 ± 0.01^{b}	48.01 ± 0.01^{a}	32.98 ± 0.01^{d}	38.85 ± 0.01^{c}
TFC (mg QE/100 g)	24.95 ± 0.04^{b}	46.23 ± 0.04^{a}	ND	24.21 ± 004^{b}
DPPH (mg GAE/100 g)	18.83 ± 0.01^{a}	19.17 ± 0.01^{a}	12.09 ± 0.01^{c}	15.97 ± 0.01^{b}
ABTS^{+} (mg GAE/100 g)	20.62 ± 0.01^{b}	28.26 ± 0.01^{a}	14.82 ± 0.02^{c}	22.92 ± 0.01^{b}

Note: N = 3 replicates ± standard deviation. Means in the same row with different superscripts are significantly different ($p < 0.05$).

Hassan et al. [32] reported a range of 6.7% to 11.6% protein in nixtamalized corn flours. The ash content of flour and tortillas varied from 1.47% to 1.61%, and similar mineral values were documented, 1.23% for maize flour and 1.04% for tortillas [33].

The TPC, TFC and antioxidant capacity of flour and tortillas are presented in table 2. There was a significant 14.28% increase ($p < 0.05$) in the TPC in NMFN with respect to NMFC. TFC increased by 85.29% in NMFN compared with NMFC. There was no significant difference ($p > 0.05$) in DPPH radical scavenging in NFMC as compared with NMFN. However, when the flours were processed into tortillas, it was clear that the nanoencapsulated bioactive compounds with antioxidant capacity had a protective effect. The capacity to scavenge the radical DPPH decreased by 35.79% in TC as compared with tortillas containing nanocapsules, in which DPPH radical scavenging decreased by only 16.69%. The value of the capacity to capture ABTS+ radicals decreased by 28.12% and 18.89% in tortillas made of control nixtamalized flours and flours containing nanocapsules, respectively. The results showed the protective effect of the nanocapsules, since the antioxidant capacity of the tortillas with the nanoencapsulated phenolic extract was maintained to a greater extent. In addition, the flavonoid compounds were highly susceptible to thermal processing at 250 °C for 89 s, used to obtain the tortillas, degrading and therefore not contributing to the DPPH and ABTS values in the tortillas. On the other hand, other types of phenolic compounds, possibly phenolic acids, stilbenes or lignans, were more stable to heat and were those that maintained the antioxidant capacity reflected in both the DPPH and ABTS values. It is worth noting that the value obtained by the ABTS^{+} radical-scavenging method is higher than that corresponding to DPPH, because it acts in both hydrophilic and hydrophobic systems, whereas for DPPH it is applied in hydrophobic systems [34].

For TPC, there was a 21.47% decrease in CT compared with NMFC and a 19.08% decrease in TN compared with NMFN. A greater effect was found for TFC, since these compounds were not detected in CT, so these compounds were lost during cooking. Also, it was observed that TFC in TN decreased in 47.63% of the original TFC present in the NMFN.

The TPC content of TN was 38.85 mg GAE/100 g, 17.79% greater than that of CT (32.98 mg GAE/100 g). No TFC was detected in CT, probably due to the sensitivity of flavonoids to heat and steam during cooking [35]. In TN, 24.21 mg QE/100 g of TFC were quantified, indicating that these compounds were protected by encapsulation.

In the literature, TPC values of 41.80 mg GAE/100 g and 38.60 mg GAE/100 g were reported for dough and tortillas, respectively [36]. Our study obtained a similar TPC content of 42.0 mg GAE/100 g in the NMFC but a lower value of 32.98 mg GAE/100 g for tortillas, partly due to the process of drying the dough and sifting to obtain flour, which could have exposed some phenolic compounds, making them more susceptible to degradation during cooking. Another study did not detect flavonoids in white corn tortillas [37], which is consistent with the present study, as these compounds were not detected in CT. Most of the phenolic acids in maize are bound, mainly to cell wall components such as proteins or carbohydrates, while flavonoids are mainly found in free or soluble form; in anthocyanins, which are also flavonoids, the bound fraction is relatively low [38], which could partly explain the degradation of these compounds during tortilla preparation in CT. García-Rojas et al. [39] reported a reduction in phenolic compounds of 16% in tortillas and 26% in tortillas containing 9% *P. agaves* flour. In the present study, there was a 21.47% decrease in TPC in CT and 19.08% in TN.

In general, the incorporation of *O. atropes* extract into nanocapsules increased the content of TPC and TFC in NMFN and TN as compared with NMFC and CT, and encapsulation protected the bioactive compounds. Also, both TN and CT lost bioactive compounds; however, the loss was lower in TN with respect to CT, indicating that encapsulation maintained the stability of the TPC, TFC, and antioxidant capacity during tortilla cooking.

Tortilla quality

Measurements were made to evaluate tortilla quality (Table 3). Puffing values of 1 (puffing completely) were obtained for both CT and TN, so the addition of nanocapsules did not affect this quality parameter. The tensile strength simulates the force of tearing with the hands, and the cutting force stimulates the force of a tooth during chewing. Tension values of 1.72 N and 1.77 N were found for CT and TN, respectively, so there was no significant difference ($p > 0.05$) between the samples. The tension values found in our study were slightly

Table 3: Quality parameters in the tortilla with the addition of NEOA.

Parameter	CT	TN
Puffing degree	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}
Tensil strength (N)	1.72 ± 0.10^{a}	1.77 ± 0.07^{a}
Cutting force (N)	3.76 ± 0.33^{a}	3.37 ± 0.16^{a}

Note: N = 3 replicates ± standard deviation. Means in the same row with different superscripts are significantly different ($p < 0.05$).

lower than the value of 1.98 N found in Jiménez et al. [40]. A similar effect was observed for cutting force, which was 3.76 N and 3.37 N, for CT and TN, respectively, where there was also no significant difference ($p > 0.05$). This means that the potential interaction between nanocapsule components such as maltodextrin and phenolic compounds with maize components did not alter the hardness of TN with respect to CT. These results were similar to those obtained in Treviño-Mejía et al. [41], who reported a cutting g force of 2.37 N and a tensile strength of 1.03 N for tortillas.

Digestion *in vitro*

Stability was evaluated based on the concentration of TPC and TFC (Table 4) and antioxidant activity in terms of DPPH and ABTS values (Figure 2) of *O. atropes* extract (EOA), nanocapsules with *O. atropes* extract (NEOA), CT, and TN under conditions simulating *in vitro* gastric and duodenal digestion stages. TPC remained stable in EOA throughout the stages of *in vitro* digestion, as there was no significant difference ($p > 0.05$) between their initial (before digestion) and final (after duodenal digestion) contents. NEOA before digestion presented a lower TPC as compared with EOA due to the nanoencapsulation process; however, the release of TCP from NEOA was observed after gastric digestion as they increased significantly ($p < 0.05$) by 48.49% and remained unchanged in concentration after the duodenal phase.

Additionally, the protective effect of nanoencapsulation was also observed as 19.5% more TPC were present in NEOA as compared with EOA after the duodenal phase. TFC in EOA decreased significantly ($p < 0.05$) by 26.29% after gastric digestion and maintained their levels after duodenal digestion, and thus there was a total decrease of approximately 24.01%. In NEOA the behavior of TFC was similar to that of TPC as flavonoids increased during gastric digestion due to their release under acidic conditions and remained stable under duodenal conditions, where a protective effect of nanoencapsulation was observed: flavonoids increased by 41.81% after duodenal digestion compared with the initial phase (before digestion). The antioxidant capacity based on the DPPH radical decreased after duodenal digestion in both EOA and NEOA, with a reduction of 76.21% and 29.16%, respectively, as compared with the antioxidant capacity of these products before digestion. The antioxidant capacity based on the ABTS^{+} radical decreased significantly ($p < 0.05$) by 23.43% in EOA from before to after duodenal digestion. By contrast, the antioxidant capacity for ABTS^{+} was lower before digestion in NEOA compared with EOA due to nanoencapsulation but increased significantly by 55.19% after duodenal digestion, which could be related to the above-mentioned increase in TPC and TFC after duodenal digestion in NEOA, attributed to the protective effect of nanoencapsulation.

In CT and TN, TPC increased after gastric digestion with respect to the initial content and then decreased significantly

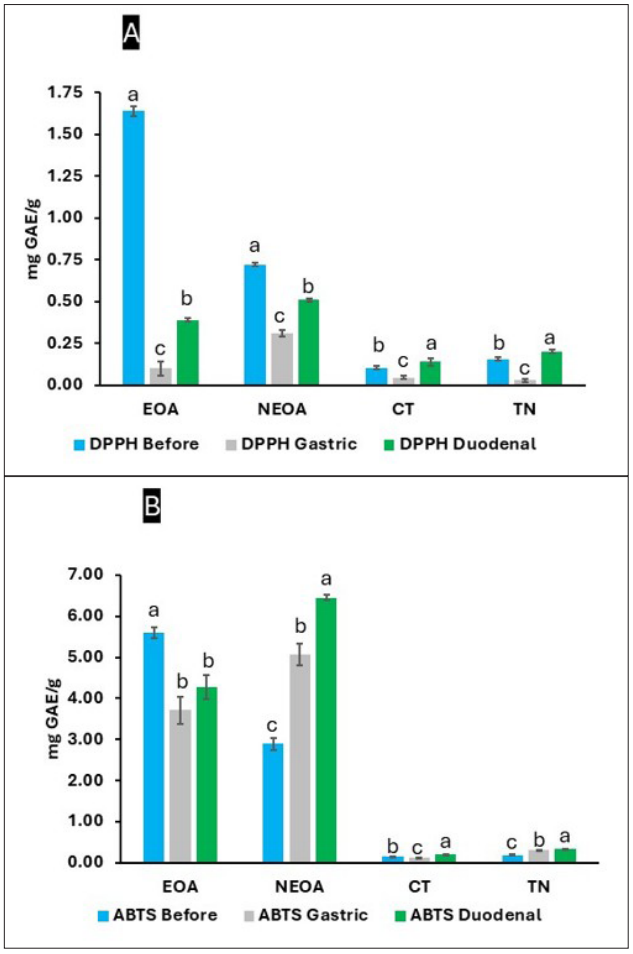


Figure 2: DPPH and ABTS values (mg GAE/g) during digestion *in vitro* of EOA, NEOA, CT, and TN products. **(A)** DPPH values and **(B)** ABTS values.

Table 4: TPC and TFC during *in vitro* digestion of EOA, NEOA, CT, and TN products.

Treatment	Parameter (dry basis)	Before *in vitro* digestion	*In vitro* gastric digestion	*In vitro* duodenal digestion
EOA	TPC (mg GAE/g)	$10.66 \pm 0.39^{a,b}$	10.95 ± 0.15^{a}	10.01 ± 0.26^{b}
NEOA	TPC (mg GAE/g)	6.30 ± 0.51^{b}	12.99 ± 0.21^{a}	12.44 ± 0.20^{a}
CT	TPC (mg GAE/g)	0.2935 ± 0.01^{c}	0.8022 ± 0.03^{a}	0.6035 ± 0.01^{b}
TN	TPC (mg GAE/g)	0.3573 ± 0.01^{c}	0.9204 ± 0.03^{a}	0.7109 ± 0.01^{b}
EOA	TFC (mg QE/g)	22.74 ± 1.05^{a}	16.76 ± 2.87^{b}	17.28 ± 1.34^{b}
NEOA	TFC (mg QE/g)	17.17 ± 0.76^{b}	28.62 ± 3.91^{a}	29.51 ± 2.67^{a}
CT	TFC (mg QE/g)	ND	ND	ND
TN	TFC (mg QE/g)	0.2213 ± 0.01^{a}	$0.2049 \pm 0.01^{a,b}$	0.1769 ± 0.01^{b}

Note: N = 3 replicates ± standard deviation. Means in the same row with different superscripts are significantly different ($p < 0.05$). Means in the same row with the same superscript are significantly equal ($p < 0.05$).

after duodenal digestion ($p < 0.05$) in comparison with gastric digestion values, in agreement with previous authors like Bermudez-Soto et al. [42], who documented a higher content of phenolic compounds after the gastric phase of digestion and a decrease after the duodenal phase tested in different fruit juices. In CT, no TFC were detected during the *in vitro* digestion phases. In TN, after duodenal digestion, 79.93% of the initial flavonoid content was preserved.

In CT and TN there was an increase in antiradical capacity (DPPH) after duodenal digestion of 24.71% and 22.28%, respectively. An increase in the antioxidant capacity in $ABTS^{+}$ was also observed for CT and TN after duodenal digestion as compared with the antioxidant capacity before digestion, this increase was 24.76% in CT and 47.90% for TN. Thus, the nanocapsules with the *O. atropes* extract in the tortilla increased the phenolic content and antioxidant capacity of the food and remained stable under gastrointestinal conditions. In another study by De Santiago et al. [43], similar results were obtained for raw or thermally processed *O. ficus-indica* cladodes subjected to *in vitro* digestion processes. These authors indicated that enzymes and digestion conditions retained or degraded flavonoids more than phenolic acids. In the present study, a similar behavior was likely to have occurred, which could also have decreased the antioxidant capacity of the *O. atropes* extract after duodenal digestion.

In the developed products CT and TN, the TPC content and antioxidant capacity increased after duodenal digestion with respect to the initial phase (before digestion). The increase was greater in TN than in CT: 15.10% in TPC, 31.75% antioxidant capacity by DPPH radical scavenging, and 40.91% antioxidant capacity by $ABTS^{+}$ radical scavenging. No increase in TFC was detected in CT, and in TN the value was 0.1769 mg QE/g. Thus, the TPC and TFC of the TN remained active during *in vitro* gastrointestinal conditions, and the antioxidant capacity was preserved.

Different *in vitro* and *in vivo* investigations have particularly associated flavonoids, such as quercetin, isorhamnetin and kaempferol derivatives present in the Opuntia genus, with the reduction of oxidative stress and the modulation of anti-inflammatory responses [18, 44], key conditions in the development of multiple chronic diseases.

Nanoencapsulation of *O. atropes* extract protects flavonoids, such as isorhamnetin and kaempferol glycosides, against environmental factors [20]. This study also demonstrated that nanoencapsulation facilitated their incorporation into foods, such as tortillas, without affecting their texture. It was also effective in preserving bioactive compounds after tortilla processing and *in vitro* digestion. Due to the preservation of flavonoids in TN and their free-radical-scavenging activity, they have the potential to become functional foods, whose regular consumption could contribute to the reduction of chronic-degenerative diseases in the population.

Conclusion

The encapsulation process was effective in preserving TPC, total flavonoid content, and antioxidant capacity. *In vitro* digestion studies showed that the nanocapsules were able to retain many phenolic compounds, mainly flavonoids, which remained present and showed a high antioxidant capacity, unlike the CT, in which even the presence of flavonoid compounds, which are more susceptible to deterioration during the heating process used in tortilla production, was not detected. Therefore, corn tortillas enriched with phenolic compounds from *O. atropes* may be a potential source of phenolic compounds and flavonoids in the diet of the Mexican population. As a future perspective, clinical studies on the intake of tortillas enriched with nanoencapsulated phenolic extract from *O. atropes* in the prevention of chronic degenerative diseases are recommended.

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Conflict of Interest

None.

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