OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLS FROM GLOBE ARTICHOKE (*CYNARA SCOLYMUS* L.) BRACTS RESIDUES USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Background. The globe artichoke (*Cynara scolymus* L.) is a rich source of phenolic compounds which may be extracted by ultrasound technology and used as a medicinal alternative. The objective of this work was to determine the radiation amplitude (%), ethanol concentration (%), and time extraction (min) required to guarantee an elevated content of polyphenol compounds.
Materials and methods. The optimal extraction conditions were assessed through the Box-Wilson design and by applying Composite Face Centered (CCFC) and total phenolic compounds (TPC) as the response variables.
Results. A quadratic model was adequate, with $R^2 = 0.993$. The optimal conditions were a radiation amplitude of 97%, an ethanol concentration of 53%, and an extraction time of 9.7 min. The optimized extract of artichoke bracts (*Cynara scolymus* L.) showed a TPC of 25.13 (±0.030) mg GAE/g, an antioxidant activity DPPH of 39.79 (±0.014) mmol Trolox equivalents (TE), and an antioxidant capacity TEAC of 33.98 (±0.03) mmol Trolox equivalents.
Conclusion. The results showed values closely related to the expected values, indicating that the models were well-developed.

Keywords: extraction, ultrasound, optimization, polyphenols, antioxidant capacity

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INTRODUCTION

The globe artichoke (*Cynara scolymus* L.) is a perennial herbaceous plant that originated from Mediterranean countries, but is also widely cultivated worldwide (Frutos et al., 2019; Zhang and Cao, 2015). The annual production of artichokes in some countries is as follows: Italy (about 458,890 t annually), Spain (239,846 t), Egypt (152,909 t), Argentina (92,672 t), and Peru (71,540 t) (FAOSTAT, 2018). In Peru, artichokes are cultivated mainly in the regions of Lima, La Libertad, Ica, Ayacucho, Huancavelica, and Junín (known as Mantaro Valley). The edible parts consist of large immature inflorescences, called *capitula* or heads (Kollia et al., 2017; Sałata et al., 2012). During industrial processing, about 60−85% of artichokes harvested are discarded (Noriega-Rodríguez et al., 2020). Artichoke waste basically consists of leaves, stems, and mainly of the external parts of the heads (known as bracts) (Noriega-Rodríguez et al., 2020). These residues generate a great economic loss and environmental contamination because they are not suitable for human consumption and are discarded into the environment. However, they may be recovered due to the fact that they are rich sources of bioactive phenolic compounds, fiber, inulin, and minerals necessary for human nutrition (Jiménez-Moreno et al., 2019; Ruiz-Cano et al., 2014). As well as this, many studies have shown that artichokes have important medicinal properties such as antioxidant, diuretic, antifungal, antibacterial, and anticarcinogenic effects, as well as aiding cholesterol reduction and weight loss and helping to prevent a wide range of disorders and degenerative diseases, etc., (D'Antuono et al., 2018; Mahboubi, 2018; Salem et al., 2015). These health benefits from artichoke consumption are primarily due to its content of polyphenols. Artichoke composition depends on many factors such as climate, the variety of artichoke, and harvest time. However, phenolic compounds such as hydroxycinnamic acids and flavonoids are found in abundance in this plant and its waste (Negro et al., 2012). For instance, Zuorro et al. (2016) found higher amounts of polyphenols in artichoke residues than in coffee and grape residues. Therefore, polyphenol extraction/recovery from artichoke waste represents a very important source of this useful resource due to its store quantity and beneficial effects on human health.

Since processes involving the use of several organic solvents (solvent extractor) are known for their undesirable environmental and biological negative impacts, new eco-friendly alternatives for the processing of substances are required. Extraction assisted by ultrasound methods is not harmful and is used in food technology to recovery high amounts of bioactive compounds like polyphenols in a shorter time and at a cheaper cost (Chemat et al., 2011). Several authors have successfully applied this technique to recover polyphenols from different byproducts such as grapes (Carrera et al., 2012), spinaches (Altemimi et al., 2015), peaches, pumpkins (Altemimi et al., 2016), artichokes (Kollia et al., 2017; Rabelo et al., 2016), and coffee beans (Al-Dhabi et al., 2017).

In Perú, the most important artichoke variety is the "Talpiot" and "A-106" in coastal regions, and the "green globe" and "imperial star" in the region of Junín-Concepcion. The last two varieties are of a high quality and have an important economic impact within the Mantaro Valley of Huancayo. However, the industries that are currently processing this plant produce about 7171 metric tons (MT) per hectare by year, of which 4302 MT are discarded as waste, with a corresponding negative environmental impact and economic loss for producers (Flores and Villanueva, 2019). The green globe artichoke is a newly improved artichoke with a good harvest of 3−4 heads, and the imperial star is a sweeter hybrid and can produce 6−8 heads per season.

Thus, the objective of this study was to optimize the ultrasound-assisted extraction (UAE) of total polyphenol compounds from artichoke bracts waste using response surface methodology.

EXPERIMENTAL PROCEDURE

Reagents and samples

Analytical standards of gallic acid, sodium carbonate, Folin-Ciocalteu, 2.2-diphenyl-1-picrylhyrazyl (DPPH), methanol, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic, 2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) refers to ABTS, and only potassium persulfate was purchased from Sigma-Aldrich (Lima, Peru). The external bracts of the artichokes used in this study were the *Cynara scolymus* L., provided by a local producer from Concepcion between May and July of 2019. Conception Province is located

at 3283 m a.s.l. in the Junín Region, Peru. The bract (fresh artichokes) samples were lyophilized (model Biobase, BK-FD10PT, Shandong, China), grounded (Model M20-S000, KIKA WERKE, Argentina), sieved (500 μm mesh), and stored in dry and closed recipients.

Extracts

Ultrasound-assisted extraction (UAE) was used to obtain the extracts using a Kisker 053275 (Kisker Ultrasonic, Steinfurt, Germany) ultrasound tank (with a tank capacity of 1.4 L, frequency 42 kHz, and 230V). Ethanol was employed as an extraction solvent. For each extract, approximately 1 g of powder sample was placed into a 100 mL Erlenmeyer flask and the corresponding solvent mixture (5 ml water : 5 mL ethanol) was added. The Erlenmeyer flask containing the solution was submitted to ultrasound following the conditions established by the design response surface. The obtained mixtures were centrifuged at 400 rpm for 15 minutes, filtered through Whatman paper no. 1 (100−200 mbar) stored in amber glass bottles under vacuum conditions, and refrigerated at −5°C until use. All analyses were performed on consecutive days to avoid any changes in the samples due to prolonged storage time.

Determination of total phenolic compounds (TPC)

The determination of total phenols was carried out using the Folin-Ciocalteu method as described by Singleton and Rossi (1965). The assay was performed using a Genesys 10 UV-Vis spectrophotometer (Thermo Fisher Scientific, UK). For this, 500 μL of the Folin-Ciocalteu reagent and 40 μL of each extract were added to a 10 mL volumetric flask and covered with aluminum foil. Then, after 10 minutes of rest, 500 μL of 10% Na_2CO_3 was added and made up to 10 mL with ultrapure water. The absorbance was measured at a wavelength of 755 nm against a blank prepared with 40 μL of ultrapure water. In order to quantify this, a calibration curve with six points (0.1; 0.2; 0.4; 0.5; 0.6; and 0.9 mg/mL) of gallic acid in water was prepared. The TPC was expressed in mg of gallic acid equivalents GAE/g dry sample.

Antioxidant activity

The measurement of solution density DPPH assay for determination of antioxidant activity was done

according to Brand-Williams et al. (1995) and conducted at 515 nm. The DPPH solution was prepared with a mixture of 80% ethanol and 20% ultrapure water. This solution was standardized. The absorbance of the samples was recorded at 540 nm in a spectrophotometer. Quantification was carried out through a calibration curve with six points (0.1, 0.2, 0.3, 0.5, 0.6, 0.7, and 0.8 mM) of Trolox in ethanol. The results were expressed in mg of Trolox equivalents (TE)/g dry sample. In all cases, the extracts were measured in triplicate.

Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay was used to measure the total antioxidant capacity. For this, the ABTS method [(2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) was used based on the method outlined by Rice-Evans et al. (1996). The absorbance was measured at 734 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific, UK). The calibration curve was prepared from a 5 mM solution of Trolox ranging from 0.1 to 0.8 mM. The results were reported in mg of Trolox equivalents (TE)/g dry sample.

Equivalent design

The experiments were carried out using the Box-Wilson design, also called the central composite design (CCD). In CCD, the central composite face-centered (CCFC) includes a total of 17 experiments (consisting of 8-factor points, 6 start points and 3 replicates at the central points to allow estimation of pure error). The three variables studied were the radiation amplitude (X_1), ethanol concentration (X_2), and extraction time (X_3). The number of experiments was computed using equation 1 (Azargohar and Dalai, 2005):

$$N = 2^n + 2n + n_c \qquad (1)$$

where:

N − the total number of experiments,

n – the number of factors,

c – the number of central points.

Significant variables were analyzed to select the optimal levels of the independent variables and the effect of the independent variable interactions using the composite face-centered (CCFC) from the central composite design (CCD). Table 1 shows the factor level and the central point of the design of the response surface methodology (low, central/medium, high)

Table 1. Level of variables selected in the factorial design and response surface methodology

Independent variable	symbol	Coded variable level		
		low	central	high
		−1	0	+1
Radiation amplitude, %	X_1	80	90	100
Ethanol concentration, %	X_2	40	50	60
Extraction time, min	X_3	5	10	15

on independent coded variables. The experimental data were adjusted through the second-order polynomial (equation 2):

$$Y = \beta_0 + \Sigma_{j=1}^k \beta_j X_j + \Sigma_{j=1}^k \beta_{jj} X_j^2 + \Sigma_i \Sigma_{<j=2}^k \beta_{ij} X_i X_j + e_i \quad (2)$$

where:

Y – the predicted response,

β_0 – the intercept,

$\beta_j, \beta_{jj}, \beta_{ij}$ – the regression coefficients for mean, linear, interactions, and quadratic terms, respectively,

X_i, X_j – independent variables or factors ranging from −1 to 1, and is the error (Maran et al., 2013).

Design-Expert version 12.0 software was employed (Trial version, Stat-Ease, Minneapolis, MN, USA).

Optimization

Derringer's desirability function method was employed to optimize responses in the UAE process, which uses functions of convenience. The general approach consists of first converting each outcome in an individual desirability function that varies along the scale ($0 \leq di \leq 1$). If the outcome is within the acceptable region, $di = 1$. In contrast, $di = 0$. And which are equal to the geometric mean of the individual desirable functions. The design variables are then chosen to maximize desirability through equation 3 (Derringer and Suich, 1980).

$$D = (d1 \cdot d2 \cdot \dots dm)^{1/m} \quad (3)$$

Model validation

The extraction of phenolic compounds from artichoke waste was optimized, considering amplitude radiation, ethanol concentration, and extraction time as independent variables. Optimal conditions were found by applying the predictive equation of the surface response methodology. The antioxidant activity DPPH was performed after polyphenolic compound extraction under optimal conditions (equation 3). Finally, the experimental and predicted values were compared to determine the validity of the model.

Statistical analysis

The coefficient values of adjusted R^2 and predicted R^2 and the coefficient of variation (CV, %) were evaluated to validate the model. The validity of each test series was checked, and the model validity was assessed by ANOVA to assess the relevance of the independent variables' influence and interactions ($P < 0.05$). The model validity was determined through the coefficient of determination (R^2), the significance (p), and the lack of adjustment test.

RESULTS AND DISCUSSIONS

Determination of process parameters in the extraction and adjustment of the model

Radiation amplitude (X_1, %), ethanol concentration (X_2, %), and extraction time (X_3, minutes) were assessed and their conditions varied in an ultrasound-assisted extraction to observe the effects on total phenolic compound (TPC), antioxidant activity (DPPH), and the Trolox equivalent antioxidant capacity (TEAC) (Table 2). The results in Table 2 show that the TPC ranged from 10.86 to 24.82 mg GAE/g, DPPH between 15.49 and 8.65 mM Trolox, and the TEAC from 12.56 to 32.52 mM Trolox.

Multiple regression analysis was applied to evaluate the adequate development of the model, and variance analysis (ANOVA) was used to assess the relevance of the independent variable's influence and interactions ($p < 0.05$). The probabilities of the significance of the effects analyzed in ANOVA and response variables are shown in Table 3. These values were recalculated excluding non-significant variables to obtain adjusted coefficients for the model. In Table 3, it can be observed that Fisher's F test values for TPC, DPPH, and TEAC were 100.93, 59.85, and 83.3 respectively, which are considered extremely high. In addition, a low $p < 0.0001$ value was produced, indicating that the model was highly significant.

Table 2. Composite face-centered (CCF) design from the three factors, three levels, and response observations under different experimental conditions

Experiment	Extraction conditions			Responses		
	X_1 %	X_2 % y/v	X_3 time	TPC mg GAE/g	DPPH mM TE	TEAC mM TE
1	90	50	10	24.28	38.42	31.37
2	90	50	10	23.92	37.68	32.28
3	80	40	15	15.91	24.20	21.87
4	90	60	10	20.72	31.22	29.82
5	80	60	15	16.59	26.24	22.48
6	90	40	10	16.13	24.52	20.57
7	90	50	10	24.82	38.65	32.52
8	100	60	5	22.99	34.39	28.39
9	100	50	10	23.73	36.93	31.82
10	90	50	15	22.04	34.76	29.64
11	100	60	15	19.89	31.05	26.28
12	80	60	5	15.85	24.88	21.56
13	100	40	15	14.60	23.50	20.82
14	90	50	5	22.04	34.00	28.41
15	80	50	10	21.77	32.37	28.89
16	80	40	5	10.87	15.50	12.57
17	100	40	5	12.58	19.80	16.25

X_1 – radiation amplitude, X_2 – ethanol concentration, X_3 – extraction time, TPC – total phenolic compounds, DPPH – Trolox equivalent antioxidant activity, TEAC.

The determination coefficient R^2 for TPC was 0.992, 0.987 for DPPH, and 0.991 for TEAC, which suggested a satisfactory correlation between the experimental and predicted values.

The Adj R-squared for TPC was 0.983, which meant that most of the TPC variations (>99%) could be predicted by the model, while only 3% could not. Likewise, the DPPH showed an Adj R-squared of 0.971, and the TEAC presented an Adj R-squared of 0.979. The lack of fit of the model was not significant. The F value of 1.92 and the p-value of 0.3775 for TPC suggested that the lack of adjustment was negligible concerning pure error due to noise. Similar results were found in DPPH (F value = 7.38 and p-value = 0.1237), and TEAC (F value = 2.48 and p-value = 0.311).

Lower values of the coefficient of variation (CV) for TCP (3.02%), DPPH (4.00%), and TEAC (3.39%) were found, clearly indicating that deviations between experimental and predicted values showed a high degree of accuracy and precision in the experiments. Our results found a signal-to-noise ratio of 29.77 for TCP, 23.98 for DPPH, and 29.09 for TEAC, confirming that this model can be used to navigate the design space.

The optimization of the extraction process was determined by applying the second-order polynomial equations (equation 4, 5, and 6), which were used to generate graphical representations of regression equation simulated by the Design-Expert Software that were represented through 3D response surface (Fig. 1, 2, and 3).

Table 3. ANOVA of the response surface quadratic polynomial model for TPC, DDPH, and TEAC

Source	Sum of squares	DF	Mean square	F-value	p-value	Prob > F
1	2	3	4	5	6	7
TPC						
Model	310	9	34.5	101	<0.0001	***
X_1-amplitude	16.4	1	16.4	47.9	0.0002	**
X_2-ethanol	67.3	1	67.3	197	<0.0001	***
X_3-time	2.2	1	2.2	6.5	0.038	*
X_1X_2	12.6	1	12.6	36.9	0.001	*
X_1X_3	5.9	1	5.9	17.4	0.004	*
X_2X_3	11.1	1	11.1	32.5	0.001	**
$X_1{}^2$	2.4	1	2.4	7.1	0.032	*
$X_2{}^2$	74.5	1	74.5	218	<0.001	***
$X_3{}^2$	7.4	1	7.4	21.6	0.0024	**
Residual	2.4	7	0.3			
Lack of fit	1.9	5	0.4	1.9	0.38	NS
Pure error	0.4	2	0.2			
Cor total	312	16				
Std. dev.	0.6					
Mean	19.3					
CV, %	3.0					
R-squared	1.0					
Adj R-squared	0.9					
Pred R-squared	0.9					
Adeq precision	29.8					
DPPH						
Model	769	9	85.5	59.9	<0.0001	***
X_1-amplitude	50.5	1	50.5	35.4	0.0006	**
X_2-ethanol	162	1	162	114	<0.0001	***
X_3-time	12.5	1	12.5	8.8	0.0211	**
X_1X_2	14.4	1	14.4	10.1	0.0156	*
X_1X_3	11.8	1	11.8	8.2	0.0240	*
X_2X_3	25.8	1	25.8	18.1	0.0038	**
$X_1{}^2$	8.7	1	8.7	6.1	0.0426	*
$X_2{}^2$	198	1	198	138	<0.0001	***
$X_3{}^2$	11.6	1	11.6	8.1	0.025	**

Table 3 – cont.

1	2	3	4	5	6	7
Residual	10.0	7	1.4			
Lack of fit	9.5	5	1.9	7.38	0.124	NS
Pure error	0.5	2	0.3			
Cor total	779	16				
Std. dev.	1.2					
Mean	29.9					
CV, %	4.0					
R-squared	0.9					
Adj R-squared	0.9					
Pred R-squared	0.9					
Adeq precision	23.9					
TEAC						
Model	566	9	62.8	83.3	<0.0001	***
X_1-amplitude	26.3	1	26.3	34.8	0.0006	**
X_2-ethanol	133	1	133	176	<0.0001	***
X_3-time	19.3	1	19.3	25.6	0.0015	**
X_1X_2	8.0	1	8.0	10.6	0.0140	*
X_1X_3	7.5	1	7.5	9.9	0.0160	*
X_2X_3	28.4	1	28.4	37.6	0.0005	**
$X_1{}^2$	5.1	1	5.1	6.8	0.0354	*
$X_2{}^2$	115	1	115	152	<0.0001	***
$X_3{}^2$	19.7	1	19.7	26.1	0.0014	**
Residual	5.3	7	0.8			
Lack of fit	4.6	5	0.9	2.5	0.3114	NS
Pure error	0.7	2	0.4			
Cor total	571	16				
Std. dev.	0.9					
Mean	25.6					
CV, %	3.4					
R-squared	0.99					
Adj R-squared	0.98					
Pred R-squared	0.93					
Adeq precision	29.1					

X_1 − radiation amplitude, X_2 − ethanol concentration, X_3 − extraction time, TPC − total phenolic content, DPPH − antioxidant activity, TEAC − antioxidant capacity in Trolox equivalent (TE). Significance level: ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, $0.05 \leq {}^{\text{fcs}}p \leq 0.1$ (factor considered significant (Rezende et al., 2017)), nsp > 0.1 (not significant).
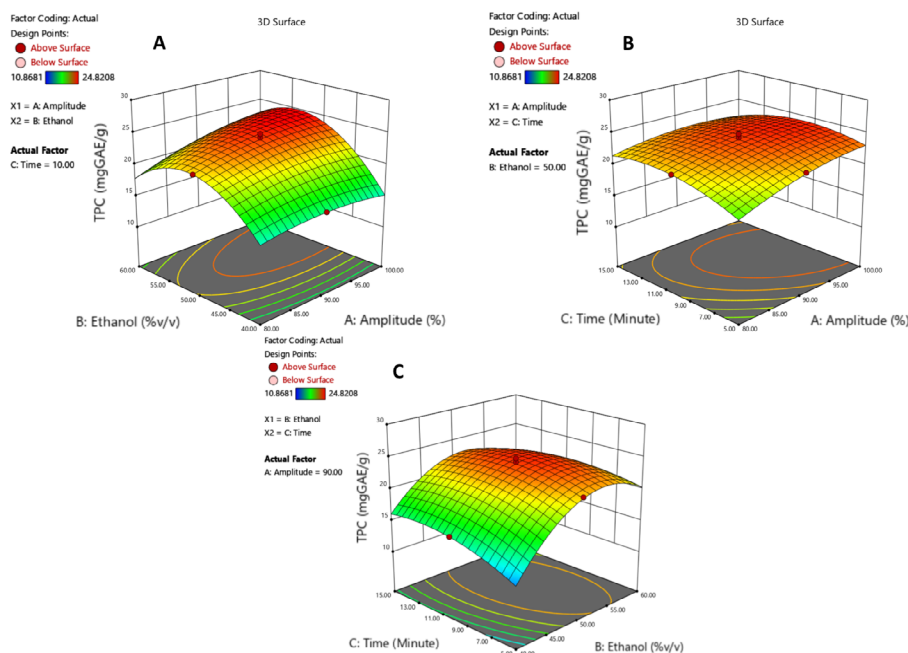
Fig. 1. Response surface graph (3D) from total phenolic compounds (TPC) based on the interaction significance between factors: A – radiation amplitude and ethanol concentration, B – ethanol concentration and extraction time, C – radiation amplitude and extraction time
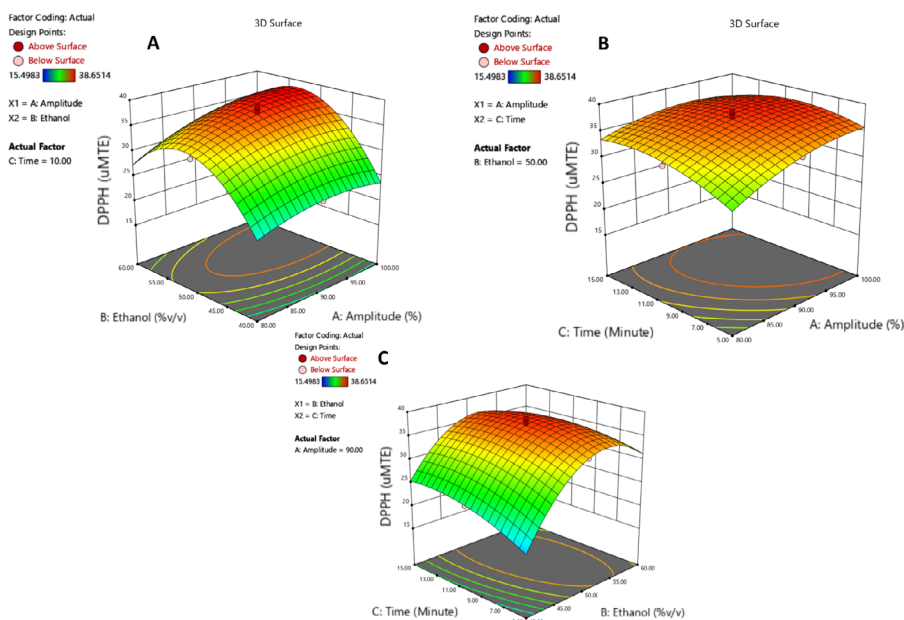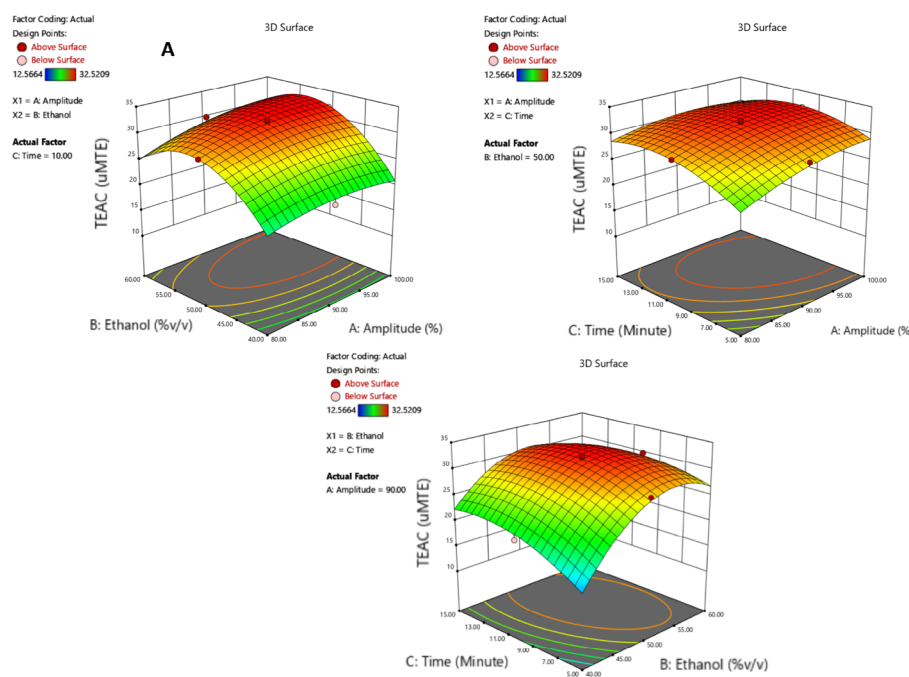

Fig. 2. Response surface graph (3D) from the radical removal activity DPPH based on the interaction significance between factors: A – radiation amplitude and ethanol concentration, B – ethanol concentration and extraction time, C – radiation amplitude and extraction time

Fig. 3. Response surface graph (3D) from the Trolox equivalent antioxidant capacity (TEAC) based on the interaction significance between factors: A – radiation amplitude and concentration of ethanol, B – concentration of ethanol and time, C – radiation amplitude and extraction time

Effects of the variables on ultrasound-assisted extraction (UAE)

Total phenolic compounds (TPC). The total phenolic compounds of the artichoke bracts ranged from 10.86 mg to 24.82 mg GAE/g (Table 2). Zuorro et al. (2014; 2016) reported similar results from total polyphenol content in bracts (24.14 mg GAE/g) using a 50:50 ethanol-water mixture as an efficient extraction method. Likewise, Mena-García et al. (2020) applying a microwave-assisted extraction and ethanol: water (50:50, v/v) as an extraction solution found that the total polyphenol content was between 9–18 mg GAE/g in bracts from artichoke residues. In contrast, Kollia et al. (2017) reported lower values of total phenolic content (TPC) in different parts (heads (0.49 ±0.03 mg GAE/g), bracts (0.41 ±0.01 mg GAE/g) and stems (0.33 ±0.01 mg GAE/g)) of artichokes using ultrasound-assisted extraction.

The interaction between the variables for the total phenolic content X_1X_2 was statistically significant with positive effects, while the terms X_1X_3, and X_2X_3 had a statistically significant but negative effect on the performance of the TPC at 99%. The second-degree terms X_{12}, X_{22}, and X_{23} increased the negative effect on TPC with a significance level of 95%.

The effect of the radiation amplitude of ultrasound power on the extraction process showed a direct effect on the polyphenol content. An increase in radiation amplitude from ultrasound facilitates cell wall rupture, increases solubility, elevates the extraction performance efficiency (Kollia et al., 2017; Maran, 2017), increases the release of specific compounds (Soria and Villamiel, 2010), and may also increase eco-chemical effects (Chemat et al., 2017). Likewise, changes in the concentration of ethanol may modify the physical properties of the solvent such as density, dynamic viscosity, and dielectric constant, as well as modifying the solubilities that influence phenolic extraction (Chaves et al., 2020).

The extraction time is also associated with input power improving the ultrasound extraction (Chemat et al., 2017). However, a longer extraction time with

ultrasound treatment could induce polyphenol degradation through equation 4 (Tiwari et al., 2009).

$$\text{Phenols} = 23.97 + 1.28X_1 + 2.59X_2 + 0.47X_3 + 12.6X_1X_2 - 0.86X_1X_3 - 1.18X_2X_3 - 0.95X_1^2 - 5.27X_2^2 - 1.66X_3^2 \quad (4)$$

Antioxidant activity (DPPH) and Trolox equivalent antioxidant capacity (TEAC). The radical removal activity DPPH and Trolox equivalent antioxidant capacity (TEAC) in bracts from artichoke waste ranged from 15.49 mM Trolox to 38.65 mM Trolox and 12.56 mM Trolox to 32.52 mM Trolox, respectively. Mena--García et al. (2020) reported similar results of DPPH (26.59 ±0.62 mg TE/g) using a mixture of ethanol/ water (50:50 v/v) and a microwave-assisted extraction from the artichoke. However, lower values of DPPH (bracts (0.11 ±0.04 mg TE/g)) and TEAC (bracts (0.66 ±0.10 mg TE/g)) from artichoke waste using ultrasound-assisted extraction were reported by Kollia et al. (2017).

Statistical analysis shows that all variables were influential in the antioxidant activity DPPH and Trolox antioxidant capacity equivalent (TEAC) and had a significant effect on the significance level of 95%. The interaction terms for the phenolic content X_1X_2 were statistically significant and with positive effects, while the terms X_1X_3 and X_2X_3 were significant but with a negative effect on the performance of TPC at 99%. The second-degree terms X_{12}, X_{22}, and X_{32} harmed the total phenolic content with a significance level of 95%. This is important in the antioxidant capacity of the polyphenols due to the ability to donate hydrogens to form stable radicals (Scalbert et al., 2005). Similar effects of the variables on TEAC and TPC was observed. Zhang et al. (2011) reported a positive correlation ($R = 0.98$, $p < 0.001$) between the total polyphenol content with the oxygen of the radical absorption capacity (ORAC) assay. Sun and Ho (2005) similarly reported that the antioxidant activity determined using the 2.2-diphenyl-1-picrilhyl (DPPH) method in extracts and total polyphenol content had a positive correlation ($R = 0.97$, $p < 0.002$). These results indicate that the total polyphenol content contributes to the antioxidant activity DPPH and the Trolox antioxidant capacity equivalent (TEAC). The radiation amplitude applied to the ultrasound-assisted

extraction process showed a better performance during the extraction of polyphenols. Zardo and Espíndola (2017) used ultrasound-assisted extraction and concluded that extraction performance depends on the nature of plant material.

The main effects related to improving releases of plant material content can be attributed to cavitation, which causes cell wall disruption, particle size reduction, intensification of plant material transfer mass, and consequently, an increase in the extraction (Wang and Weller, 2006). The results obtained showed that the optimal concentration of ethanol was equal to 50% for antioxidant capacity (Table 2).

This concentration was found within the values reported by Maran (2017) for polyphenol performance and antioxidant capacity from artichoke residues extracted using ultrasound.

The use of hydro-alcoholic ethanol mixtures was raised in this study because several authors agree that it is one of the most suitable systems for extraction thanks to the different polarities of the polyphenols and the acceptability of this system for human consumption (Alonso-Riaño et al., 2020). The performance of antioxidant activity DPPH and Trolox antioxidant capacity equivalent (TEAC) increased when the time remained between 5 and 10 minutes but decreased slowly when the time continued to extend (Fig. 2B and 3B). Most polyphenols in broken cells were released in the initial period of 10 minutes due to ultrasound improving the release. Besides this, the number of micro-bubbles created by ultrasound increased as the time extended (>10 minutes), which could undermine surfaces and damage the substance in solution. The structure of the polyphenols and the antioxidant capacity were destroyed, thus their stability decreased due to the continued collapse of microbubbles (Maran et al., 2018).

Determination and validation of optimal conditions. Derringer's function methodology through model equations to predict optimal response values was tested under the following conditions: radiation amplitude to 95%, ethanol concentration at 53%, and an extraction time of 9.7 minutes. The experiments were carried out under optimal conditions to compare the experimental results with the predicted values through the empirical equations developed (equations 4, 5, and 6).

$$DPPH = 37.23 + 2.25X_1 + 4.03X_2 + 1.12X_3 + 1.34X_1X_2 - 1.21X_1X_3 - 1.80X_2X_3 - 1.81X_1^2 - 8.59X_2^2 - 2.08X_3^2 \quad (5)$$

$$ABTS = 31.87 + 1.62X_1 + 3.65X_2 + 1.39X_3 + 1.00X_1X_2 - 0.97X_1X_3 - 1.88X_2X_3 - 1.38X_1^2 - 6.54X_2^2 - 2.71X_3 \quad (6)$$

The experiments were conducted in triplicate and average values are shown in Table 4. These values obtained from TPC, DPPH, and TEAC were compared with the predicted values. The experimental values were found to be similar to the predicted values and indicated the suitability of the developed quadratic models (Fig. 4). The optimal results found were 25.13 ±0.030 mg GAE/g for TPC, 39.79 ±0.014 mM TE for DPPH, and 33.98 ±0.03 mM TE for Trolox equivalent antioxidant capacity (TEAC) (Table 4).
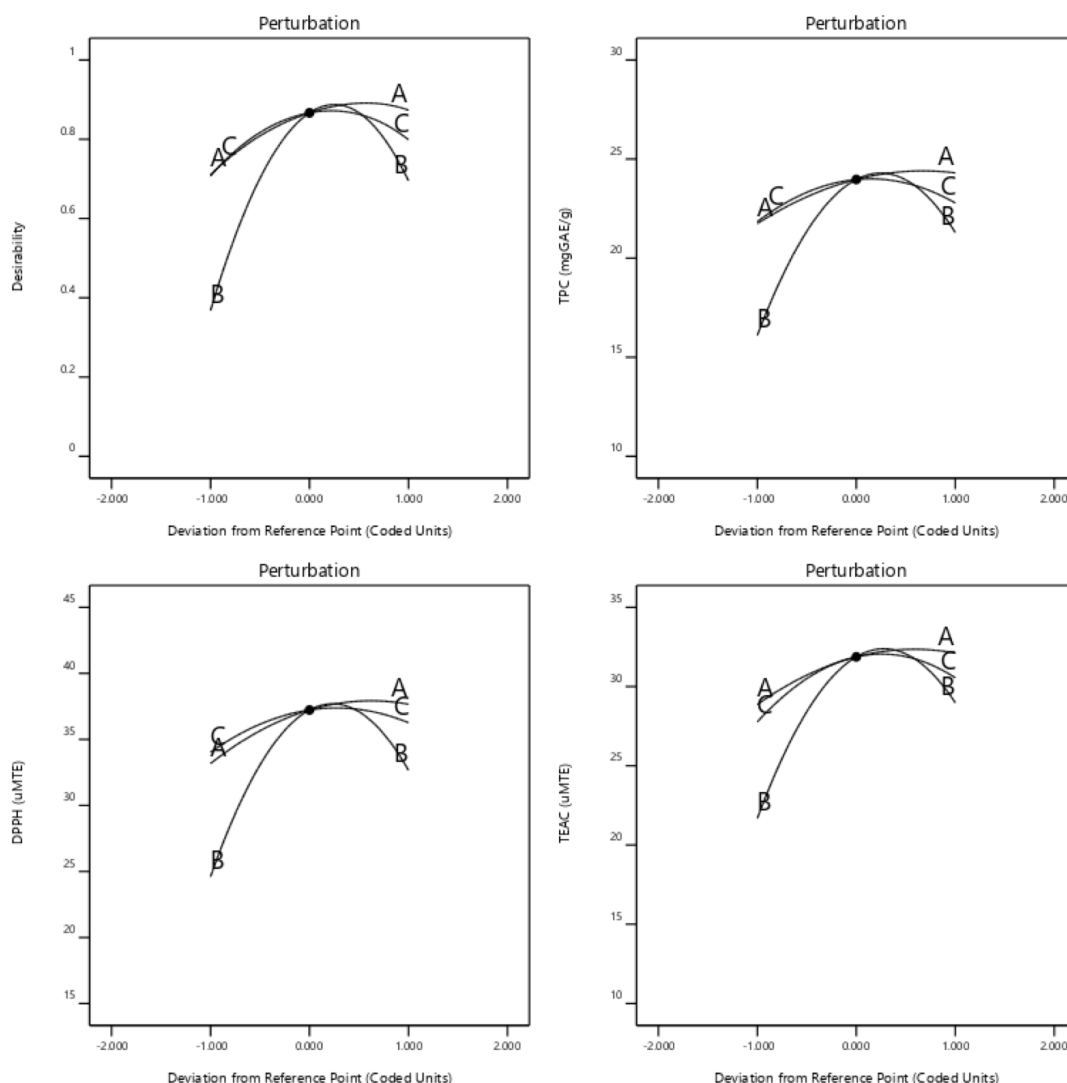

Fig. 4. Desirability function for the abundance of total phenolic compounds (TPC), radical removal activity DPPH, and the Trolox equivalent antioxidant capacity (TEAC) of the extracts from artichoke residues in function of radiation amplitude, %, ethanol concentration, % v/v, and time extraction, min

Table 4. Predicted and experimental values based on optimal conditions

Optimum levels	Predicted			Experimental		
	TPC mg GAE/g bracts	DPPH mM TE	TEAC mM TE	TPC mg GAE/g bracts	DPPH mM TE	TEAC mM TE
$X_1 = 97\%$	25.0	38.6	33.0	25.13 ±0.03	39.79 ±0.01	33.98 ±0.03
$X_2 = 53\%$						
$X_3 = 9.7$ min						

TPC, DPPH, and TEAC are expressed as a mean of three determinations ±standard deviation (SD).

CONCLUSION

The CCFC design was successfully used to optimize and study the individual and interactive effects of ultrasound-assisted extraction (UAE) process variables, showing values closely related to the expected values, which demonstrates the suitability and validation of the models. Through the analysis of surface response methodology, the factor levels which provide the maximum theoretical content were a radiation amplitude of 97%, an ethanol concentration of 53%, and an extraction time of 9.7 min. The polynomial model presented the best fit with an R^2 value of 0.992, 0.987, and 0.991 for TPC, DPPH, and TEAC, respectively. The results were validated experimentally reaching values similar to those that were predicted. Therefore, these results provide valuable information on the extraction process referred to as antioxidant phenols in artichoke bract extract.

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