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EFFECT OF MICROWAVE PRETREATMENT ON BIOACTIVE COMPOUNDS EXTRACTION FROM XOCONOSTLE (*Opuntia joconostle*) BY-PRODUCTS

EFECTO DE UN PRETRATAMIENTO CON MICROONDAS EN LA EXTRACCIÓN DE COMPUESTOS BIOACTIVOS DE RESIDUOS DE XOCONOSTLE (*Opuntia joconostle*)

G. Dávila-Hernández[1], M.E. Sánchez-Pardo[1], G.F. Gutiérrez-López[1], H. Necoechea-Mondragon[2], A. Ortiz-Moreno[1]*

[1]*Instituto Politecnico Nacional, National School of Biological Sciences, Department of Biochemical Engineering. Unidad Profesional Adolfo Lopez Mateos, Av. Wilfrido Massieu esq. Cda. Miguel Stampa s/n, C. P. 07738. Gustavo A. Madero, Mexico City, Mexico.*

[2]*Coordination of Network Operation of Research and Postgraduate of the Instituto Politecnico Nacional. Miguel Bernard s/n Colonia La Escalera, C. P. 07320. Gustavo A. Madero, Mexico City, Mexico.*

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Abstract

Xoconostle is a common feature of the Mexican landscape in semi-arid regions. Its mesocarp is processed into jellies, sauces and other products but the endocarp is discarded as waste; in spite of containing appreciable amounts of bioactive compounds with antioxidant activity. This study aimed to evaluate the effect of microwave pretreatment on the extraction of phenolic and flavonoids compounds from xoconostle's endocarp and to determine their antioxidant capacity and microstructural changes. According to the surface response methodology applied, the optimum conditions for phenolic compounds and flavonoid extraction from endocarp using microwave pre-treatment (297 W for 5.5 min) were: 0% ethanol, 5 °C and 10 min of stirring. The total phenolic, total flavonoid, betalains and ascorbic acid contents, as well as the antioxidant capacity were higher in the microwave pretreated samples compared with non-pretreated ones. The following phenolic acids and flavonoids were identified in the endocarp extracts obtained under optimal conditions: gallic, caffeic, protocatechuic, coumaric and, vanillic acids, epicatechin and catechin. Our results suggested that the application of a microwave pretreatment improves compounds extraction in xoconostle's endocarp, which could be an alternative source of bioactive substances with high antioxidant capacity.

Keywords: xoconostle, phenolic compounds, microwaves, extraction, antioxidant capacity, microstructure.

Resumen

El xoconostle (*Opuntia joconostle*) es distintivo del paisaje mexicano en regiones semiáridas. Su mesocarpio se procesa en gelatinas, salsas y otros productos, sin embargo, el endocarpio se descarta como desecho. Este endocarpio contiene cantidades apreciables de compuestos bioactivos con capacidad antioxidante. El objetivo del estudio fue evaluar el efecto de un pretratamiento con microondas en la extracción de compuestos fenólicos y flavonoides del endocarpio de xoconostle y determinar su capacidad antioxidante y cambios en su microestructura. De acuerdo con la metodología de superficie de respuesta, las condiciones óptimas para la extracción de compuestos fenólicos y flavonoides de endocarpio con pretratamiento con microondas (297 W durante 5,5 min) fueron: 0% de etanol, 5 °C y 10 min de agitación. El contenido de compuestos fenólicos totales, flavonoides totales, betalaínas, ácido ascórbico y la capacidad antioxidante fueron más altos en las muestras pretratadas con microondas. Se identificaron los siguientes ácidos fenólicos y flavonoides en los extractos de endocarpio obtenidos en condiciones óptimas: gálico, cafeico, protocatecúico, cumarico y vanílico, epicatequina y catequina. Nuestros resultados sugieren que aplicar un pretratamiento con microondas mejora la extracción de compuestos bioactivos presentes en el endocarpio de xoconostle, que podrían utilizarse como fuente de sustancias con alta capacidad antioxidante.

Palabras clave: xoconostle, compuestos fenólicos, microondas, capacidad antioxidante, microestructura.

* *Corresponding author. E-mail*: ortizalicia@hotmail.com
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1 Introduction

Nowadays, cactus pear cultivation for fruit production takes place in at least 18 countries in semi-arid areas in both hemispheres on more than 100 000 ha. Mexico has the larger cultivated area of this cactus fruit (51,112 ha.) with approximately 10,000 tons produced in 2015; other countries such as Italy, Chile, South Africa, Argentina and Israel, also commercially cultivate the fruit (FAO, 2017). Cactus pear, also known as prickly pear, is a fruit produced by several species of *Opuntia* genus belonging to the family of Cactaceae, commonly found in the arid parts of the World (El Kharrassi *et al*., 2016; Santos *et al*., 2017). Cactus pear are one of the few crops that can be cultivated in areas which offer minimal growth possibility for conventional fruits and vegetables (Yeddes *et al*., 2013). The fruits are used for the manufacture of food products such as juices, alcoholic beverages, jams and natural liquid sweeteners (Melgar *et al*., 2017). Different color varieties of *Opuntia* fruits are available due to significant genetic variability. The fruit of *Opuntia joconostle* has become popular due to its nutritional value and health benefits including: antioxidant effects against oxidative stress (Osorio-Esquivel *et al*., 2013), reduction in cholesterol and blood triglycerides (Pimienta-Barrios *et al*., 2008). Its beneficial properties can be attributed to the high content of polyphenols, flavonoids, betalains and vitamin C (Guzmán-Maldonado *et al*., 2010; Cortez-García *et al*., 2015).

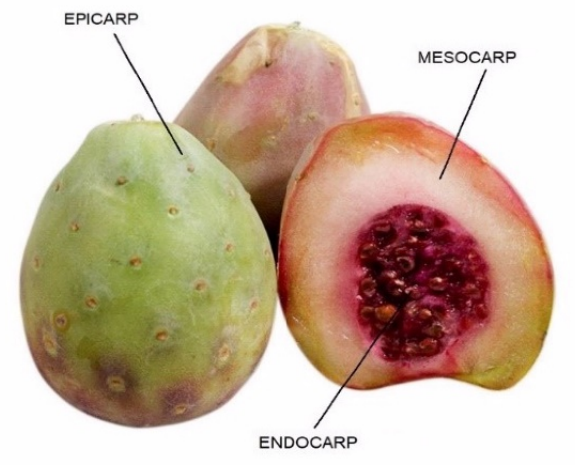

Fig. 1. Xoconostle fruit (*Opuntia joconostle*). Parts: epicarp (skin), mesocarp (pulp) and endocarp (mucilage and seeds).

Since these compounds are considered important in human diet given their antioxidant capacity, the designing of health-directed products using environmentally friendly processes to extract them from natural sources, constitute a good alternative. The xoconostle has a pink-colored pericarp, a succulent mesocarp and a red colored endocarp that contains small brown seeds (Osorio-Esquivel *et al*., 2011) (Figure 1). However, processed products are manufactured mainly by using the mesocarp (70% of the fruit), and the endocarp (mucilaginous part and seeds) is discarded (Morales *et al*., 2012). This by-product contains appreciable amounts of bioactive compounds (Morales *et al*., 2014). Recently, the extraction of bioactive compounds from the by-products of processed food has attracted the attention of researchers, since these processes help to reduce the environmental problems caused by their disposal (Yeddes *et al*., 2013).

The selection of the optimum solvent for the extraction of bioactive compounds is based on several factors, such as their physicochemical properties and processing temperature (Boonchu & Utama-Ang, 2015; Metrouh-Amir *et al*., 2015). However, issues related to environmental aspects, toxicity, presence of solvent residues, safety and cost issues help to finally deciding the best solvent for a given task, so that solvent used in practical application, may be different to the statistically optimum combination of the solvents when yields are relatively close (Tiwari, 2015). In previous studies, extractions of biocompounds from fruits have been carried out by using solvents such as water, methanol and ethanol in different proportions to obtain higher extraction rates (Sanchez-Gonzalez *et al*., 2013; Koubaa *et al*., 2015; Muruganandam *et al*., 2017).

Microwave heating has gained popularity in food processing due to its capacity to reach high heating rates, significant decrease in cooking time, uniform heating, safe handling, easiness of operation and low maintenance costs (Li & Jiang, 2010; Chandrasekaran *et al*., 2013; Dorta *et al*., 2013; Quiroz-Reyes *et al*., 2013). Moreover, microwave heating might improve the extraction of valuables substances (such as phenolic compounds, vitamins, etc.) (Lima *et al*., 2017; Roncero-Ramos *et al*., 2017). Different researchers have reported that microwave heating increases the rates of bioactive compound extractions from fruit and vegetables more than other heating methods, saving time and energy (Chandrasekaran *et al*., 2013). Microwave heating disrupts cells and membrane walls, helping to release biocompounds

from the food matrix (Guo *et al*., 2017; Jiang *et al*., 2017).

This work aimed to evaluate the effect of microwave pretreatment on phenolic compounds and flavonoid extraction from xoconostle's endocarp and to determine their antioxidant capacity and microstructure changes.

2 Materials and methods

2.1 Samples preparation

Fresh *Opuntia joconostle* fruits (xoconostles) weighing approximately 50 g each, with a uniform shape and maturity, were obtained during the months of August-September from a commercial orchard in San Martin de las Piramides, Mexico, located at 19° 44' 0" N (North) latitude and 98° 49' 0" W (West) longitude, in 2017. A voucher specimen number 15721 of this fruit is deposited at the Herbarium of Centro Medico Siglo XXI, Mexico City. The fruits were washed with tap water and disinfected with Citrus 21 (20 mL per liter). The fruits were stored in refrigeration at 4-6 °C until further use.

2.2 Microwave pretreatment

The endocarp was obtained by cutting the fruit into halves and manually extracting the endocarp without following a special technique. The bioactive compound-containing endocarps were crushed and mix to achieve a particle size of approximately 0.5 mm. The lot was divided into two lots. The first lot (about 400 g of xoconostle's endocarp) was poured onto a glass plate and heated in a microwave oven (LG M51744XT Busan, South Korea) at 297 W for 5.5 min according to previous optimization research conducted by Cortez-García *et al*. (2015). The second lot remained non-treated.

2.3 Optimization for the extraction of total phenolic content (TPC) and total flavonoid content (TFC)

In order to determine the optimal conditions for TPC and TFC xoconostle's endocarp extraction with and without microwave pretreatment, a response surface methodology and a three factorial, two-level and centroid design were used and supported by the Design Expert version 11 software (Stat-Ease, Inc. Minneapolis) (Table 1).

The solvent ratio, stirring time and extraction incubation temperature were independent variables, while the TPC and TFC were the response variables. A second-order polynomial equation was used to fit the experimental data of the studied variables. The generalized second-order polynomial model used in the response surface analysis is shown in Equation (1):

$$Y = \beta_0 + \sum_{i=1}^{n}\beta_i X_i + \sum_{i=1}^{n}\beta_{ii}X_i^2 + \sum_{i=1}^{n}\cdot\sum_{j=i+1}^{n}\beta_{ij}X_iX_j + \beta_{ijk}X_iX_jX_k \quad (1)$$

where Y is the response variable, β_0 is the intercept, $\beta_i, \beta_{ii}, \beta_{ij}$ and β_{ijk} are linear, quadratic and interaction coefficients respectively, and X_i, X_j and X_k are the coded independent variables. The significance of the model was evaluated through the P value and the determination of the regression coefficient (R^2) generated by the analysis of variance (ANOVA). The significance of the model terms was established with a confidence level of 95%.

For TPC and TFC extraction from the pretreated and non-pretreated endocarp with microwaves, 1 g of the endocarp mixture was weighed and diluted in Nalgene tubes containing 10 mL of different water: ethanol ratios and stirred at 200 rpm (Thermo scientific mod. 135935, China). Different extraction temperatures were then used following the experimental design presented in Table 2. Afterwards, the extracts were centrifuged (Hermle Z326, Germany) at 10,000 rpm at 4 °C for 15 min. The extracts obtained were collected in amber flasks for subsequent determinations. Betalain and ascorbic acid contents as well as antioxidant capacity were analyzed in the samples by using the extraction conditions of those runs that resulted in higher extractions of TPC and TFC with and without microwave pretreatment.

Table 1. Experimental parameters for the extraction of the bioactive compounds of the xoconostle endocarp.

Variable	Low level	High level
X_1 = percentage of solvent in water (%, v/v)	0	80
X_2 = extraction temperature (°C)	5	30
X_3 = extraction time	10	30

The samples pretreated and non-pretreated with microwaves under the optimal extraction conditions were analyzed by HPLC to identify content changes in the primary phenolic compounds.

2.4 Determination of total phenolic content

The measurement of the total phenolic compounds content was conducted by using the spectrophotometric method of Folin-Ciocalteu (Osorio-Esquivel *et al*., 2011). An aliquot of 100 μL of each extract was added to 900 μL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) and allowed to stand for 5 minutes at room temperature. Then, 750 μL of a 7% sodium bicarbonate (Reasol, Mexico) solution was added, and the sample was vortexed for 30 seconds. The mix was then incubated at room temperature for 90 minutes. The absorbance was measured at 725 nm by means of a Jenway 6705/UV-VIS spectrophotometer (Staffordshire, UK). Gallic acid was used as a standard and its calibration curve was used to extrapolate the total phenolics content present in the sample. The values were expressed as mg Gallic Acid Equivalents (GAE)/g dry weight (DW).

2.5 Determination of total flavonoid content

The measurement of the total flavonoid content was conducted by using a colorimetric method (Osorio-Esquivel *et al*., 2011). Briefly, 250 μL of the sample were mixed with 1250 μL of distilled water and followed by the addition of 75 μL of a $NaNO_2$ (Reasol, Mexico) solution. After 6 minutes, 150 μL of a 10% $AlCl_3$ (Caledon Ltd, Canada) solution was added, and the sample was left to stand for another 5 minutes before 500 μL of 1 M NaOH (Hycel de Mexico, Mexico) were added. The mixture was brought to a final volume of 2.5 mL with distilled water and vortexed for 30 seconds. The absorbance was immediately measured at 510 nm by means of a Jenway 6705/UV-VIS spectrophotometer (Staffordshire, UK) and these measurements were interpolated into a standard curve of catechin. The results were expressed as mg Catechin Equivalents (CE)/g dry weight (DW).

2.6 Quantification of betalains

The measurement of the betalains content was conducted using a modified version of the method described by Stintzing & Carle (2004). A sample of 1.5 mL of each extract was used to perform a wavelength scan (450-550 nm) by means of a Jenway 6705/UV-VIS spectrophotometer (Staffordshire, UK). The quantification of betalains was carried out using an extinction coefficient of ε = 60,000 at 535 nm. The results were expressed as mg of betanin/100 g dry weight (DW).

2.7 Quantification of ascorbic acid

The measurement of ascorbic acid was conducted by using the xylene extraction method reported by Ranganna (2008). Briefly, 10 mL of each extract was diluted to 100 mL with 0.3% metaphosphoric acid (Meyer, Mexico). An aliquot of 2 mL was then placed in a stoppered conical flask and, 2 mL of an acetate buffer solution, 3 mL of a solution of 2,6-dichlorophenolindophenol (Sigma, USA) and 15 ml of xylene (J.T. Baker, USA) were added in rapid succession. The flask was stoppered and shaken for 10-15 seconds. An absorbance reading was taken on a Jenway 6705/UV-VIS spectrophotometer (Staffordshire, UK) at a wavelength of 520 nm. The results were compared with a standard ascorbic acid curve and expressed as mg ascorbic acid/100 g sample DW.

2.8 Antioxidant capacity

Antioxidant capacity was determined by using three different methods: 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Ferric reducing antioxidant power (FRAP). Briefly, the DPPH method was determined as previously reported by Brand-Williams *et al*. (1995), each sample extract (50 μL) was mixed with 1950 μL of DPPH solution and left in darkness at room temperature for 60 min, and the absorbance reading was taken at 515 nm. The ABTS test was carried out according to the modified method of Ozgen *et al*. (2006). For measuring antioxidant capacity, 20 μL of the extract were mixed with 3 mL of ABTS solution, and the absorbance was monitored at 734 nm after 6 minutes. The FRAP method was conducted according to Benzie & Strain (1996). A solution of 10 mM TPTZ and 20 mM ferric chloride was diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Samples (50 μL) were added to 1.5 mL of the TPTZ solution, and the absorbance at 595 nm was determined. The samples were then warmed to 37°C for 20 minutes.

A Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard calibration curve was elaborated for DPPH, ABTS and FRAP methods. The results of the three methods were expressed as mmol Trolox Equivalents (TE)/100 g DW.

2.9 *HPLC analysis for phenolic compounds*

Phenolic compound content in the extracts was determined by using an Agilent 1260 HPLC with a diode array detector and automatic injection. Chromatograms were obtained at 280 nm with an injection volume of 20 μL and a reverse-phase column at constant a temperature of 30 °C was used. The mobile phase A consisted of 3% (v/v) formic acid in water, and 100% acetonitrile was used as eluent B. The flow rate was 0.8 mL/min with a linear gradient from 5% to 30% B over 35 min (Osorio-Esquivel *et al*., 2011).

2.10 *Optical microscopy and scanning electron microscopy (SEM) analysis*

Pretreated and non-treated microwave xoconostle's endocarp were examined using optical microscopy and scanning electron microscopy (SEM). Endocarp pretreated and non-treated samples, were dyed with methylene blue to facilitate the tissue components observation. Optical microscopy observation was performed in an optical microscope coupled to a digital camera (VELAB Co, model VE-BC1, USA). Magnification was 100x. Pretreated and non-treated samples of xoconostle's endocarp were observed by using SEM for morphological characterization. Samples were examined at 200x in a scanning electron microscope JSM 5800LV (Jeol Inc., USA), equipped with a program for digital image capture.

2.11 *Statistical analysis*

The results were expressed as the mean values ± standard deviations. Significant differences were determined at 95% confidence by Tukey's test by using GraphPad Prism software v. 5.0 (CA, USA, 2015). The optimal conditions were estimated through three-dimensional response surface analysis of the three independent variables and for each dependent variable. An ANOVA analysis was performed to determine the models significance and which factors had more influence in the response variables.

3 Results and discussion

3.1 *Extraction optimization of bioactive compounds*

The total phenolic content (TPC) and total flavonoid content (TFC) of xoconostle's endocarp extracts from the seventeen runs determined by the experimental model with and without microwave pretreatment are shown in Table 2.

A second-order polynomial model ($p < 0.05$) was obtained through the regression analysis for TPC and TFC extracted from xoconostle's endocarp pretreated with microwaves (equation 2 and 3) and a similar model was obtained for xoconostle's endocarp without pretreatment (equations 4 and 5):

$$TPC = 9.32 - 0.7385A - 0.6227B + 0.5231AB \qquad R^2 = 0.930 \tag{2}$$

$$TFC = 2.63 - 0.9906A - 0.1136B + 0.0833BC + 1.59A^2 \qquad R^2 = 0.997 \tag{3}$$

$$TPC = 9.15 - 0.1631A - 0.1969B - 0.1754C + 0.2491AB - 0.4616A^2 - 0.1667C^2 \qquad R^2 = 0.966 \tag{4}$$

$$TFC = 2.72 - 0.5385A + 0.1110C - 0.1207AC + 1.16A^2 \qquad R^2 = 0.991 \tag{5}$$

where A = solvent, B = temperature, C = time, AB = solvent-temperature interaction, AC = solvent-time interaction, BC = temperature-time interaction, A^2B^2C = quadratic effects and ABC = solvent, temperature and time interaction.

From the ANOVA analysis, it is possible to observe that the models in Eq 2-5 had a good fit (all R^2 were above 0.9).

Table 2. TPC and TFC content of xoconostle endocarp extracts obtained with and without microwave pretreatment according to the experimental design.

Independent variables				Response variables MPT		NMPT	
Run	X_1	X_2	X_3	TPC mg GAE/g	TFC mg CE/g	TPC mg GAE/g	TFC mg CE/g
1	40	17.5	20	9.23±0.01	2.62±0.05	9.15±0.02	2.93±0.13
2	80	5	10	9.80±0.09	3.44±0.12	8.34±0.08	3.33±0.14
3	0	30	30	9.84±0.06	5.12±0.11	8.41±0.04	4.90±0.07
4	0	30	10	9.58±0.04	5.14±0.13	8.28±0.04	4.14±0.13
5	80	5	30	9.16±0.07	3.31±0.13	8.48±0.04	3.35±0.08
6	40	17.5	36	10.02±0.04	2.78±0.06	9.13±0.03	2.82±0.13
7	40	17.5	20	8.97±0.01	2.65±0.06	9.14±0.11	2.64±0.03
8	40	17.5	20	9.27±0.10	2.55±0.08	9.15±0.04	2.68±0.14
9	40	17.5	20	9.76±0.06	2.68±0.05	9.15±0.28	2.74±0.05
10	**0**	**5**	**10**	**12.92±0.10**	**5.64±0.13**	9.01±0.07	4.20±0.13
11	40	17.5	3.18	9.53±0.01	2.64±0.11	8.22±0.03	2.50±0.11
12	80	30	10	9.24±0.03	3.15±0.14	8.45±0.09	3.34±0.13
13	40	17.5	20	9.07±0.03	2.71±0.03	9.16±0.26	2.72±0.08
14	80	30	30	9.32±0.01	3.34±0.03	8.58±0.06	3.33±0.13
15	40	38.5	20	9.23±0.06	2.60±0.12	9.13±0.09	2.84±0.09
16	40	17.5	20	9.59±0.01	2.54±0.08	8.93±0.04	2.60±0.03
17	**0**	**5**	**30**	11.09±0.05	5.27±0.07	**9.46±0.06**	**4.42±0.13**

x_1 = percentage of ethanol in water (%); x_2 = extraction temperature (°C); x_3 = extraction time (min). MPT= microwave pretreatment, NMPT= non-microwave pretreatment, TPC= total phenolics content, TFC= total flavonoid content, GAE= gallic acid equivalents, CE= catechin equivalents.

Also, it is possible to point out, that the factors that most affected the response variables (TPC and TFC content) for endocarp treated with microwaves were: solvent concentration followed by the temperature while the extraction time was not significant for the phenolic compounds and flavonoids extraction (equations 2 and 3). Regarding the xoconostle's endocarp non-pretreatment with microwave, for TPC the three variables; solvent concentration, temperature and time, had a very similar significance. For TFC, the solvent concentration had a greater influence on the extraction, while the extraction time had four-fold less influence than solvent concentration and the temperature was not significant (equations 4 and 5).

The resulting range of TPC in xoconostle's endocarp extracts pretreated with microwaves (8.97-12.92 mg GAE/g), was significantly ($p < 0.05$) higher than the range of the extracts without microwave pretreatment (7.88- 9.46 mg GAE/g) (Table 2). Gao *et al*. (2012), Karami *et al*. (2015), and Liu *et al*. (2016) reported that when microwave energy is applied to fruits' tissues, a disruption of the tissue is provoked, releasing the phenolic compounds from the matrix. Microwave heating also dissociates some phenolic compounds, and turning insoluble phenols into more soluble forms, which allows an easier extraction (Lima *et al*., 2017; Roncero-Ramos *et al*., 2017).

The results showed that TPC extraction was enhanced by increasing the solvent polarity, independently to the extraction method. Both the microwave pretreated and non-pretreated extracts presented better TPC extraction when water was used as extraction solvent than by using other solvent mixtures as by García-Márquez *et al*. (2012), Drosou *et al*. (2015), Flores-Martínez *et al*. (2016), Castro-Lopez *et al*. (2017) and Felix *et al*. (2018) suggested. These results could be attributed to the high-water polarity index compared with other solvents or solvents mixtures (Ammar *et al*., 2015; Hossain *et al*., 2016; Ribeiro *et al*., 2018). Water presents high dielectric constant and cohesive energy compared with other solvents which provide strong bonding between water molecules and polar compounds from the solute, producing its dissolution (Ammar *et al*.,

2015). Moreover, water helps in swelling of the plant matrix and enlarges contact surface area between the solvent and plant matrix resulting in increased extraction efficiencies (Simsek *et al*., 2012; Alara *et al*., 2017).

The average TPC in endocarp extracts without using microwaves pretreatment in this study (8.64 mg GAE/g) was 50% higher than the values obtained by Cejudo-Bastante *et al*. (2014) (4.0 mg GAE/g). However, this result was 50% lower than the value reported by Osorio-Esquivel *et al*. (2011) of 17.28 mg GAE/g DW for *Opuntia joconostle*; this lower concentration of phenols obtained in the present research may be due to: the extracts preparation method, the season in which the fruits were acquired, the agronomic conditions and the specific location in which the fruit was grown (Osorio-Esquivel *et al*., 2011; Cortez-García *et al*., 2015). In contrast, the number of phenolic compounds found in the present study was 5-fold higher than the concentration of phenols reported by Guzmán-Maldonado *et al*. (2010) in the xoconostle's endocarp variety *Opuntia matudae* (1.68 mg GAE/g DW).

The average concentration of TFC in xoconostle's endocarp extracts with and without microwave pretreatment was 5.64 mg CE/g, and 4.42 mg CE/g respectively. The increase in the flavonoid content in endocarp extracts pretreated with microwaves could be mainly due to the release of phytochemicals from the matrix during microwave heating due to the disruption of cell membranes and cell walls, so releasing bioactive compounds that increase the pool of polyphenols (Lima *et al*., 2017). Our results clearly show that microwave pretreatment increased the content of phenolic compounds in 27% (12.92 mg GAE/ g vs. 9.46 mg GAE/g) and 22% for flavonoid content (5.64 mg CE/g vs. 4.42 mg CE/g).

Based on the Equations 2-5, Figures 2 and 3 were constructed and represent the response surface graph as a function of the extraction factors and their interactions over the TPC and TFC in xoconostle's endocarp with and without microwave pretreatment.

According to Figure 2, the optimum extraction conditions of TPC and TFC for endocarp extracts with microwave pretreatment were: 15% ethanol, 5 °C and 10 minutes; under these conditions, the predicted response for this sample was TPC: 11.94 mg GAE/g and TFC: 4.15 mg CE/g. The predicted total phenolic content for the microwaved sample was confirmed and validated by performing an experiment (in triplicate) under the optimum conditions, obtaining a value for TPC of 12.92 mg GAE/g and a value for TFC of 5.64 mg CE/g. Considering that run 10 presented extraction values close to the optimum and uses 100% water for the extraction thus being an environmental friendly process (Tiwari, 2015), the conditions of sample 10 were selected for the pretreated sample.

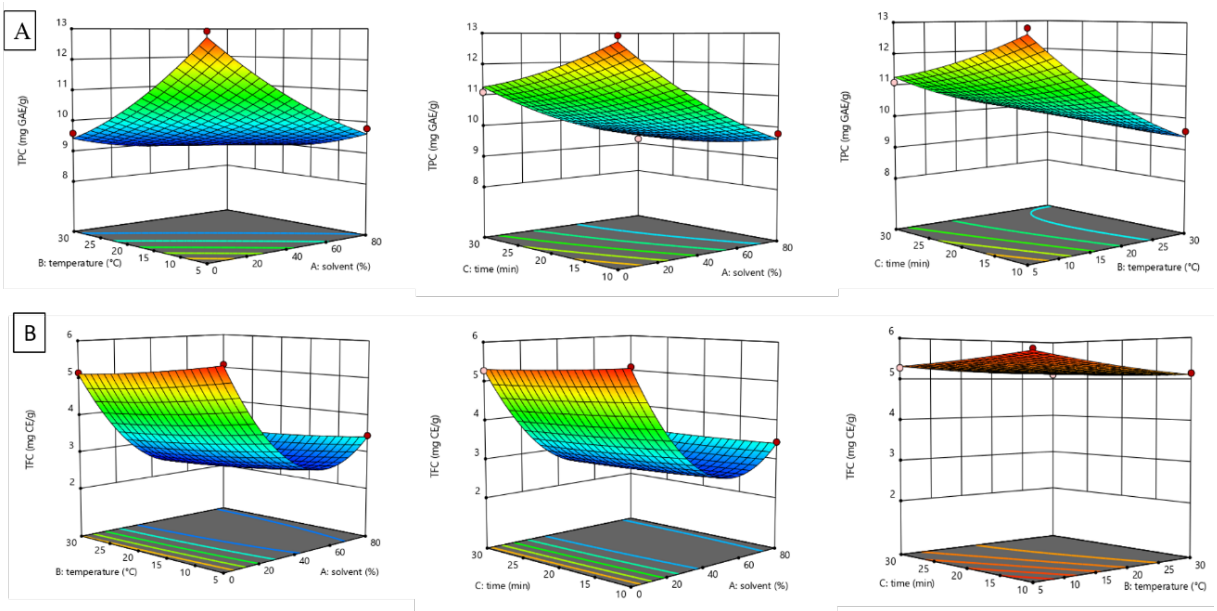

Fig. 2. Extraction of (A) TPC and (B) TFC from xoconostle's endocarp pretreated with microwaves as a function of time, temperature and solvent.

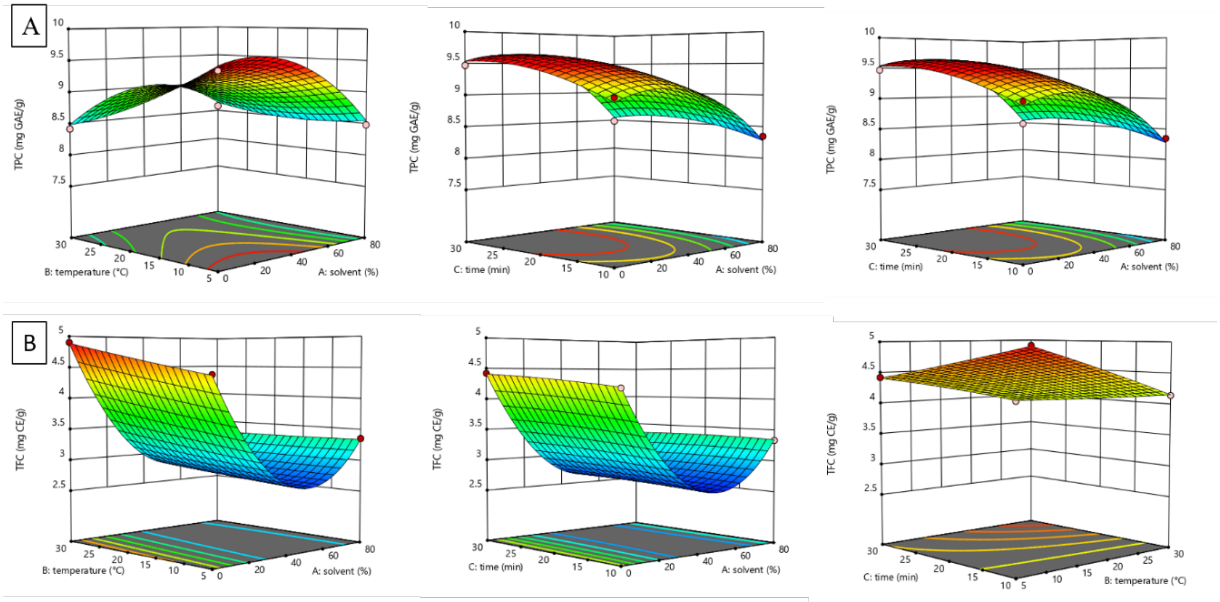

Fig. 3. Extraction of (A) TPC and (B) TFC from xoconostle's endocarp non- pretreated as a function of time, temperature and solvent.

Table 3. Bioactive compounds and antioxidant capacity in xoconostle endocarp extracts with and without microwave pretreatment.

Optimal extraction conditions	Betalains (mg betanin 100 g)	Ascorbic acid (mg/100 g)	DPPH mmol ET 100 g	ABTS mmol ET 100 g	FRAP mmol ET 100 g
0%,5 °C, 10 min pretreatment MPT	$(8.47\pm0.03)^a$	$(13.01\pm0.04)^a$	$(6.72\pm0.31)^a$	$(10.28\pm0.49)^a$	$(10.25\pm0.06)^a$
0%,5 °C, 30 min NMPT (control)	$(5.22\pm0.00)^b$	$(8.17\pm0.08)^b$	$(6.20\pm0.32)^b$	$(8.51\pm0.82)^b$	$(9.87\pm0.15)^b$

Data expressed as the mean ± standard error and analysed by ANOVA and Tukey's test. Means of triplicates in the same column with different letters are significantly different at $P \leq 0.0001$. MPT: microwave pretreatment, NMPT: non- pretreatment (control).

For the non-pretreated sample, the optimum extraction conditions of TPC and TFC for endocarp extracts were: 3.5% ethanol, 6 °C and 30 minutes. Under these conditions, the predicted response was 9.46 mg GAE g and TFC: 4.42 mg CE/g. There was no difference between the optimal values and the run 17. The conditions of run 17 were preferred over the optimal due to the same reason mentioned above for the run 10 in the pretreated sample.

3.2 Betalains, ascorbic acid content and antioxidant capacity

The betalains content of the microwave pretreated and non-pretreated endocarp aqueous extracts are shown in Table 3. The sample pretreated with microwaves presented a higher betalains content than that sample non-pretreated (8.47 ± 0.03 mg/100 g and 5.27 mg/100 g, respectively).

The values obtained for betalains in the aqueous extract without pretreatment were comparable to those reported by Osorio-Esquivel *et al.* (2011) of 7.57 mg/100 g, who extracted these pigments using acetone. However, the betalain concentration in this study was 6-fold higher than those found by Guzmán-Maldonado *et al.* (2010) for the *Opuntia matudae* variety of 0.49-1.30 mg/100 g, who made a methanolic extraction of these pigments. These differences in the concentrations could be associated to the cultivation and processing extraction conditions, the degree of ripeness of the fruit and the fruit variety (Sanchez-Gonzalez *et al.*, 2013; Yeddes *et al.*,

2013). The content of ascorbic acid in the microwaved endocarp aqueous extracts was higher than that in the extracts without microwaving (13.01 ± 0.04 and 8.21 ± 0.08 mg/100 g, respectively). These values are comparable to those reported by Guzmán-Maldonado *et al*. (2010) of 9 mg/100 g in *Opuntia matudae* fresh fruits.

The results of the antioxidant capacity of the xoconostle's endocarp extract obtained under the optimal conditions with and without pretreatment with microwaves, measured by DPPH, ABTS, and FRAP methods are presented in Table 3. The values obtained showed that the xoconostle's endocarp extracts pretreated with microwaves (DPPH: 6.72 ± 0.31 mmol TE/100 g, ABTS: 10.28 ± 0.43 mmol TE/100 g and FRAP: 10.25 ± 0.06 mmol TE/100 g) had higher antioxidant activities than the non-treated extracts (DPPH: 6.20 ± 0.32 mmol TE/100 g, ABTS: 8.51 ± 0.31 mmol TE/100 g and FRAP: 9.87 ± 0.10 mmol TE/100 g). These results could be attributed to the microwave positive effects mentioned for TPC and flavonoid extraction (Guo *et al*., 2017). A similar effect regarding the increase of antioxidant capacity was reported by Lima *et al*. (2017), Cavdar *et al*. (2017), and Ribeiro *et al*. (2018), after applying microwave treatment to mango peels, pomegranate seeds, and sour cherry pomace.

3.3 Identification of phenolic compounds

The microwave pretreated samples (Figure 4A) and the non-pretreated samples (Figure 4B) were analyzed by HPLC, to identify the primary phenolic compounds.

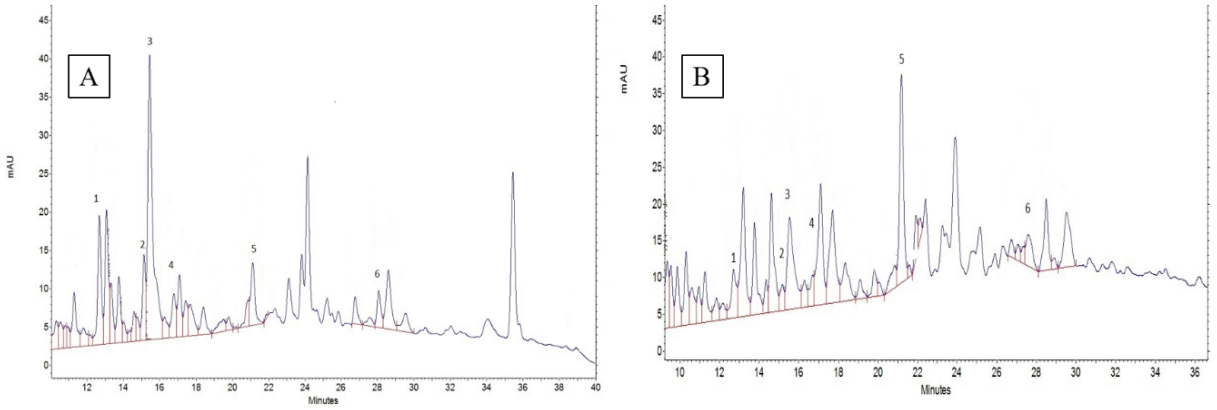

Fig. 4. The HPLC chromatogram showing the individual phenolic compounds detected at 280 nm in: (A) xoconostle's endocarp extract pretreated with microwaves and (B) xoconostle's endocarp non-pretreated. (1) catechin, (2) vanillic acid, (3) caffeic acid, (4) epicatechin, (5) coumaric acid, and (6) protocatechuic acid.

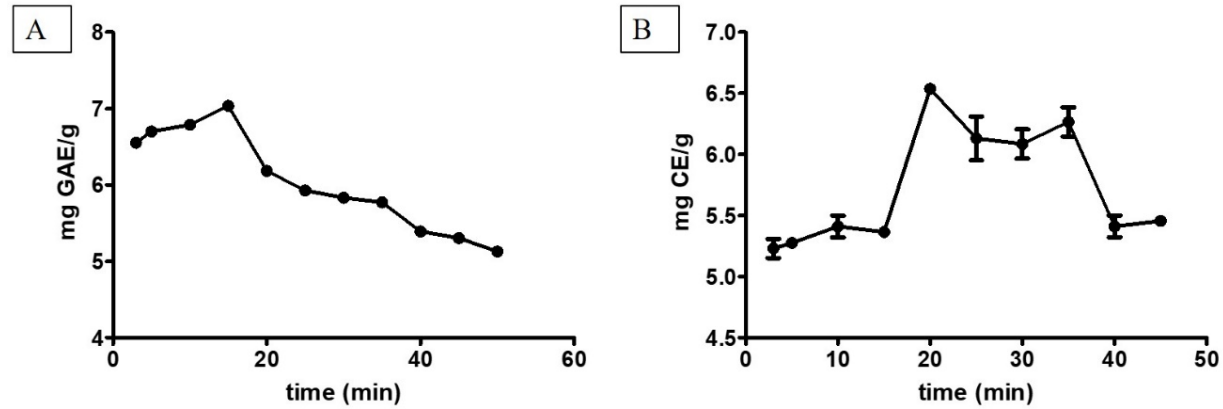

Fig. 5. Extraction kinetics for A) polyphenols and B) flavonoids with microwave pretreatment: 100% water and 5 °C.

The following acids and flavonoids were identified comparing the standards (Sigma-Aldrich, USA) with the significant peaks in the HPLC-DAD analysis: protocatechuic, vanillic, coumaric and caffeic acids and catechin and epicatechin flavonoids. The main differences found between pretreated and non-pretreated samples were: catechin 6.15 mg/L vs. 3.25 mg/L, caffeic acid 2.72 mg/L vs. 1.15 mg/L and epicatechin 10.69 mg/L vs.7.19 mg/L respectively. It is clear the microwave pretreatment resulted in an increment of 47% in catechin, 57% in caffeic acid and 32% in epicatechin in the xoconostle's endocarp. These results are comparable to those reported by Guzmán-Maldonado *et al*. (2010), Osorio-Esquivel *et al*. (2011), Morales *et al*. (2014) and Cortez-García *et al*. (2015), who suggested that xoconostle and their residues could be considered a potential source of bioactive substances.

3.4 Mass transfer phenomenon during the TFC and TFC extraction

Fick's first law of diffusion (Cussler, 1986) could be applied to describing extraction of components from vegetable tissues. However, given the nature of the vegetable tissue under study, certain limitations must be considered before applying conventional mass transfer principles. Such limitations are related to the structure of tissues and heat damage of extracted compounds which substantially affect mass transfer characteristics of the system. This leads to the application of lumped mass transfer coefficients (Treybal, 1981) and proper choosing of concentration gradients and system boundaries. Such coefficients will necessarily include tissue structure, physicochemical and morphometric characteristics (i.e. the fractal dimension) of an increasingly disrupted tissue (Lima *et al*., 2017). The main limitations to the application of mass transfer theory are: it has been reported that, microwave processing disrupts the integrity of vegetable tissues by means of selective heating of structures depending on their water content (Chandrasekaran *et al*., 2013). In the case of our experiments, the extraction of TPC and TFC was favoured by such treatment as shown in Table 2, since, conditions corresponding to run 10 (including microwave treatment) gave place to the maximum extraction of these components. Also, these extraction conditions included a shorter extraction time as compared to those reached by means of run 17 in which, by using 30 min extraction time, a 73% and 78% of TPC and TFC respectively, in relation to those achieved by the use of microwaves were reached (Table 2). Theory indicates that the diffusion of a certain component increases with temperature (Treybal, 1981). However, in the case of this work, a low temperature (5°C) favoured extraction given the marked heat-sensibility of extracted compound (Dahmoune *et al*., 2015). The above considerations were reflected in extraction kinetics for polyphenols (Figure 5A) and flavonoids (Figure 5B). Regarding extraction of polyphenols, it is possible to observe that, during the first 15 minutes of extractions at the conditions of run 10, an increase in extraction time produced higher extractions while after this time, extraction started to decrease, due to the lower concentration of these compounds in the vegetable matrix. Regarding the extraction of flavonoids, a different behaviour was observed. In Figure 5B, it is possible to observe that during the first 20 minutes of extraction time, the release of these compounds increased up to a maximum of mg CE/g and then the extraction was maintained relatively constant for 15 minutes, after which, extraction decreased due to the lower residual concentration of flavonoids in the vegetable tissue after this time.

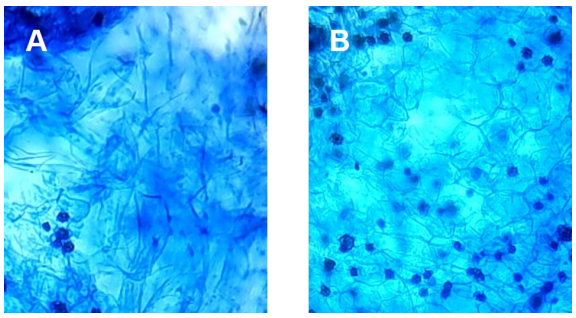

Fig. 6. Optical microscopy images obtained from A) xoconostle's endocarp pretreated with microwave and B) xoconostle's endocarp non-pretreated.

The images of non-treated and pretreated xoconostle's endocarp, under the optical microscope are presented in Figure 6A and 6B.

3.5 Optical and scanning electron microscopy (SEM)

In Fig. 6A we can observe, that the non-treated endocarp exhibited relative regular and well-defined shapes of intracellular spaces with homogeneous size cells and intact cell walls. In contrast, xoconostle's endocarp pretreated with microwave, presented changes in the morphological structure of the cells, observing opened, empty expanded cells and without

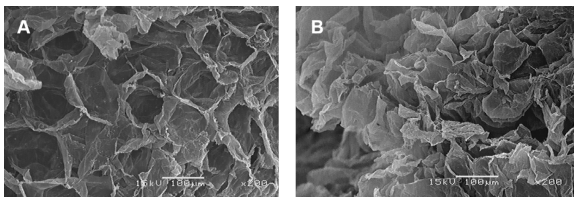

Fig. 7. Scanning electron micrographs of: A) xoconostle's endocarp pretreated with microwaves and B) xoconostle's endocarp non-treated at magnification 200x.

turgor (Fig. 6A).

This results suggest that microwave action affected the physical structure of the cell due to molecular movement leading to speedy heating of the solvent (Dahmoune *et al*., 2014). Moreover, the microwave reduces heating time through the mechanical shock of internal disruption, which promotes the extractability of the bioactive compounds from cells (Nistor *et al*., 2017).

These structural findings were similar to those reported by Lee *et al*. (2018) and Paciulli *et al*. (2016) after microwave treatment, where carrots' cells looked open and empty, with a slight loss of turgor. The microstructure of xoconostle's endocarp with and without microwave pretreatment is shown in Figures 7A and 7B. Figure 7A presents the image of non-treated endocarp and Figure 7B shows endocarp pretreated in microwave oven. In Figure 7A xoconostle's endocarp maintains some original parts, well-defined and preserved structures of cell walls with alternating filled rings. The smooth internal surfaces were observed as the dominant architectural element in non-treated microstructures. In contrast, after samples preparation and microwave pretreatment, the endocarp lost their original structure due to the cell stress (Figure 7B).

After microwave pretreatment, the surface of sample was disrupted, and the texture was crumbled; the cells seem ripped and empty due to the electromagnetic waves effect reported by Latorre *et al*. (2013), Dahmoune *et al*. (2015) and Mustapa *et al*. (2015). These authors, found that microwave treatment in myrtle leaves, red beet root and snake grass, produced high vapor pressure inside the cells (as a consequence of the sudden temperature rise), accelerating cell rupture and improving the release of solutes from the plant material, as we observed in the micrograph 7B.

Conclusions

This investigation shows that xoconostle´s endocarp is a rich source of antioxidant compounds such as phenolic compounds. Our results showed that microwave pretreatment improved the extraction of antioxidant substances such as: polyphenols, flavonoids, betalains and ascorbic acid from xoconostle's endocarp fruit. The best conditions for total phenolics compounds extraction and maximum antioxidant activity were: 100% water as a solvent, 10 min of agitation and 5 ºC of incubation with microwaves pretreatment. The consumption of antioxidants from xoconostle or its byproducts, can help to improve human health. Further studies have to be conducted in order to scale up and model large-scale extraction and determine the cost analysis.

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