Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Original Article

Effect of extraction conditions on total phenolic content and antioxidant capacity of bagasse Mexican oregano

Efecto de las condiciones de extracción sobre el contenido fenólico total y la capacidad antioxidante del orégano mexicano de bagazo

Rojo-Gutiérrez, E.[1] **, Ordoñez-Cano, A.J.**[1] **, Ochoa-Reyes, E.**[1] **, Tafolla-Arellano, J.C.**[2] **, Romeo Rojas**[3] **, Buenrostro-Figueroa, J.J.**[1,*]

[1] Grupo de Bioprocesos y Compuestos Bioactivos, Laboratorio de Biotecnología y Bioingeniería, Centro de Investigación en Alimentación y Desarrollo, A.C. 33089, Cd. Delicias, Chihuahua, México.
[2] Laboratorio de Biotecnología y Biología Molecular, Departamento de Ciencias Básicas, Universidad Autónoma Agraria Antonio Narro, 25315, Saltillo, México.
[3] Universidad Autónoma de Nuevo León, Facultad de Agronomía, General Escobedo, 66050, Nuevo León, México.

ABSTRACT

Mexican oregano (*Lippia graveolens* Kunth) is one of the most important species worldwide due to its beneficial human health properties and high economic value. Oregano leaves are primarily used for essential oil extraction, generating substantial agro-industrial waste known as bagasse (OB). It has been reported that OB still contains several bioactive compounds with high antioxidant activity. This study aimed to evaluate the extraction conditions to recover phenolic compounds and assess their antioxidant capacity in OB extracts obtained through ultrasound. A 3^k Box-Behnken design was employed to explore the effects of temperature, mass/volume ratio, and [EtOH]. Total phenolic content and antioxidant capacity against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals were determined. The increase in temperature and m/v ratio promoted the extraction of phenolic compounds, with strong correlations to antioxidant activity by DPPH$^{\cdot}$ (0.81) and ABTS$^{\cdot+}$ (0.82) assays. A quadratic effect by [EtOH] was observed, with a maximum value of 50 % ethanol. The highest value of total phenolic content (35.66 mgGAE/gdm), and antioxidant capacity against DPPH$^{\cdot}$ (100.94 mgTE/gdm) and ABTS (93.02 mgTE/gdm) radicals were achieved in treatment 13 (0.02 mg/mL, 50 % EtOH, and 90°C). The OB is a potential source of bioactive compounds with potential applications in functional food development.

Keywords: agroindustrial waste; oregano bagasse; bioactive compounds; sustainability; valorization.

RESUMEN

El orégano mexicano (*Lippia graveolens* Kunth) es una de las especies más importantes a nivel mundial por sus propiedades benéficas para la salud humana y su alto valor económico. Las hojas de orégano se utilizan principalmente para la extracción de aceite esencial, generando un importante residuo agroindustrial conocidos como bagazo (BO). Se ha reportado que el BO aún contiene varios compuestos bioactivos con alta actividad antioxidante. El objetivo del estudio fue evaluar las condiciones de extracción para recuperar compuestos fenólicos y evaluar su capacidad antioxidante en extractos de BO obtenidos por ultrasonido. Se empleó un diseño Box-Behnken 3^k para explorar el efecto de la temperatura, relación masa/volumen y [EtOH]. Se determinó el contenido de fenoles totales y la capacidad antioxidante frente a los radicales DPPH$^{\cdot}$ and ABTS$^{\cdot+}$. El aumento de la temperatura y de la relación masa/volumen favoreció la extracción de compuestos fenólicos, con fuertes correlaciones con la actividad antioxidante mediante el ensayo DPPH$^{\cdot}$ (0.81) y ABTS$^{\cdot+}$ (0.82). Se observó un efecto cuadrático por [EtOH], con un valor máximo al 50 % de etanol. El valor más alto de contenido fenólico total (35.66 mgEAG/gms), y de capacidad antioxidante frente a los radicales DPPH$^{\cdot}$ (100.94 mgET/gms) y ABTS$^{\cdot+}$ (93.02 mgET/gms) se alcanzó en el tratamiento 13 (0.02 mg/mL, 50 % EtOH y 90°C). El BOes una fuente potencial de compuestos bioactivos con aplicaciones potenciales en el desarrollo de alimentos funcionales.

Palabras clave: residuos agroindustriales; bagazo de orégano; compuestos bioactivos; sostenibilidad; valorización.

INTRODUCTION

Mexican oregano (*Lippia graveolens* Kunth or *Lippia berlandieri* Schauer, Verbenaceae) is one of the non-timber forest products of high economic importance in Mexico, since it is the second largest oregano exporter country and contributes 35 -40 % of worldwide oregano production (Cid-Pérez *et al.*, 2019), satisfying about half of its consumption in the United States (Orona Castillo *et al.*, 2017).

The commercial value of Mexican oregano resides in its food, cosmetic, and pharmaceutical uses (Calvo-Irabien, 2018; Stashenko *et al.*, 2018). Leaves are widely used as seasoning or for infusion teas, as well as raw material for extracting its essential oil (EO). *L. graveolens* is enriched with a variety of flavonoids (*pinocembrin*, *quercetin O-hexoside*) (Lin *et al.*, 2007; Du *et al.*, 2018), and terpenes, such as thymol and carvacrol, which are the major phytochemical components of its EO (Reyes-Jurado *et al.*, 2019). Additive, antioxidant, antibacterial, antifungal, analgesic, anti-inflammatory, anticancer, and antidiabetic properties of *L. graveolens* EO have been reported in the literature, among others (Alagawany *et al.*, 2015; González-Trujano *et al.*, 2017; Gutiérrez-Grijalva *et*

*Author for correspodece: J. J. Buenrostro Figueroa
e.mail: jose.buenrostro@ciad.mx
Received: November 4, 2024
Accepted: January 8, 2025
Published: February 7, 2025

DOI: 10.18633/biotecnia.v27.2494

al., 2017). Extraction of EO by the industry generates large amounts of the total weight of the plant (Martínez-Natarén *et al.*, 2013). This agroindustrial waste could be valorized since several amounts of bioactive compounds are still contained in the oregano bagasse (OB); however, it is commonly discarded.

Cid-Pérez *et al.* (2019) characterized and evaluated Mexican oregano (*Lippia longiflora*) EO and OB antioxidant and antimicrobial activities. The GC-MS analysis showed that the carvacrol and thymol relative % content in OB (non-polar subfraction of an ethyl acetate extract), was 1.8 and 1.5 times higher, respectively, compared to the values exhibited by the EO. However, bioactivity information about *L. graveolens* OB is still scarce. Besides, there is no information on the analysis of the extraction process to obtain the most suitable operational parameters for the extraction of bioactive compounds. Therefore, this study aimed to evaluate the extraction conditions (mass/volume ratio, temperature, [EtOH]) to recover phenolic compounds and assess their antioxidant capacity in OB extracts obtained through ultrasound.

MATERIAL AND METHODS

Plant material

The OB was provided by an oregano essential oil plant (ORE®, Saucillo, Chihuahua, Mexico). OB was dried at 60 °C for 48 h (Shel lab model 1380FX; Sheldon Manufacturing, Inc., Oregon, US) and milled (Hamilton Beach model 80350R). Dried powder (<500 µm) was stored in black bags at room temperature.

Extraction of phenolic compounds

Ultrasound-assisted extraction (UAE) was conducted by a set of 15 treatments composed of different values of mass/volume (m/v) ratio, temperature, and [EtOH], as shown in Table 1. Samples were placed in an Erlenmeyer flask (125 mL), and 25 mL of solvent (ethanol or distilled water) were added. UAE was conducted in a VWR sonicator (150 D; VWR International, West Chester, PA) at 50 - 60 Hz. After 30 min of extraction, samples were centrifuged (Eppendorf Centrifuge model 5804R) at 4300×*g* for 10 min, and extracts were obtained by decantation. Samples were placed in amber vials (1.5 mL) and stored at - 20 °C until its analysis.

Analytical procedures

Total phenolic content

The amount of total phenolic content (TPC) was determined according to the methodology reported by Wong-Paz *et al.* (2015). Twenty µL of the Folin-Ciocalteu reagent were mixed with 20 µL of the sample and allowed to stand for 5 min at room temperature (28±2ºC). Subsequently, the mixture sample was reacted with 200 µL of Na_2CO_3 (0.01 M) for 5 min. Finally, the sample was diluted with 125 µL of distilled water. The absorbance was measured at 730 nm using a UV-VIS microplate reader (Multiskan Go, Thermo Scientific). The standard calibration (0 - 200 µg/L) curve was plotted using gallic acid. The TPC was expressed as mg of gallic acid equivalents per gram of dry matter (mgGAE/gdm). Each treatment was conducted by triplicate.

Antioxidant capacity by DPPH$^{\cdot*}$ assay

The DPPH$^{\cdot}$ (2,2-difenil-1-picril-hidracil) antioxidant activity assay was conducted following the methodology described by Meléndez *et al.* (2014). Seven µL of extract sample were added to 193 µL of free radical DPPH$^{\cdot}$ solution (60 µM). The solution mixture was allowed to react in the dark for 30 min. The absorbance was measured using a UV-VIS microplate reader at 517 nm. The standard calibration (0 - 200 µg/L) curve was plotted using Trolox as a reference. The antioxidant capacity against DPPH$^{\cdot}$ radical was reported as mg of Trolox equivalents per g of dry matter (mgTE/gdm). Each treatment was conducted by triplicate.

Antioxidant capacity by ABTS$^{\cdot+}$ assay

Antioxidant capacity against radical ABTS$^{\cdot+}$ (2,2'-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]) was determined according to a procedure reported by López-Cárdenas *et al.* (2023). ABTS$^{\cdot+}$ free radical was obtained by mixing 12.5 mL of $K_2S_2O_8$ (2.45 mM) with 25 mL of ABTS (7 mM) and left for 16 h in the dark at room temperature (28ºC). After that, ABTS$^{\cdot+}$ was diluted with ethanol to get an absorbance value of 0.7±0.01 at 734 nm. ABTS$^{\cdot+}$ scavenging activity assay was performed by mixing 190 µL of ABTS$^{\cdot+}$ with 10 µL of the sample. The mixture was left to react for 1 min, and its absorbance was measured and registered using a microplate spectrophotometer (UV-VIS). Ethanol (100 %) was employed as blank, and an ethanolic ABTS$^{\cdot+}$ solution as the control. The standard calibration (0 - 200 µg/L) curve was plotted using Trolox as a reference. The antioxidant capacity against ABTS$^{\cdot+}$ radical was reported as mg of Trolox equivalents per g of dry matter (mgTE/gdm). Each treatment was conducted by triplicate.

Experimental design and data analysis

A 3^k Box Behnken design (BBD) was used to evaluate the influence of extraction conditions on the extraction yield of TPC and antioxidant capacity of extracts from OB by UAE (Table 1). Temperature (40, 50, and 60 °C), m/v ratio [0.1(1:10), 0.04 (1:25), and 0.02 (1:50) mg/mL] and [EtOH] (0, 50 and 100 %) were the extraction variables evaluated. ANOVA test was used for data analysis, and when needed, means of treatments (three replicates) were compared by the Tukey-test ($p < 0.05$) using STATISTICA 7.0 software (Stat Soft, Tulsa, OK, USA). Also, the Pearson correlation coefficient was used to determine the linear correlation among response variables.

The experimental data were integrated into a second-order model to determine the relationship between the independent variables and the TPC release from OB. The best conditions for maximizing TPC release were estimated using the regression coefficient generated for each assayed term and its combination at a significance level of 0.05. Equation 1 provides an outline of the regression response model.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \sum \beta_{ij} x_i x_j \quad (1)$$

where Υ is the predicted response (TPC), k is the number of variables evaluated; X_i and X_j represent the independent variables; β_o, β_{ii}, β_i, and β_{ij} are the coefficients of regression to the constant, linear, quadratic, and interaction effects, respectively. The best conditions for TPC release from OB (temperature, m/v ratio, and ethanol concentration) were obtained from the predictive surface response equation. Finally, the experimental results were statistically compared with the predicted values to validate the model.

RESULTS AND DISCUSSION

The effect of extraction parameters, was evaluated by the influence of the interaction among the three main variables (temperature, m/v ratio, and [EtOH]) on the extraction yield of total phenolic compounds using the BBD. The results of TPC and AC obtained for the treatments conducted are presented in Table 1, showing a significant effect of the evaluated parameters ($p < 0.01$), with a recovery rate ranging from 8.40 to 35.66 mgGAE/gdm for TPC, as well as 18.37 to 107.61 and 9.93 to 98.2 mgTE/gdm for DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, respectively.

Treatment 13 showed the highest extraction of phenolic compounds (35.66 mg GAE/g dm) and antioxidant capacity values (107.61 and 98.02 mg TE/g dm of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, respectively), whereas treatment 5 showed the lowest. Treatment 13 (60 ºC, 0.02 m/v ratio, 50 % [EtOH]) exhibits a 4.3-fold increase in TPC content and enhances antioxidant activity by 5.9- and 9.9 - fold against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, respectively, compared to treatment 5. Phenolic compounds are known for neutralizing free radicals by donating hydrogen atoms, electrons, or metallic cations. These interactions with free radicals are due to their unique structure, specifically the arrangement and quantity of hydroxyl groups and the substitutions that occur within the aromatic rings (Becerril-Sánchez *et al.*, 2021). Therefore, an increase in TPC is usually followed by an increase in antioxidant capacity. The latter supports the obtained results, as a significant correlation ($p < 0.01$) was observed between TPC and antioxidant capacity (DPPH$^{\bullet}$, ABTS$^{\bullet+}$) based on Pearson's coefficient (Table 2). This indicates that the enhanced antioxidant capacity is due to the increased release of TPC. Consequently, only the results related to the extraction yield of TPC are presented.

Table 1. Box-Behnken design matrix and response variables for the extraction yield of total phenolic (TPC) and antioxidant capacity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) of extracts from OB by UAE.

Tabla 1. Matriz de diseño Box-Behnken y variables de respuesta para el rendimiento de extracción de fenoles totales (TPC) y capacidad antioxidante (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) de extractos de BO por EAU.

Treatment	A	B	C	TPC[1]	DPPH$^{\bullet}$[2]	ABTS$^{\bullet+}$[2]
1	60	0.10	50	26.34±1.14^{bc}	54.62±0.79^{ef}	35.69±0.07^{fg}
2	60	0.04	100	20.43±3.34^{de}	46.97±2.35^{fg}	58.64±1.98^{d}
3	40	0.04	0	15.54±1.45^{ef}	60.55±3.54^{e}	44.34±3.44^{ef}
4	50	0.04	50	29.75±1.22^{b}	109.01±7.54^{a}	80.57±0.87^{b}
5	40	0.04	100	8.40±0.97^{g}	18.37±2.42^{h}	9.93±1.88^{h}
6	50	0.02	0	24.02±2.19^{bcd}	85.41±2.07^{d}	71.33±6.02^{bc}
7	40	0.02	50	24.33±1.71^{bcd}	96.53±4.25^{bc}	76.16±6.50^{bc}
8	60	0.04	0	21.13±2.72^{cde}	56.59±3.75^{ef}	53.72±3.93^{de}
9	50	0.02	100	13.61±2.46^{fg}	39.65±0.67^{g}	29.86±2.43^{gh}
10	50	0.1	100	10.08±1.33^{fg}	22.72±5.37^{g}	23.63±1.29^{h}
11	40	0.1	50	22.62±1.09^{cd}	56.48±.013^{ef}	35.94±0.03^{fg}
12	50	0.1	0	14.57±0.50^{f}	48.85±1.41^{fg}	34.56±0.58^{fg}
13	**60**	**0.02**	**50**	**35.66±2.36^{a}**	**107.61±3.72^{a}**	**98.02±1.51^{a}**
14	50	0.04	50	28.92±2.08^{b}	105.98±3.42^{ab}	77.63±0.93^{bc}
15	50	0.04	0	29.38±1.79^{b}	94.77±1.74^{cd}	69.47±6.48^{c}

A: Temperature (°C); B: m/v ratio (mg/mL); C: [EtOH] (%); [1]mgGAE/gdm; [2]mgTE/gdm. *The same letters among columns indicate a significant difference ($p < 0.05$). Values are the mean ± standard error (n = 3).

Table 2. Pearson correlation analysis among TPC and antioxidant capacities. *means significant correla-tion at 0.01 level. TPC, total phenolic content; DPPH, DPPH radical antioxidant capacity; ABTS, ABTS radical antioxidant capacity.

Tabla 2. Análisis de correlación de Pearson entre TPC y capacidades antioxidantes. *significa correlación significativa al nivel 0.01. TPC, contenido fenólico total; DPPH, capacidad antioxidante frente al radical DPPH; ABTS, capacidad antioxidante frente al radical ABTS.

Factor	TPC	DPPH$^{\bullet}$	ABTS$^{\bullet+}$
TCP	1.00	0.88*	0.86*
DPPH$^{\bullet}$	---	1.00	0.93*
ABTS$^{\bullet+}$	---	---	1.00

Significant factors for yield extraction of TPC

To evaluate the influence of temperature, m/v ratio, and [EtOH], the absolute values of standardized effects were estimated, with their behaviors shown in Fig. 1. Temperature, [EtOH], and temperature–m/v ratio interaction were the most influential ($p < 0.05$). In contrast, m/v ratio showed no significant effect (Fig. 1a).

An increase in temperature was observed to enhance the extraction of TPC (Figure 1b). It is known that temperature plays a crucial role in the efficiency of extracting phenolic compounds through UAE. Increasing the temperature facilitates the breaking of sample analyte links and reduces the solvent's viscosity. This enhances the diffusivity and solubility of polyphenols, resulting in improved mass transfer and higher extraction yields (Machado *et al.*, 2015; Osorio-Tobón, 2020). Based on the reviewed literature by Osorio-Tobón (2020), the temperature extraction values of phenolic compounds generally ranged between 20 and 90 ºC, whereas the optimal temperatures for many polyphenol extractions are between 40 and 60 ºC (Chávez-González *et al.*, 2020). The latter follows our results since the highest extraction yield of TPC was obtained at 60 ºC. Other researchers, such Pandey *et al.* (2018), Wojeicchowski *et al.* (2021), Shehata *et al.* (2021), Brahmi *et al.* (2022), have also observed similar behavior in the extraction of phenolic compounds by UAE from *Rheum moorcroftianum*, rosemary, orange peels, and *Opuntia ficus-indica*, respectively.

Contrary to the effect exerted by temperature, an increase in m/v ratio tends to decrease the TPC extraction yield from OB (Figure 1c), as well as the antioxidant activity of the

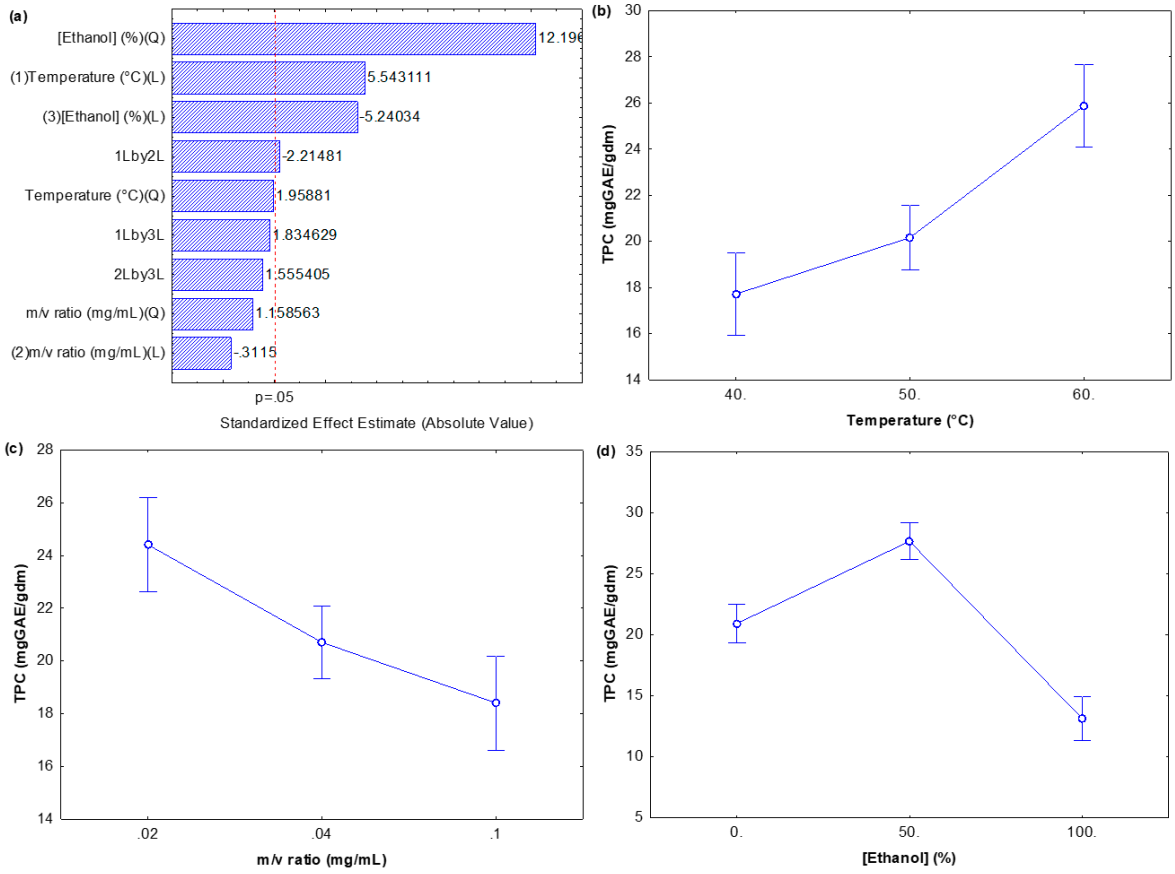

Fig. 1. Pareto chart of estimated effect (a), and influence of (b) temperature, (c) ratio m/v, and (d) [EtOH] on extraction yield of total phenolic content in oregano bagasse extract obtained by ultrasound-assisted extraction.
Fig. 1. Diagrama de Pareto del efecto estimado (a) y de la influencia de la (b) temperatura, (c) relación m/v, y (d) [EtOH] en el rendimiento de extracción del contenido total de fenoles en el extracto de bagazo de orégano obtenidos mediantela extracción asistida por ultrasonido.

resulting extracts. As the sample amount increases, less solvent can penetrate the plant cells, limiting cell wall rupture and reducing the TPC extraction yield (Uysal *et al.*, 2019). Additionally, increasing the m/v ratio reduces the concentration gradient created by the diffusion of the sample into the solvent. This also results in minimal material swelling, which decreases the contact area between the solvent and the sample, ultimately lowering the TPC extraction yield (Goula *et al.*, 2017). The results suggest that the TPC extraction yield increases with a higher solvent volume and decreases when less solvent is used. This effect has been previously reported in studies where TPC was extracted from red algae (Putra *et al.*, 2022) and spent coffee grounds (Ferreira *et al.*, 2021; Solomakou *et al.*, 2022). Despite this decreasing trend, no significant differences were observed among the three level points in the Box-Behnken design (Fig. 1c).

As shown in Fig. 1d, a quadratic effect of ethanol concentration was observed. The maximum TPC extraction yield occurred at the center value (50 %), while lower (0 %) and higher (100 %) ethanol concentrations resulted in a decreased yield. Specifically, the TPC extraction yield increased by an average of 47.4 % as the ethanol concentration rose from 0 % to 50 %, then reduced by an average of 47.33 % as the ethanol concentration increased from 50 % to 100 %. The phenolic profile of the plant material or food matrix will determine the solubility of TPC. Therefore, the extraction yield is highly influenced by the polarity of the employed solvent since it affects the solubility of the bioactive compounds of interest (Romero-Díez *et al.*, 2018). In that matter, results showed that using a 50/50 aqueous ethanol mixture allowed us to achieve the maximum TPC yield, indicating that the phenolic compounds found in the bagasse Mexican oregano are partially polar. The obtained results are in accordance with several studies which reported that the use of a bi-component solvent system (water-ethanol) was the most efficient in promoting the extraction yield of TPC on dried chokeberry, mangiferin, grape pomace, spices, and herbs (Ćujić *et al.*, 2016; Lim *et al.*, 2019; Drevelegka and Goula, 2020; Muzolf-Panek and Stuper-Szablewska, 2021). Moreover, the maximum yields of TPC were obtained when the [EtOH] ranged between 40–60 %. Any lower o higher proportion of [EtOH] resulted in lower TPC yields.

To analyze the combined effects of the independent variables and their mutual interaction on the extraction yield of TPC and AC, three-dimensional surface profiles with multiple non-linear regression models were plotted. The corresponding contour plots of these profiles are presented in Fig. 2.

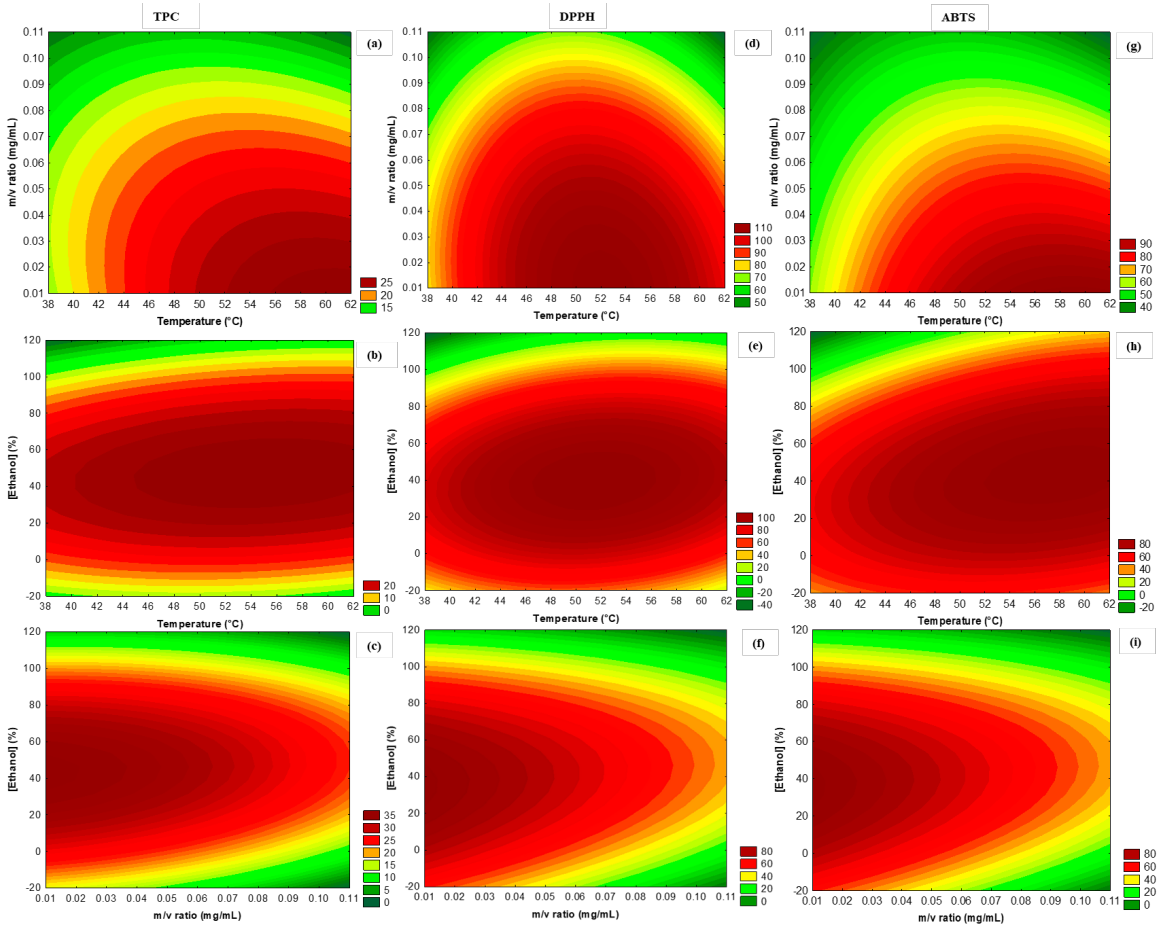

Fig 2. Interactive effects of temperature, m/v ratio and [ethanol] on extraction yield of total phenolic content (a,b,c) and antioxidant capacity (DPPH$^{\cdot}$ (d,e,f) and ABTS$^{\cdot+}$ (g,h,i)) from oregano bagasse extract obtained by ultrasound-assisted extraction.
Fig. 2. Efectos interactivos de la temperatura, relación m/v y [etanol] sobre el rendimiento de extracción de TPC (a,b,c) y la capacidad antioxidante (DPPH$^{\cdot}$ (d,e,f) y ABTS$^{\cdot+}$ (g,h,i)) del extracto del bagazo de orégano obtenidos mediante la extracción asistida por ultrasonido.

Increasing the temperature from 40 to 60 ºC and decreasing the m/v ratio from 1:10 (0.1 mg/mL) to 1:50 (0.02 mg/mL), increased the TPC extraction yield (Fig. 2a). The same effect was observed over the antioxidant capacity against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals (Figure 2d and 2g, respectively). Maximum yield extraction of TPC and AC were obtained at center values of temperature and [EtOH] (Fig. 2b), like the observed in AC against DPPH$^{\cdot}$ and ABTS$^{\cdot}$ radicals (Figures 2e and 2h). Increasing the [EtOH] from the low to the central point value, an average increase of 68.66 and 30.52 % were registered in DPPH$^{\cdot}$ and ABTS$^{\cdot}$, respectively. As the [EtOH] increased from the central to the highest level, the DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ activity decreased by an average of 30.12 and 37.77 %, respectively. Considering the interactive effect of [EtOH] and m/v ratio, the best yield extraction of TPC and AC were observed at center values of [EtOH] and low values of m/v ratio (Fiures 2c, 2f and 2i).

These findings are supported by Ferreira *et al.* (2021), who reported that the m/v ratio (1:30 – 1:100) did not present any significant effect in extracting PC from *Psidium guajava* coproduct, employing ionic liquid UAE. However, the combined effect of temperature and m/v ratio did show a positive influence ($p < 0.05$) in the extraction of TPC and antioxidant activity (DPPH$^{\cdot}$, ABTS$^{\cdot+}$). Frías-Zepeda and Rosales-Castro (2021) analyzed through a 3^2 factorial the impact of m/v ratio (1:10, 1:20, and 1:30) and solvent (30, 50, and 80 % [EtOH]) on the extraction of phenolic compounds in oregano residues, through the recovery of TPC with the maceration method at room temperature (25 ºC). The maximum yield was achieved with a m/v ratio of 1:30, while 1:10 extractions yielded the lowest ($p < 0.05$) TPC value. They reported no significant difference between 1:20 (central level) and 1:30 (top-level) m/v ratios. The results obtained in the present work partially agree with these findings, since the yield values obtained from the extraction 1:25 (central level) and 1:60 (top-level) m/v ratios were not statistically different. However, contrary to their results, the extraction values of TPC employing a 1:10 m/v ratio did not show any statistical difference from those obtained using the rest of the m/v ratios (1:25, 1:50). A feasible explanation could be that in the current study, different temperatures, and extraction technology (UAE) were used. Contour plots showed a tendency to improve the extraction yield of TPC and antioxidant capacity by DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ assays, within the temperature range of 56 to 62

°C, [EtOH] of 30 to 60 %, and m/v ratio of 0.01 to 0.02 mg/mL (Fig. 2). A second order polynomial model (Eq. 1), along with experimental data and regression coefficients derived from multiple linear regressions (Eq. 2), were used to identify the best conditions for TPC extraction from OB by UAE.

$$TPC = -45.76 + 2.32x - 0.02x^2 + 236.91y - 957.75y^2 - 4.58xy \quad (2)$$

Under ideal conditions, the simplified model (Eq. 2) predicted a maximum extraction yield of 37.11 mg/gdm. When applying the model to treatment 13 conditions (60 °C, 0.02 mg/mL and 50 % ethanol), the experimental yield value (35.66 mg/gdm) was 2 % higher than the model's prediction (34.90 mg/gdm), but still within the model's error of significance. The study of extraction conditions resulted in a 4.3-fold increase in TPC extraction yield and 5.9-fold and 9.9-fold increases in the AC against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals, respectively.

CONCLUSIONS

This study focused on temperature, [EtOH], and m/v ratio, which significantly influence the extraction yield of TPC and AC from OB using UAE. The best extraction conditions were found at 60 °C, 50 % ethanol, and an m/v ratio of 0.02 mg/mL, which resulted in the highest TPC yield (35.66 mg GAE/gdm) and AC against DPPH and ABTS$^{\cdot+}$ radicals. These conditions led to a 4.3-fold increase in TPC extraction and 5.9- and 9.9-fold improvements in AC against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals, respectively. The extraction process was enhanced with higher temperatures and lower m/v ratios, with a quadratic effect observed for ethanol concentration, where 50 % ethanol provided the best results. The findings suggest that OB, often considered waste, is a potential source of bioactive compounds and could serve as an important ingredient in functional food products or other industrial applications.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Alagawany, M., Elgammal, M., Farag, M., Tiwari, R. and Dhama, K. 2015. Biological effects and modes of action of carvacrol in animal and poultry production and health - A review. *Advances in Animal and Veterinary Sciences,* 3, 73-84. http://dx.doi.org/10.14737/journal.aavs/2015/3.2s.73.84

Becerril-Sánchez, A.L., Quintero-Salazar, B., Dublán-García, O. and Escalona-Buendía, H.B. 2021. Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants*, 10 (11), 1700. https://doi.org/10.3390/antiox10111700

Brahmi, F., Blando, F., Sellami, R., Mehdi, S., De Bellis, L., Negro, C., Haddadi-Guemghar, H., Madani, K. and Makhlouf-Boulekbache, L. 2022. Optimization of the conditions for ultrasound-assisted extraction of phenolic compounds from *Opuntia ficus*-indica [L.] Mill. flowers and comparison with conventional procedures. *Industrial Crops and Products,* 184, 114977. https://doi.org/10.1016/j.indcrop.2022.114977

Calvo-Irabien, L.M. 2018. Native Mexican aromatic flora and essential oils: Current research status, gaps in knowledge and agro-industrial potential. *Industrial Crops and Products,* 111, 807-822. https://doi.org/10.1016/j.indcrop.2017.11.044

Chávez-González, M.L., Sepúlveda, L., Verma, D.K., Luna-García, H.A., Rodríguez-Durán, L.V., Ilina, A. and Aguilar, C.N. 2020. Conventional and emerging extraction processes of flavonoids. *Processes,* 8 (4), 434. https://doi.org/10.3390/pr8040434

Cid-Pérez, T., Avila-Sosa, R., Ochoa Velasco, C.E., Rivera-Chavira, B. and Nevarez-Moorillon, G. 2019. Antioxidant and antimicrobial activity of Mexican oregano (*Poliomintha longiflora*) essential oil, hydrosol and extracts from waste solid residues. *Plants,* 8, 22. https://doi.org/10.3390/plants8010022

Ćujić, N., Šavikin, K., Janković, T., Pljevljakušić, D., Zdunić, G. and Ibrić, S. 2016. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry,* 194, 135-142. https://doi.org/10.1016/j.foodchem.2015.08.008

Drevelegka, I. and Goula, A.M. 2020. Recovery of grape pomace phenolic compounds through optimized extraction and adsorption processes. *Chemical Engineering and Processing - Process Intensification,* 149, 107845. https://doi.org/10.1016/j.cep.2020.107845

Du, G., Ma, Y., Song, J., Li, L., Niu, Z., Wu, S. and Lv, Y. 2018. *Applications of pinocembrin in preparing anti-cerebral hemorrage medicament*. China patent application WO/2018/090358.

Ferreira, B.L., Junior, T.K., Block, J.M., Granato, D. and Nunes, I.L. 2021. Innovative approach for obtaining phenolic compounds from guava (*Psidium guajava* L.) coproduct using ionic liquid ultrasound-assisted extraction (IL-UAE). *Biocatalysis and Agricultural Biotechnology,* 38, 102196. https://doi.org/10.1016/j.bcab.2021.102196

Frías-Zepeda, M.E. and Rosales-Castro, M. 2021. Efecto de las condiciones de extracción sobre la concentración de compuestos fenólicos en residuos de orégano mexicano (*Lippia graveolens* Kunth). *Revista Chapingo Serie Ciencias Forestales y del Ambiente,* 27, 367-381. https://doi.org/10.5154/r.rchscfa.2020.10.066

González-Trujano, M.E., Hernández-Sánchez, L.Y., Muñoz Ocotero, V., Dorazco-González, A., Guevara Fefer, P. and Aguirre-Hernández, E. 2017. Pharmacological evaluation of the anxiolytic-like effects of *Lippia graveolens* and bioactive compounds. *Pharmaceutical Biology,* 55, 1569-1576. https://doi.org/10.1080/13880209.2017.1310908

Goula, A.M., Ververi, M., Adamopoulou, A. and Kaderides, K. 2017. Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils. *Ultrasonics Sonochemistry,* 34, 821-830. https://doi.org/10.1016/j.ultsonch.2016.07.022

Gutiérrez-Grijalva, E.P., Angulo-Escalante, M.A., León-Félix, J. and Heredia, J. B. 2017. Effect of in vitro digestion on the total antioxidant capacity and phenolic content of 3 Sspecies of oregano (*Hedeoma patens*, *Lippia graveolens*, *Lippia palmeri*). *Journal of Food Science,* 82, 2832-2839. https://doi.org/10.1111/1750-3841.13954

Lim, Y.P., Pang, S.F., Yusoff, M.M., Abdul Mudalip, S.K. and Gimbun, J. 2019. Correlation between the extraction yield of mangiferin to the antioxidant activity, total phenolic

and total flavonoid content of Phaleria macrocarpa fruits. *Journal of Applied Research on Medicinal and Aromatic Plants,* 14, 100224. https://doi.org/10.1016/j.jarmap.2019.100224

Lin, L.Z., Mukhopadhyay, S., Robbins, R.J. and Harnly, J.M. 2007. Identification and quantification of flavonoids of Mexican oregano (*Lippia graveolens*) by LC-DAD-ESI/MS analysis. *Journal of Food Composition and Analysis,* 20, 361-369. https://doi.org/10.1016/j.jfca.2006.09.005

López-Cárdenas, F., Ochoa-Reyes, E., Baeza-Jiménez, R., Tafolla-Arellano, J.C., Ascacio-Valdés, J.A. and Buenrostro-Figueroa, J.J. 2023. Solid-state fermentation as a sustainable tool for extracting phenolic compounds from cascalote pods. *Fermentation,* 9, 823. https://doi.org/10.3390/fermentation9090823

Machado, A.P.D.F., Pasquel-Reátegui, J.L., Barbero, G.F. and Martínez, J. 2015. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International,* 77, 675-683. https://doi.org/10.1016/j.foodres.2014.12.042

Martínez-Natarén, D., Parra-Tabla, V., Dzib, G., Acosta-Arriola, V., Canul-Puc, K. and Calvo-Irabién, L. 2013. Essential oil yield variation within and among wild populations of Mexican oregano (*Lippia graveolens* H.B.K.-Verbenaceae), and its relation to climatic and edaphic conditions. *Journal of Essential Oil Bearing Plants,* 15, 589-601. https://doi.org/10.1080/0972060X.2012.10644093

Meléndez, N.P., Nevrez-Moorillón, V., Rodríguez-Herrera, R., Espinoza José, C. and Aguilar, C.N. 2014. A microassay for quantification of 2,2-diphenyl-1-picrylhydracyl (DPPH) free radical scavenging. *African Journal of Biochemistry Research,* 8, 14-18. https://doi.org/10.5897/AJBR2013.0669

Muzolf-Panek, M. and Stuper-Szablewska, K. 2021. Comprehensive study on the antioxidant capacity and phenolic profiles of black seed and other spices and herbs: effect of solvent and time of extraction. *Journal of Food Measurement and Characterization,* 15, 4561-4574. https://doi.org/10.1007/s11694-021-01028-z

Orona Castillo, I., Salvador Almazán, A.J., Espinoza Arellano, J.D.J. and Vázquez, C. 2017. Recolección y comercialización del orégano (*Lippia* spp) en el semidesierto mexicano, un caso de estudio: Reserva ecológica municipal Sierra y Cañon de Jimulco, México. *Revista Mexicana de Agronegocios,* 41, 684-695. http://dx.doi.org/10.22004/ag.econ.266423

Osorio-Tobón, J.F. 2020. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology,* 57, 4299-4315. https://doi.org/10.1007/s13197-020-04433-2

Pandey, A., Belwal, T., Sekar, K.C., Bhatt, I.D. and Rawal, R.S. 2018. Optimization of ultrasonic-assisted extraction (UAE) of phenolics and antioxidant compounds from rhizomes of *Rheum moorcroftianum* using response surface methodology (RSM). *Industrial Crops and Products,* 119, 218-225. https://doi.org/10.1016/j.indcrop.2018.04.019

Putra, V.G.P., Mutiarahma, S., Chaniago, W., Rahmadi, P., Kurnianto, D., Hidayat, C., Carrera, C., Palma, M. and Setyaningsih, W. 2022. An ultrasound-based technique for the analytical extraction of phenolic compounds in red algae. *Arabian Journal of Chemistry,* 15, 103597. https://doi.org/10.1016/j.arabjc.2021.103597

Reyes-Jurado, F., Cervantes-Rincón, T., Bach, H., López-Malo, A. and Palou, E. 2019. Antimicrobial activity of Mexican oregano (*Lippia berlandieri*), thyme (*Thymus vulgaris*), and mustard (*Brassica nigra*) essential oils in gaseous phase. *Industrial Crops and Products,* 131, 90-95. https://doi.org/10.1016/j.indcrop.2019.01.036

Romero-Díez, R., Rodríguez-Rojo, S., Cocero, M.J., Duarte, C.M.M., Matias, A.A. and Bronze, M.R. 2018. Phenolic characterization of aging wine lees: Correlation with antioxidant activities. *Food Chemistry,* 259, 188-195. https://doi.org/10.1016/j.foodchem.2018.03.119

Shehata, M.G., Abd El Aziz, N.M., Youssef, M.M. and El-Sohaimy, S.A. 2021. Optimization conditions of ultrasound-assisted extraction of phenolic compounds from orange peels using response surface methodology. *Journal of Food Processing and Preservation,* 45, e15870. https://doi.org/10.1111/jfpp.15870

Solomakou, N., Loukri, A., Tsafrakidou, P., Michaelidou, A.-M., Mourtzinos, I. and Goula, A.M. 2022. Recovery of phenolic compounds from spent coffee grounds through optimized extraction processes. *Sustainable Chemistry and Pharmacy,* 25, 100592. https://doi.org/10.1016/j.scp.2021.100592

Stashenko, E., Duran García, D.C., Martinez Morales, J.R., Cordoba Campo, Y., Arias Velandia, A.J., Mejia Medina, J.J. and Tavera Reyes, C. A. 2018. *Method for making full use of Lippia origanoides*. Colombia patent application 16/474,470.

Uysal, S., Cvetanović, A., Zengin, G., Zeković, Z., Mahomoodally, M.F. and Bera, O. 2019. Optimization of maceration conditions for improving the extraction of phenolic compounds and antioxidant effects of *Momordica charantia* L. leaves through response surface methodology (RSM) and artificial neural networks (ANNs). *Analytical Letters,* 52, 2150-2163. https://doi.org/10.1080/00032719.2019.1599007

Wojeicchowski, J.P., Marques, C., Igarashi-Mafra, L., Coutinho, J. a. P. and Mafra, M.R. 2021. Extraction of phenolic compounds from rosemary using choline chloride – based Deep Eutectic Solvents. *Separation and Purification Technology,* 258, 117975. https://doi.org/10.1016/j.seppur.2020.117975

Wong-Paz, J.E., Contreras-Esquivel, J.C., Rodríguez-Herrera, R., Carrillo-Inungaray, M.L., López, L.I., Nevárez-Moorillón, G.V. and Aguilar, C.N. 2015. Total phenolic content, in vitro antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. *Asian Pacific Journal of Tropical Medicine,* 8, 104-111. https://doi.org/10.1016/S1995-7645(14)60299-6