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Ultrasound-assisted extraction of polyphenols from mandarin peel (C. reticulata Blanco)

Extracción de polifenoles asistida por ultrasonido de la cáscara de mandarina (C. reticulata Blanco)

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Abstract

Mandarin peel (Citrus reticulata Blanco) contains significant amounts of bioactive compounds, which can add value to this fruit's residues. Different bioactive compound extraction methods have been implemented as an alternative to conventional ones, achieving high yields in the recovery of these compounds with a shorter extraction time and less solvent use. In this research, polyphenols were extracted from mandarin peels using an ultrasound-assisted extraction (UAE) technique. Folin-Ciocalteu and DPPH colorimetric methods were used to determine polyphenol content and antioxidant capacity. The effects of amplitude percentage, temperature, extraction time on total polyphenol content (TPC), and antioxidant capicity (AC) were evaluated using a central composite design (CCD) – Response surface methodology (RSM). In addition, the desirability composite was used to calculate the optimal process conditions. The results showed maximum TPC and AC values of 211.82 mg GAE/100g dw and 19.16 mM TE/100g dw. The optimized UAE conditions were 28% amplitude, 33.3 °C, and 30.8 min, with a total polyphenol content of 205.60 mg GAE/100g dw and an antioxidant capacity of 18.98 mM TE/100g dw. Finally, the TPC and AC of the peel extracts obtained in the UEA optimization were compared with the results of a conventional extraction (Soxhlet method). The results obtained from the extraction yield of the optimized UAE (87.74 %) showed a higher efficiency than the Soxhlet method, and presented the UAE as a good alternative for extracting polyphenols from mandarin peel.

Keywords: Total Phenolic Content, Antioxidant Capacity, Ultrasound.

Resumen

La cáscara de mandarina (Citrus reticulata Blanco) contiene cantidades significativas de compuestos bioactivos que pueden añadir valor a los residuos de esta fruta. Se han implementado diferentes métodos de extracción de compuestos bioactivos como alternativa a los convencionales, logrando altos rendimientos en la recuperación de estos compuestos con un menor tiempo de extracción y menor uso de solventes. En esta investigación, se extrajeron polifenoles de la cáscara de mandarina mediante una técnica de extracción asistida por ultrasonidos (EAU). Se utilizaron los métodos de Folin-Ciocalteu y colorimétrico DPPH para determinar el contenido de polifenoles y la capacidad antioxidante. Los efectos del porcentaje de amplitud, la temperatura y el tiempo de extracción sobre el contenido total de polifenoles (TPC) y la capacidad antioxidante (AC) se evaluaron mediante un diseño central compuesto (DCC) - metodología de superficie de respuesta (RSM). Además, se utilizó el compuesto de deseabilidad para calcular las condiciones óptimas del proceso. Los resultados mostraron valores máximos de TPC y AC de 211.82 mg GAE/100g dw y 19.16 mM TE/100g dw. Las condiciones optimizadas de EAU fueron 28% de amplitud, 33.3 °C y 30.8 min, con un contenido total de polifenoles de 205.60 mg GAE/100g dw y una capacidad antioxidante de 18.98 mM TE/100g dw. Finalmente, el TPC y AC de los extractos de cáscara obtenidos en la optimización UEA se compararon con los resultados de una extracción convencional (método Soxhlet). Los resultados obtenidos del rendimiento de extracción de la EAU optimizada (87.74 %) mostraron una mayor eficiencia que el método Soxhlet, y presentaron a la EAU como una buena alternativa para la extracción de polifenoles de la cáscara de mandarina.

Palabras clave: Contenido Total Fenólico, Capacidad Antioxidante, Ultrasonido.

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Introduction

Colombia is one of the largest producers of fruits and vegetables in the Andean region thanks to the great diversity of topographic and climatic zones, allowing year-round harvests. Fruit production in Colombia grew 20.6% in the last five years, reaching 6.7 million tons in 2019 (1). In the case of citrus, total production was 1.4 million tons in 2020 which 27% corresponds to mandarin (2), being the second most produced citrus fruit. Mandarins are part of the Citrus genus and have a flattened, spherical shape, an intense aroma, a pleasant taste, and a thin peel. They are primarily composed of water and have fewer calories than other fruits in the same genus due to their lower sugar content. Mandarins have a similar nutritional value to oranges, as they are rich in citric acid, potassium, and magnesium. (3)

In recent years, citrus consumption has generated high demand. About 60% of citrus fruits are consumed in the fresh market, and approximately 40% are processed. This causes significant organic waste, including peels, seeds, stems, and leaves. Such waste is often inappropriately discarded and is part of the solid waste that is deposited in dumps or landfills, and due to its decomposition, contributes to environmental pollution by generating CH_4 methane gas. (4, 5)

Therefore, as an alternative to reduce the environmental impact and add value to these wastes, citrus peel is mainly used to produce essential oils, compost, animal feed, and gasification. However, it has been demonstrated that citrus residues, such as peel, contain components that could be used in the pharmaceutical, cosmetic, and food industries. An example of these compounds is phenolic compounds with biological activity, which could prevent the development of chronic diseases such as diabetes, cardiovascular disease, and neurodegenerative disease. (6, 4)

Phenolic compounds are secondary metabolites containing more than one phenol group and are particularly antioxidants (7). They are generally extracted by solid-liquid extraction mechanisms, where an organic solvent is used as the extraction medium (7). These extraction methods can be classified into two categories: conventional and non-conventional. Conventional methods are techniques based on the extraction capacity of different solvents with the application of heat or agitation. Soxhlet extraction is one of the most well-known conventional methods for concentrating target compounds. It involves cycling a solvent through the sample, with the siphon dropping it into a vessel, and repeating the process until saturation is reached (8). However, these types of methods, although simple and inexpensive, have long extraction times, low yields, and use high contaminating solvents. (9)

Non-conventional extraction methods have emerged as a response to the limitations of conventional methods, including the need for more environmentally friendly techniques, high yields of bioactive substances, and shorter extraction times. Ultrasound-assisted extraction (UAE) is a non-conventional method for extracting polyphenols from plant matrices. By using ultrasonic waves and the cavitation mechanism, UAE has been shown to produce better results than conventional methods in biocompounds extraction. (9–11).

The total polyphenol content that can be extracted by UAE depends on a proper selection of process conditions such as temperature, percentage amplitude, and time. Therefore, the objectives of this study were 1) To evaluate UAE to obtain polyphenols from mandarin

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peels, 2) Evaluate the impact of UAE operating conditions, including temperature, percentage of amplitude, and time, on the total polyphenol content and antioxidant capacity of the extract, 3) To compare to the optimal operating conditions obtained from the conventional Soxhlet extraction method and 4) validation of models and optimization of extraction parameters to ensure the accuracy and reliability of the results.

Materials and methods

Plant material

Mandarin peels (C. reticulata Blanco) were obtained as a residue from juice extraction in a local restaurant located in Santiago de Cali, Colombia. The state of commercial maturity of mandarin was selected according to Pássaro and Londoño (12). The authors state that mandarin peel coloration should be one-third of that type of its variety and add, as a minimum requirement, a color index (CI) for the domestic market of -9 < IC < 6, in this case, peels with an IC of -3 were used.

Methods

Mandarin peel conditioning

The mandarin peels were washed with tap water, and the remaining mesocarp was removed. Subsequently, the peels were cut into pieces of approximately 1x1 cm and dried in a hot air-drying oven (Dies- 7M 53, Colombia) at 60°C for 2 h. The dried peels were ground (CGOLDENWALL High-speed Multi-function Comminutor blade mill, USA) and sieved (Sieve set, W.S Tyler, USA) to obtain a homogeneous powder with a particle size of 300 µm according to Nipornram et al. (13).

Ultrasound-assisted extraction (UAE)

The methodology proposed by Anticona et al.(14) was used with some modifications. 5 g of sample were mixed with 50 mL of 80% (v/v) ethanol (Fisher Scientific, USA) in an amber flask. The sample with ethanol was taken to an ultrasonic bath (Elmasonic TI-H-15, Germany) at amplitudes of 15, 20, and 25% at 30, 40, and 50°C for 20, 30, and 40 min according to the experimental design. After the extraction, the samples were cooled at room temperature (25°C). The extracts were centrifuged (LABWE BT16 Centrifuge, China) at 9610 rpm for 10 min. Finally, the supernatant was filtered through Millipore paper (0.75 mm) to obtain the final extract.[14] The extraction yield (EY) was determined using Equation 1:

$$EY\ (\%) = \frac{EW}{SW} * 100 \quad \text{Eq. (1)}$$

where EW is the extract weight (g), and SW is the sample weight (g).
The samples obtained were stored at 4°C until further analysis.

Soxhlet extraction

The Soxhlet extraction was performed using the methodology proposed by Ramírez (16) with some modifications. 5 g of the sample was loaded onto filter paper and placed inside the Soxhlet extraction chamber. Then, 75 mL of the solvent (80% (v/v) ethanol) was added to a flask and joined to the chamber. The extraction was carried out for 8 h at 60°C. Finally, the extract obtained was purified using a rotary evaporator (R-3001CE, China) at 60°C to a volume of approximately 30 mL, and the percentage yield of the extraction was determined by Equation 1. The extract was stored at 4°C for subsequent analysis. This extraction served as the control sample.

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Total polyphenol analysis

The total polyphenol content (TPC) of mandarin peel extracts was evaluated using the Folin-Ciocalteu assay, adapted from Tarazona (17) with some modifications. A stock solution of gallic acid ($C_7H_6O_5$) (ALDRICH, USA) was prepared at 5 mg/mL for polyphenol quantification.

Calibration curve preparation

Gallic acid (GA) solutions were prepared at 0.05, 0.1, 0.15, 0.25, and 0.5 mg/mL concentrations. Subsequently, 0.04 mL of each solution was added to 1.56 mL of distilled water, followed by 0.1 mL of Folin-Ciocalteu's reagent. The resulting solutions were then shaken, and 0.3 mL of 10% sodium carbonate (Na2CO3) was added. After incubating the solutions for 1 hour at room temperature in the dark, the absorbance was measured at 765 nm using a spectrophotometer (Labscient UV 1800, USA) (14, 17).

Sample preparation

From the extracts obtained, 0.04 mL of extract was utilized to perform the calibration curve standards using the same procedure. Triplicate samples were prepared, and the results were expressed as mg of gallic acid equivalent per 100g of dry weight (dw). (17) The total polyphenol content was quantified by measuring the absorbance at 765 nm using a spectrophotometer (Labscient UV 1800, USA).

Antioxidant capacity analysis

Total antioxidant activity was determined using the DPPH - Radical scavenging activity method described by Meneses et al. (18) with some modifications. 3.9 mg of DPPH reagent was added in a 100 mL volumetric flask with methanol. The mixture was shaken and incubated in the dark for 1 hour to allow the reaction to occur. Subsequently, the absorbance was measured at 517 nm in a spectrophotometer (Labscient UV 1800, USA).

Calibration curve preparation

Initially, a stock solution of Trolox at 1 mM in ethanol was prepared. Subsequently, Trolox solutions at concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.7 % (v/v) were prepared in 25 mL flasks and were volumetrically diluted with distilled water. In each test tube, 0.3 mL of each Trolox solution was mixed with 3.7 mL of DPPH reagent (0.1 mM) and incubated for 30 min in the dark at room temperature. Finally, the absorbance was read at 517 nm in a spectrophotometer (Labscient UV 1800, USA).

Sample preparation

0.3 mL of the extracts obtained were used for analysis, following the same procedure as the calibration curve standards. The antioxidant capacity was expressed as mM TE/100g dry weight (dw) and analyzed by measuring the absorbance at 517 nm in a spectrophotometer (Labscient UV 1800, USA). Each sample was prepared in triplicate.

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Experimental design

The study used a 3-factor composite central design (CCD) with high and low levels, including central and axial points, to apply a response surface methodology (RSM). The axial points were included to obtain a better estimation of curvature. The independent variables investigated were percent amplitude (X_1, %), temperature (X_2, °C), and extraction time (X_3, min) in the UAE method. Table 1 presents the design summary, including factor levels, center, and axial points. The experimental design consisted of twenty combinations, with 6 replicates at the center point for error estimation. The response variables analyzed were TPC (total polyphenol content, mg GAE/100g dw) and AC (antioxidant capacity, mM TE/100g dw).

Table 1. Experimental design for UAE

Factor		**Levels**				
		-1.68	-1	0	1	1.68
Percentage of amplitude (%)	X_1	11.6	15	20	25	28.4
Temperature (°C)	X_2	23.2	30	40	50	56.8
Time (min)	X_3	13.2	20	30	40	46.8

Linear, interaction and quadratic models were used for the regression analyses to estimate the response variables. The models are shown below:

Linear model,

$$y = \sum_{i=1}^{k} \beta_i X_i \quad \text{Eq. (2)}$$

interaction model,

$$y = \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k-1} \sum_{i=i+1}^{k} \beta_{ii} X_i X_i \quad \text{Eq. (3)}$$

full quadratic model.

$$y = \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k-1} \sum_{i=i+1}^{k} \beta_{ii} X_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i{}^2 \quad \text{Eq. (4)}$$

Where y is the predicted response (TPC or AC), X_i are the factors, and β_i the coefficients.

Analysis of Variance (ANOVA) was performed to assess the fitness of the model and the significance of the model components using a p value ≤0.05. The “backward elimination regression” procedure was implemented by eliminating insignificant interactions from the best model fitting. The present study uses the desirability compound approach to determine the optimal values. By employing this method, the study was able to determine the optimal combination of predictor variables that maximized TPC and AC. The statistical analyses were conducted using MINITAB version 19.

In addition, the regression coefficients, R^2 and R^2 adjusted, and the root mean square error (RMSE) were calculated to evaluate the quality of each model and its accuracy in predicting the response. The following equations were used for these calculations:

$$R^2 = 1 - \frac{\sum_{i=1}^{N} (y-\hat{y})^2}{\sum_{i=1}^{N} (y-\bar{y})^2} \quad \text{Eq. (5)}$$

$$R^2 adjusted = 1 - \frac{(1-R^2)(N-1)}{N-P-1} \quad \text{Eq. (6)}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N}} \quad \text{Eq. (7)}$$

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where, y is the observed value of the response, $\hat{y}$ the average of the data, P is the number of predictors, N is the number of experiments.
These coefficients must fulfill the condition that 0 < adjusted $R^2 \leq R^2$ < 1.0, and for the model to fit the data, the R^2 adjusted value must be greater than 0.7. The adjusted R2 is especially important for models with many terms since its value varies according to the significance of each term on the response. (19)

Finally, the optimal condition was experimentally validated. To determine the significance of any differences between the optimal condition and the conventional Soxhlet extraction method, a one-way completely randomized design was utilized. The experimental results were then analysed using an analysis of variance (ANOVA). In cases where significant differences were found, a Tukey post-ANOVA test was conducted to determine the differences between the two extraction methods.

Results and discussion

Effect of ultrasound conditions on total polyphenol content

The factors of amplitude percentage (X_1, %), temperature (X_2, °C), and time (X_3, min) were analyzed for their linear, interaction, and quadratic models. The independent and dependent variables were fitted to the different models proposed in Table 1. The predicted models are shown in Equations S1, S2 and S3 of the supplementary material.

Table 2 presents the predicted responses for each model based on the operating conditions. The R^2 values for the linear, interaction, and full quadratic models are 0.67, 0.69, and 0.90, and the R^2 adjusted values are 0.61, 0.54, and 0.81, respectively. The full quadratic model is considered the best fitting model based on these results, as it exhibits the highest regression coefficients. The calculated RMSE also indicates that the full quadratic model has the closest proximity between the predicted and experimental responses, with a lower value obtained (6.61) compared to the other models.

Table 2. Experimental and predicted response according to the proposed models for TPC.

Exp. Number (N)	Components			TPC (mg GAE/100g dw)			
					Models		
	X_1	X_2	X_3	Observed	Linear	Interactions	Quadratic
1	20	40	30	137.53	153.45	143.72	148.74
2	20	40	30	155.03	153.45	143.72	148.74
3	20	40	30	147.57	153.45	143.72	148.74
4	20	40	30	147.39	153.45	143.72	148.74
5	20	40	30	149.75	153.45	143.72	148.74
6	20	40	30	154.02	153.45	143.72	148.74
7	15	30	20	116.68	130.53	119.34	127.23
8	15	30	40	149.95	148.59	132.32	147.65
9	25	30	20	166.85	167.01	162.74	170.72
10	25	30	40	186.38	185.07	178.12	193.62
11	20	40	13.2	129.39	138.28	134.78	117.04
12	20	40	46.8	148.31	168.62	152.66	147.48
13	15	50	20	128.75	121.83	122.36	130.32
14	15	50	40	138.73	139.89	128.26	143.66
15	25	50	20	146.13	158.31	149.16	157.23

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16	25	50	40	174.81	176.37	157.46	173.05
17	20	23	30	175.12	160.76	151.13	167.48
18	20	56	30	158.50	146.14	136.31	152.80
19	28.4	40	30	211.82	184.09	174.21	203.32
20	11.6	40	30	146.98	122.81	113.23	142.10
R^2					0.67	0.69	0.90
R^2 adjusted					0.61	0.54	0.81
RMSE					12.13	15.60	6.61

To improve the fit of the quadratic model, the "backward elimination regression" procedure was applied to the non-significant interactions, and only variables with a p-value < 0.05 were selected. Table S1 (see Supplementary material) shows the summary of the ANOVA before and after the backward elimination regression and the corresponding values of the regression coefficients (R^2 and R^2-adjusted) and RMSE. The results showed that the X_1X_3 and X_2X_3 terms were eliminated because they had no significant effect (p-value > 0.05) and not affect the final model fit. The similarity in the values obtained for R^2 and R^2-adjusted, and RMSE confirm the effectiveness of this procedure in keeping the fit of the quadratic model and decreasing the model complexity.
The statistical analysis also showed that the p-value < 0.05 of the final models demonstrates a strong correlation between the response and independent variables and provides a prediction accuracy of 90%. Although the temperature terms (X_2) of the linear and quadratic model are not significant, which implies that statistically, it does not affect the TPC, in the literature, it is reported that temperature is a parameter that influences the efficiency in polyphenol extraction.(10, 20, 21)

According to the above, the equation that best predicts TPC considering the operating conditions evaluated is presented in Equation 8.

$$y = 147.9 - 6.62X_1 - 2.01X_2 + 4.41X_3 + 0.34X_1^2 + 0.04X_2^2 - 0.06X_3^2 - 0.08X_1X_2$$

Eq. (8)

Contour plots were generated using the model to visualize the effects of the operating conditions on the TPC (see Figure 1). The contour plots showed that the highest response was observed at high percentage amplitude (> 24%), temperatures below 35°C, and extraction times greater than 20 minutes. Specifically, for the effect between temperature and percent amplitude together (Figure 1.a), the total polyphenol content increases at high power and low temperature. This phenomenon can be attributed to the increased collapse of cavitation bubbles that leads to greater rupture of plant tissues, thereby increasing the penetration and diffusivity of the solvent,(8) and by using low extraction temperatures, the degradation of phenolic compounds is avoided.

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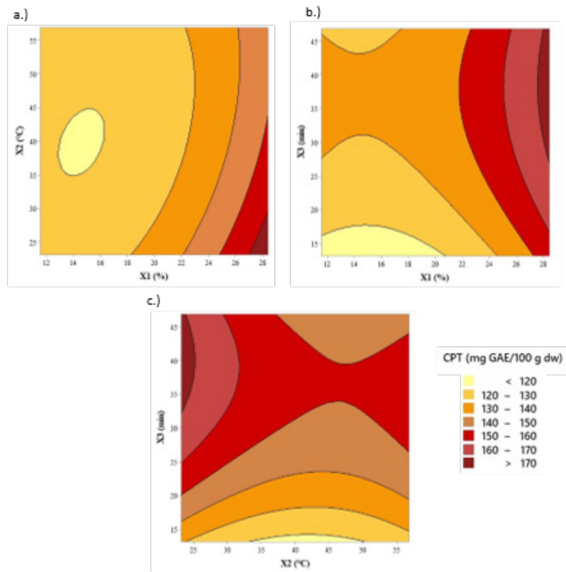

Figure 1. Contour diagrams of the effect of X2 vs X1 (a), X3 vs X1 (b) y X3 vs X2 (c)

Similarly, Figure 1.c shows that a higher number of total polyphenols is obtained at low temperatures (20 and 30 °C) and prolonged times (30 and 45 min). This effect is also related to the increased fragmentation and pore formation in the plant tissue matrix, which helps solvent penetration and subsequent extraction of the target compound, due to the longer exposure time of the waves.(10,11) This can also be observed in Figure 1.b, where the more prolonged the extraction time, the higher the number of total polyphenols. Increases in polyphenol content according to extraction time were also observed by Khan et al.(22), who evaluated optimal extraction parameters in the UAE on orange (C. sinensis L.) peel with times of 5, 10, 20, and 30 min, to investigate the extraction time needed to obtain the maximum yield. The authors concluded that the best extraction results were achieved at 30 min for all cases. On the other hand, they also mention that at temperatures above 50 °C and prolonged times, instability of phenolic compounds occurs, thus decreasing the total polyphenol content of the extracts. According to Ma et al. (23) the higher the temperature, the lower the extraction efficiency due to a higher concentration of solvent molecules in the cavitation bubble, which dampens the collapse and decreases the forces acting on the peel surface. The temperature is considered a sensitive ultrasonic variable for the extraction of phenolic compounds from citrus peel.

Considering the data recorded in Table 2, the experimentally obtained TPC values are in the range of 116.68 - 211.82 mg GAE/100g dw. The highest number of polyphenols was observed at temperature conditions of 40°C, an amplitude of 28.4%, and a time of 30 min. The results obtained are similar to those reported by Al-Juhaimi(24) for Kinnow mandarin peels with 169.54 mg GAE/100g dw, but significantly lower than those reported by Singanusong et al.(25) and Anticona et al.(14), which report TPCs of 2456.71 and 1540

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mg GAE/100g dw, respectively. These differences are associated with the direct influence of process conditions, and those related to the physical properties of the sample and fruit, such as particle size, state of maturity, and geographical origin, are added. Zhang et al. (26) states that Citrus reticulata Blanco tissues have phenolic concentrations that vary according to geographical origin and relative air humidity, which affect flavonoid accumulation by altering the actions of activators and enzymes.

Khan et al.(22) consider particle size as one of the critical factors that can affect extraction efficiency. In their study conducted from orange peel, they mention that lower extraction yields were observed with particle sizes smaller than $2\ cm^2$, because the particles tend to remain on the surface of the solvent during extraction, thus limiting their exposure to ultrasonic waves during the process. In contrast, Romero Bayona(27) establishes the relationship between the maturity stage of mandarin peel (Citrus reticulata) and the content of phenolic compounds and total flavonoids by ultrasound-assisted extraction. The authors concluded that the highest content of total polyphenols (50 mg GAE/g dw) was observed in the samples at the maturity stage. Therefore, fruit ripening favors the formation of a greater amount of metabolites of interest. Zhang et al.(26) states that Citrus reticulata Blanco tissues have phenolic concentrations that varied according to geographical origin and relative air humidity, which affect flavonoid accumulation by altering the actions of activators and enzymes.

Effect of ultrasound conditions on antioxidant capacity

Table 3 shows the predicted response for AC according to the operating conditions for each model (see equations S4, S5 y S6 in supplementary material). The model that best fits the data is the full quadratic model since it has the highest magnitude in R^2 and R^2-adjusted with 0.78 and 0.58, respectively. The RMSE validates that the full quadratic model is the one that best predicts the response because its value is lower (0.16) compared to the other models.

The “backward elimination regression” procedure was applied for the quadratic model. Table S2 (see Supplementary Material) presents the summary of the ANOVA before and after backward elimination regression and the corresponding values of the regression coefficients (R^2 and R^2-adjusted) and RMSE. The results showed that the X_1X_3 and X_2X_3 terms in the original model have a p-value > 0.05 and were therefore removed. The linear terms, are kept because it is a hierarchical model, and all lower-order terms should be included. (19)

Table 3. Experimental and predicted response according to the proposed models for AC.

Number exp. (N)	Factors			AC (mM TE/100 g dw)			
					Models		
	X_1	X_2	X_3	Observed	Linear	Interactions	Quadratic
1	20	40	30	19.14	18.53	18.53	17.10
2	20	40	30	18.87	18.53	18.53	17.10
3	20	40	30	19.16	18.53	18.53	17.10
4	20	40	30	18.80	18.53	18.53	17.10
5	20	40	30	18.84	18.53	18.53	17.10

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6	20	40	30	18.68	18.53	18.53	17.10
7	15	30	20	18.25	18.56	18.45	16.22
8	15	30	40	17.91	18.52	18.34	16.11
9	25	30	20	18.46	18.58	18.71	13.77
10	25	30	40	18.54	18.55	18.75	13.82
11	20	40	13.2	18.39	18.55	18.56	16.54
12	20	40	46.8	18.55	18.50	18.50	16.48
13	15	50	20	18.33	18.50	18.70	20.04
14	15	50	40	18.26	18.47	18.58	19.93
15	25	50	20	18.20	18.53	18.34	16.98
16	25	50	40	18.02	18.49	18.38	17.02
17	20	23,2	30	18.38	18.57	18.58	14.08
18	20	56,8	30	18.34	18.48	18.48	19.97
19	28,4	40	30	18.61	18.55	18.55	14.49
20	11.6	40	30	18.78	18.50	18.51	18.98
R^2					0.01	0.10	0.78
R^2 adjusted					0.00	0.00	0.58
RMSE					0.33	0.31	0.16

The final model proposed for AC ($\widehat{y_2}$) is shown in Equation 9.

$$\widehat{y_2} = 8.74 + 0.33X_1 + 0.26X_2 + 0.12X_3 - 0.005X_1^2 - 0.002X_2^2 - 0.002X_3^2 - 0.003X_1X_2 \quad \text{Eq. (9)}$$

The effects of the operating conditions on the antioxidant capacity are shown in the contour plots made from Equation 9. In general, it is shown that in the central region of all the plots the value of AC is higher (see Figure 2).

Figure 2. Contour diagrams of the effect X2 vs X1 (a), X3 vs X1 (b) y X3 vs X2 (c).

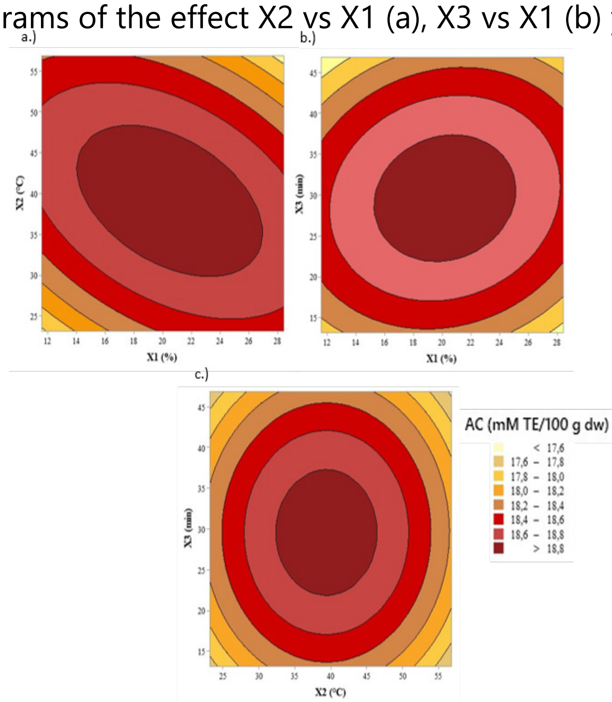

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Figure 2 shows that the regions of values greater than 18.2 mM TE/100g dw involve the temperatures (30, 40, and 50 °C), amplitude percentages (15, 20, and 25 %), and time (20, 30, and 40 min), indicating that with these operating conditions, it is possible to obtain an antioxidant capacity greater than 18.2 mM ET/100g dw, as evidenced by the experimental data recorded in the AC of the extracts evaluated is significantly higher than those reported by other authors in the literature. Zhang et al. (28) analyzed freeze-dried powdered peels of 14 Chinese wild mandarins (Citrus reticulata Blanco), after the extraction process with 80% methanol and obtained results from 29.04 to 50.46 µmol TE/g dw. In another study, Mei et al.(29) determined the optimal conditions for obtaining bioactive compounds from mandarin (Citrus reticulata) by ultrasound method and reported antioxidant capacity values of 87.88 mg TE/ g dw. These differences can be attributed to the different variables involved in the extraction method, the region of fruit production, the cultivation method, and the maturity stage at which the fruit was found. (30)Vitamin C present in mandarin is one of the main contributors to its antioxidant capacity and is higher in the peel than in the edible parts, although it decreases as it matures. (31) According to the above, the AC results obtained are due to the state of maturity of the mandarin peels at the time of extraction, which allowed the extract to have a high antioxidant capacity.

Ferreira et al.(32) state that the peels of C. reticulata Blanco present a potential source of antioxidant activity due to the high content of flavonoids, such as hesperidin, naringin, tangeritin, and rutin, as they represent 86% of the total phenols in the peels. In addition, flavonoids are chemical compounds that are less degraded by AUE than other phenolic compounds, allowing better stability in a solvent (33). Other antioxidants are derived from fruit peel oil, such as terpenes (γ-terpinene and terpinolene), which are responsible for 75.8% and 64.2% of the high antioxidant activity measured by DPPH, because they have highly activated methylene groups in their structure. (34)

Comparison of extraction methods

The determination of the optimal UAE condition was performed using the desirability composite to maximize both response variables (TPC and AC). The optimal operating conditions are amplitude percentage of 28.23%, the temperature of 33.37 °C, and time of 30.85 min. According to the equipment used, approximations were made to the optimal extraction conditions values as follows: amplitude percentage of 28%, temperature of 33.3 °C, and time of 30.8 min. The models and the optimal conditions predict a total polyphenol content of 211.83 mg GAE/100g dw and an antioxidant capacity of 18.67 mM TE/100g dw. These results were experimentally verified, and TPC of 205.60 mg GAE/100g dw and AC of 18.98 mM TE/100g dw were obtained.

Comparison of extraction methods

The result of the comparison of TPC and AC by UAE and Soxhlet is shown in Table 4.

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Table 4. Comparison of extraction methods.

Extraction method	TPC (mg GAE/100g dw)	AC (mM TE/100g dw)
Soxhlet	146.37 ± 27.27[a]	18.55 ± 0.01[a]
UAE	205.60 ± 4.41[b]	18.98 ± 0.25[b]

The UAE resulted in a significantly higher TPC compared to the traditional Soxhlet method, with a 1.4- fold increase. Moreover, the optimal conditions for UAE also showed a significantly higher AC of the extract, with a 1.02-fold increase compared to the Soxhlet method.

Guntero et al. (35) have reported that UAE is a faster and more efficient extraction method due to the increased surface contact between the solid and liquid phases and the disruption of cell walls caused by acoustic cavitation. Similarly, Gao et al. (36) found higher total polyphenol extraction yields from Empetrum nigrum parts using optimal conditions for UAE compared to conventional extraction methods, with a 1.29 to 1.44 fold increase in polyphenol content.

The extraction yield for UAE was estimated at 87.74%, nearly twice as much as the yield obtained with the Soxhlet method (46.67%). Additionally, the TPC obtained by UAE in 32.3 minutes was significantly higher than Soxhlet's 8 hours process. This shows that UAE can produce higher extraction yields in shorter periods and less solvent, thereby reducing energy consumption and solvent volumes compared to the Soxhlet method.

Conclusion

This study highlights an innovative approach in the UAE for polyphenol recovery, using a response surface methodology and optimizing the UAE operating conditions. The methodology used allowed us to find optimal temperature values, amplitude percentage, and time to maximize the extraction of total polyphenols from mandarin peel. However, the limitation of small-scale experimentation suggests the need for future scaling process studies to fully realize the technique's potential. Additionally, there is a need to identify the bioactive components extracted, utilizing specialized methodologies such as high-performance liquid chromatography (HPLC), to fully understand and exploit the potential of UAE.

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