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Valorisation of goldenberry calyx: ultrasound-assisted extraction of phenolic compounds

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Abstract

The extraction of value-added compounds from agro-industrial wastes is important to reduce their environmental impact. In this research, the time for ultrasound-assisted extraction of phenolic compounds obtained from goldenberry (*Physalis peruviana* L.) calyx was first chosen. Subsequently, response surface methodology was used to optimise the effect of wave amplitude, liquid:solid ratio, and particle size on total phenol content and *in vitro* antioxidant capacity. Then, antioxidant capacity (DPPH, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and ferric reducing antioxidant power) was measured in the extract obtained under optimal conditions, and rutin flavanol was identified and quantified by HPLC-DAD. Analysis of polynomial models indicated adequate fit ($p \leq 0.05$) for phenolic content (R^2=0.98) and antioxidant capacity (R^2=0.91). Optimal amplitude (54%), liquid:solid ratio (33 mL/g), and particle size (213 µm) maximised the response of phenolic content (53 mg GAE/g) and antioxidant capacity (167 µmol TE/g). The *in vitro* antioxidant capacity of the extract was demonstrated, and a high rutin concentration (19 mg/g) was found in the extract obtained under optimum conditions. High extraction yields of phenolic compounds were found using the shortest time (*i.e.*, 10 min), intermediate particle diameters, a low amount of solvent, and low energy consumption.

Keywords: *Physalis peruviana* L.; circular economy; valorisation strategy; fruit by-product; rutin; antioxidant activity.

Practical Application: This study shows the suitability of ultrasound-assisted extraction as a technology with high extraction yields of phenolic compounds in short times, intermediate particle diameters, low amount of solvent, low energy consumption, and solvents generally recognised as safe. This is a clear indication that the technique could favour technology transfer or industrial scale-up.

1 INTRODUCTION

Agricultural and food residues are the main organic waste produced worldwide, which could generate between 6 and 10% of greenhouse gas emissions (Teigiserova *et al.*, 2020). In this context, the circular economy emerges as a strategy that seeks to mitigate the environmental impact generated by agro-industrial waste, giving it a new application in productive chains (León-Roque *et al.*, 2023; Mesquita *et al.*, 2023), for example, the extraction of bioactive compounds (Tirado *et al.*, 2019).

Colombia is the world's largest producer and exporter of goldenberry (*Physalis peruviana* L.), which is an edible climacteric berry with waxy yellow-orange skin and juicy pulp that is protected by a paper-like shell called calyx (Ballesteros-Vivas *et al.*, 2019). Goldenberry is recognised for its high nutritional value and content of bioactive compounds such as polyphenols (Embaby *et al.*, 2022), and during its commercialisation, the calyx must be removed from the fruit due to export policies. Just in 2021, Colombia produced almost 17,000 tonnes and exported roughly 8,000 tonnes of goldenberries (Ministerio de Agricultura, 2022), which meant that the country produced about 1.2 tonnes of calyx, a by-product that currently has no commercial use despite its high nutritional potential due to its high content of bioactive compounds (Ballesteros-Vivas *et al.*, 2019).

Ultrasound-assisted extraction is a low-cost and environmentally sustainable technology. It is based on the application of high-frequency sound waves between 20 and 100 kHz, generating a cavitation effect (Demirci *et al.*, 2022; Xu *et al.*, 2022). Several studies demonstrated its effectiveness as an emerging non-thermal technology during the extraction of phenolic compounds (Dadi *et al.*, 2019; Dal Prá *et al.*, 2015; Görgüç *et al.*, 2022; Guandalini *et al.*, 2019; Monteiro *et al.*, 2022; Pradal *et al.*, 2016), such as rutin, a flavanol characterised by its antioxidant capacity but whose bioavailability is low due to its low water solubility, poor chemical and metabolic stability, and limited membrane permeability (Fan *et al.*, 2022; Zitha *et al.*, 2022).

Considering the environmental and economic relevance reflected in the demand for compounds obtained from agro-industrial waste, the objective of this study was to maximise the response of phenolic content and antioxidant capacity during the ultrasound-assisted extraction from goldenberry calyx.

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2 MATERIALS AND METHODS

2.1 Materials

Goldenberries used in this study were collected from farms of the Universidad de Nariño (San Juan de Pasto, Colombia) at a commercial maturity stage of roughly six and categorised by colour according to the Colombian Technical Standard 4580 (ICONTEC, 1999). Calyxes were removed from the fruit by hand, disinfected with sanitiser, and dried (UN 110, Memmert, Germany) at 40±1°C for 24 h to 8% *w/w* humidity. The particle size was then reduced in an electric mill (IKA-Werke A11, United States) with two cycles of 7 min and subsequently sieved with No. 60, 70, 80, and 100 mesh (Standard Physis, Analytica Ltda., Colombia) using an electric sieve (PS-35, series 1329, PINZUAR LTDA, Colombia). The mean particle diameter was determined according to the methodology reported by Zhao *et al.* (2014). The resulting material was stored at 3°C in dark.

2.2 Ultrasound-assisted extraction: optimal extraction time

Ultrasound-assisted extractions were performed using a sonicator (Ref. 5059600 INVYMEN, Equipos y Laboratorio de Colombia, Colombia) coupled with an electric probe. The equipment specifications were a frequency of 20 kHz, external dimensions of 22 cm × 19 cm × 33 cm, power supply voltage of 220 V/50–60 Hz, a total power input of 500 W, and a weight of 6.7 kg. The processing parameters were a 1/2" diameter tip, ethanol (60% *w/w*) at about 30°C, a processed volume of 36 mL, a probe immersion depth of 15% of the mixture, and a maximum power intensity of 82.5 W/cm^2. The temperature was controlled using an ice bath during sonication. Subsequently, the extracts were centrifuged at 8,000 rpm for 10 min (Universal 320R, Hettich, Germany). The supernatant was used directly for the quantification of phenolic content. The variables evaluated were sonication time (1, 5, 10, 20, and 30 min), amplitude (20, 40, 50, and 60%), liquid:solid ratio (10, 20, 30, 30, and 40 mL/g), and particle size (110, 150, 210, and 270 µm), using independent experimental designs. The ranges of the variables evaluated were selected based on values found in the literature (Mrkonjić *et al.*, 2021; Zhou *et al.*, 2017) and preliminary tests performed by our research group (data not shown).

2.2.1 Total phenolic content analysis

The TPC of goldenberry calyx extracts was determined according to Aadil *et al.* (2013). For this, each sample was incubated with 60 µL of Folin-Ciocalteu reagent diluted with ultrapure water (1:10) and 180 µL of a 7% sodium carbonate solution. They were then kept in the dark at 30°C for 30 min. The absorbance was measured at 750 nm in a microplate reader (Biotek Lx800, United States). The TPC was determined from a calibration curve using gallic acid as a standard (2–128 mg/L) and expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

2.2.2. Oxygen radical absorbance capacity

ORAC was determined according to the methodology reported by Yarce *et al.* (2020). For this, fluorescein and 2,2'-azobis(2-amidinopropane) dihydrochloride solutions were prepared in phosphate-buffered saline (pH 7.4) at 0.02 and 59.8 mg/mL, respectively. Likewise, the standard antioxidant Trolox® at 7.5, 15.0, 30.5, and 61.0 µg/mL was used to prepare the calibration curve. Fluorescence intensity was monitored every 5 min for 120 min at 37°C (excitation and emission wavelengths of 485 and 528 nm, respectively) in a Synergy H1 microplate reader (Biotek, United States). The fluorescence intensity versus time data was expressed as the average antioxidant efficiency (micromoles of Trolox® equivalent per gram of extract; µM TE/g) calculated by Equation 1.

$$\frac{\mu M\ TE}{g} = \left[\frac{AUC_S - AUC_C}{AUC_T - AUC_C}\right] * f \quad (1)$$

Where:

AUC_S: the area under the fluorescence decrease curve for extract;

AUC_C: the area under the fluorescein decrease curve for the negative control;

AUC_T: the area under the fluorescein decrease curve for Trolox®;

f: the sample dilution factor.

2.3 Response surface design: TPC and ORAC optimisation

Data analysis was performed using a Box-Behnken design with 3 factors and 3 replicates at the central point for a total of 15 experiments and 2 response variables: TPC (mg GAE/g) and ORAC (µM TE/g). The factors studied were (see Table 1): amplitude (X_1), liquid:solid ratio (X_2), and particle size (X_3). From the phenolic compound extraction, the optimal time was fixed.

The results were fitted to a second-order polynomial equation, as shown in Equation 2. An analysis of variance (ANOVA) was used with a confidence level of 95% ($p \leq 0.05$). The statistical models were evaluated in terms of lack of fit and the coefficients of determination in individual linear, quadratic, and interaction terms.

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} {X_{ii}}^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon \quad (2)$$

Where:

β_0: the model constant;

β_i: the dependent term or linear effect of the input factor X_i;

Table 1. Actual and coded factors in the Box-Behnken design.

Factor	Code	Levels		
		-1	0	1
Amplitude (%)	X_1	40	50	60
Liquid:solid ratio (mL/g)	X_2	20	30	40
Particle size (µm)	X_3	150	210	270

β_{ii}: the quadratic effect of input factor X_i;

β_{ij}: the linear-linear interaction effect between input factor X_i and X_j.

Statistical analysis of the Box-Behnken design was performed using R language.

2.4 Validation of the optimised extraction

2.4.1 DPPH assay

DPPH was determined according to Castañeda-Valbuena *et al.* (2021). For this, in each microplate, 60 µL of the sample was mixed with 140 µL of 100 ppm DPPH dilution. The microplate was kept in the dark at room temperature for 30 min, vortexed, and its absorbance measured at 515 nm. For the determination of antioxidant capacity, a calibration curve was prepared using Trolox® as a standard at different concentrations (16–512 µM). The results were expressed as µM TE/g according to Equation 3.

$$\mu\text{moles TE/g} = \left(\frac{(A-b) \times f \times V}{m \times M}\right) \quad (3)$$

Where:

A: the corrected absorbance of the sample;

b: the intercept of the equation of the calibration line;

m: the slope of the equation of the calibration line;

f: the dilution factor of the sample;

V: the original volume of the sample (L);

M: the mass (g) of the dry material used during the extraction.

2.4.2 ABTS radical scavenging activity

The extract was mixed with potassium persulfate (2.45 mM) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 7 mM) in equal proportions and kept in the dark at 4°C for 16 h. Trolox® was used to prepare the calibration curve. The absorbance was measured at 734 nm, and the data were expressed in µM TE/g.

2.4.3 Ferric reducing antioxidant power FRAP

The extract was shaken with 1 mL of ferric reducing antioxidant power (FRAP) reagent previously prepared from 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl + 20 mM $FeCl_3$ aqueous solution. A calibration curve was performed using a 1024 µM $FeSO_4$ solution, and from this, serial dilutions were performed. Using a microplate assay, 60 µL of the ethanol-diluted extracts were added to the wells as appropriate and then 140 µL of FRAP reagent was added to induce the reaction. The absorbance was measured at 630 nm after 20 min of incubation, as indicated in the methodology reported by Tlili and Sarikurkcu (2020). The results were expressed in micromoles of ferrous ions per gram of extract (µM Fe^{2+}/g).

2.4.4 Identification and quantification of rutin

The method used for rutin identification was based on a previous study on the extraction of this compound from plant extracts (Gullón *et al.*, 2017). Samples were filtered through a 0.20 µm membrane, and 20 µL were injected into a chromatograph (Shimadzu®, Japan) equipped with a binary pump (ref. 1525, Waters, United States) and a diode array detector (DAD 1200 G1315, Agilent, USA). The stationary phase consisted of a C18 column (4.6×150 mm) at 30°C. The mobile phase consisted of the combination of solvents A (0.1% formic acid) and B (acetonitrile in 0.1% formic acid) according to the following gradient: 10–60% A (0–10 min), 60% A (10–15 min), 60–80% A (15–20 min), and 90% A (25–30 min). Reversed-phase separation was performed, and the system pressure was 104.7±0.7 bar (90% solvent A, 1 mL/min, 30°C). Chromatograms were recorded at 260 nm. The rutin standard was prepared by dissolving 10 mg in 10 mL of 10% methanol, and from this solution, different dilutions were prepared in a concentration range of 0.01–0.1 mg/mL.

2.5 Statistical analysis

The experiments were repeated three times. Analyses were performed in duplicate for each replicate (n=3×2). An analysis of variance with a 95% ($p \leq 0.05$) confidence interval was used, and statistical analysis was performed with the R language.

3 RESULTS AND DISCUSSION

In this section, we initially reported the total phenol content of extracts obtained by ultrasound-assisted extraction as a function of time (min), amplitude (%), solid:liquid ratio (mL/g), and particle size (µm). Based on the highest phenol extraction yield, an optimal extraction time was determined. Subsequently, to optimise TPC and ORAC, response surface methodology was employed to analyse amplitude (%), solid:liquid ratio (mL/g), and particle size (µm), with the extraction time fixed at the optimal value. Then, antioxidant capacity in terms of DPPH, ABTS, and FRAP was measured in the extract obtained under optimal conditions, and rutin flavanol was identified and quantified by HPLC-DAD. The most commonly employed techniques for measuring the *in vitro* antioxidant capacity of plant extracts involve the use of DPPH, ABTS, FRAP, and ORAC assays, which were performed in this study. While no *in vitro* antioxidant test can accurately indicate the full antioxidant capacity, these spectrophotometric methods can provide an estimate of the phenolic content/total antioxidant capacity of the extract. However, to fully evaluate the potential of the extract, additional *in vitro* biological assays and animal studies are needed after this initial screening is completed (Tlili and Sarikurkcu, 2020).

3.1 Phenolic compound extraction

3.1.1 Effect of sonication time

As can be seen in Figure 1A, time had an influence ($p \leq 0.05$) on the total phenol content in extracts obtained from goldenberries by ultrasound-assisted extraction. Figure 1A shows that

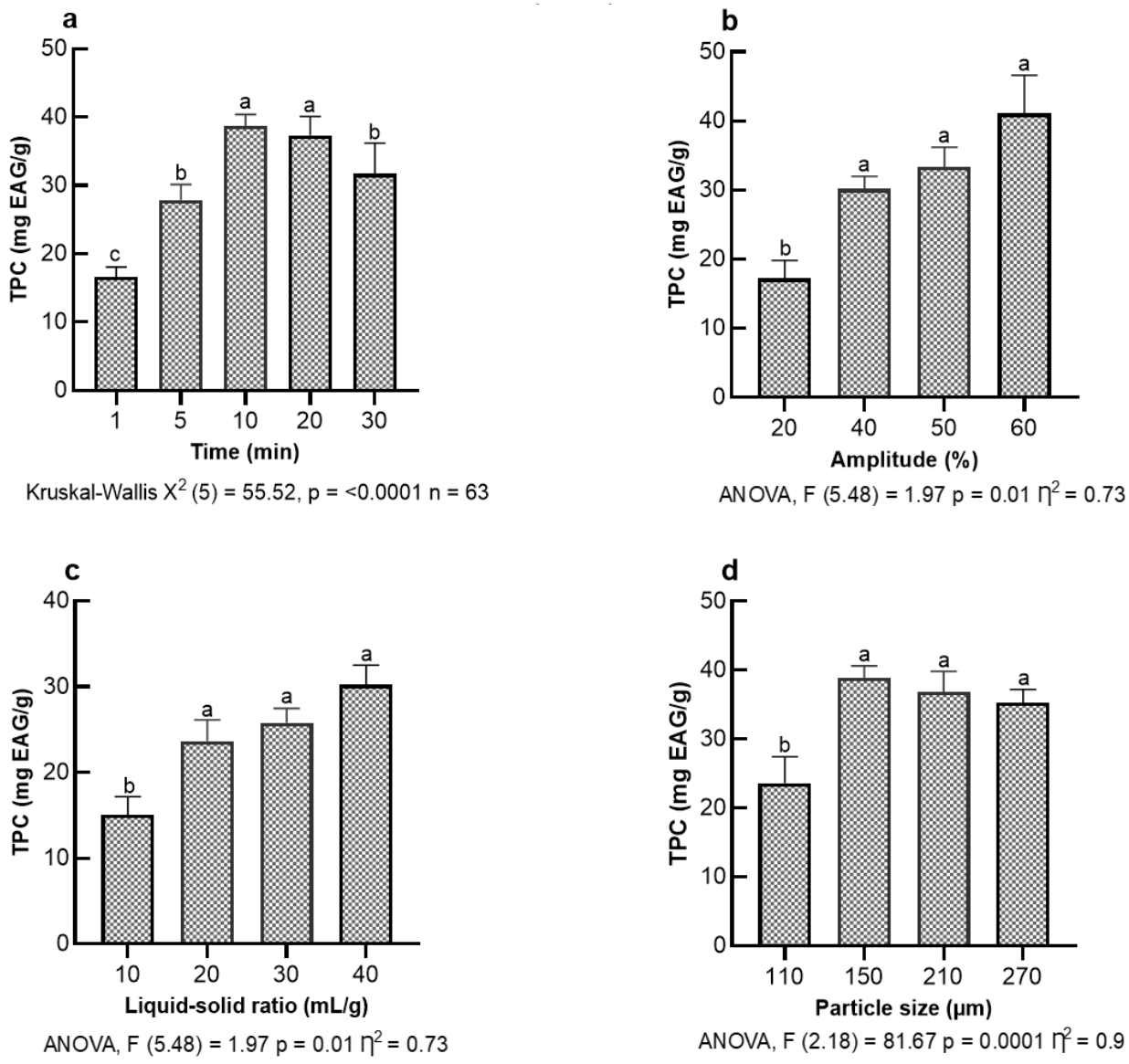

Figure 1. Phenolic content of goldenberry calyx extract obtained by ultrasound-assisted extraction: effect of (A) sonication time, (B) amplitude, (C) liquid:solid ratio, (D) particle size.

the treatments were divided into three main groups that differed statistically from one another ($p \leq 0.05$). On the one hand, we found the 1-min treatment. There was also the group consisting of 5 and 30 min treatments, and finally, the group integrated with 10 and 20 min treatments.

Figure 1A shows that, at first, the phenolic content in extracts increased directly with time (*i.e.*, from 1 to 10 min; $p \leq 0.05$), reaching the maximum yield at 10 min. Applying twice the treatment time (*i.e.*, 20 min) did not represent a significant change in phenol content in the extracts ($p > 0.05$). Even applying more time (*i.e.*, 30 min) led to a decrease in phenol content ($p \leq 0.05$), which showed no significant difference ($p > 0.05$) with 5 min. The above results can be explained by the fact that during the first minutes (*i.e.*, 1–10 min), the extraction was controlled by convective mechanisms due to the high availability of the extract since it was just starting to be extracted. After the maximum extraction yield (*i.e.*, 10 min), diffusive mechanisms began to mediate, which is why there were no significant differences between the extractions at 10 and 20 min (Milićević *et al.*, 2021). After this, the contribution of external energy became more important due to the high shear and cavitation forces generated by the ultrasound (Torres-Gallo *et al.*, 2022). After 20 min, there was an overexposure of the vegetal material to the ultrasound, which could have caused non-enzymatic oxidation processes and the formation of complexes by chemical reactions, along with a possible overheating that degraded the phenolic material (Wen *et al.*, 2018).

From the above results, it was clear that the optimum extraction time was 10 min, which was set for the upcoming optimisation analysis (see Section 3.2). All the above highlights

the short times that are necessary with ultrasound-assisted extraction compared to conventional solvent extractions (*i.e.*, 4–10 h), which is a great advantage for the commercial use of the ultrasound technology.

3.1.2 Amplitude effect

As can be seen in Figure 1B, the amplitude also influenced TPC. There were significant differences ($p \leq 0.05$) between 20 and 40%, and no significant differences ($p > 0.05$) among 40, 50, and 60%. However, the highest phenolic content was found at the maximum amplitude (*i.e.*, 60%). This was because, during the ultrasound-assisted extraction, the pressure generated in the expansion and rarefaction phases of the ultrasonic waves broke through the attractive force between the molecules and the solvent, leading to the formation of cavitation bubbles. The cavitation bubbles influenced by higher amplitude increased in size and collapsed, thus generating collisions between particles and creating a region of high pressure that allowed the fragmentation of the intracellular structure of the plant material, facilitating mass transfer and thus a higher extraction of phenol-rich extracts (Dash *et al.*, 2021).

3.1.3 Effect of liquid:solid ratio

The effect of the liquid:solid ratio on TPC is shown in Figure 1C. Significant differences ($p \leq 0.05$) were found between 10 and 20 mL/g, and no differences ($p > 0.05$) were found after this last liquid:solid ratio. The highest phenolic content was related to the highest liquid:solid ratio (*i.e.*, 40 mL/g), which allowed inferring that the increased solvent may have enhanced diffusion processes in cell tissues (Bouafia *et al.*, 2021).

The ethanol-water combination in the solution was important in the extraction of phenolic compounds since water swells the cell of the plant material while ethanol dissolves the compounds of interest present in the vacuoles (Garcia-Castello *et al.*, 2015). It could be deduced from Figure 1C that there was an enhanced molecular interaction (mainly through hydrogen bonds) between the phenols contained in the cell and the solvent as the fraction of ethanolic solution increased (Lezoul *et al.*, 2020).

3.1.4 Effect of particle size

According to Figure 1D, particle size also affected TPC extraction. This variable had an influence similar to the amplitude and the liquid:solid ratio. However, it was time that showed the greatest influence.

Significant differences ($p \leq 0.05$) were observed between 110 and 150 μm, and no differences were found among 150, 210, and 270 μm, with the highest TPC at 150 μm. Accordingly, mass transfer during ultrasound-assisted extraction from goldenberry calyx was dependent on the transport rate within the particle. Particles with a diameter larger than 150 μm could have hindered solvent penetration and solute solubilisation (Tirado *et al.*, 2019). Conversely, particles smaller than 150 μm could have resulted in the formation of preferential channels and/or blockages during extraction, hindering cavitation mechanisms (Niknam *et al.*, 2021). It is worth mentioning that particle size is a solid condition that must be optimised considering how energy-intensive particle size reduction processes are, which could jeopardise the economic viability of the process.

3.2 Optimisation

The response variables obtained from the Box-Behnken design are shown in Table 2. TPC values were between 22 and

Table 2. Box-Behnken experimental design and response variables*.

Run	Amplitude (%)	Liquid:solid ratio (mL/g)	Particle size (μm)	Coded values			TPC (mg GAE/g)	ORAC (μM TE/g)
				X_1	X_2	X_3		
1	50	20	270	0	-1	1	32±0	132±12
2	60	30	270	1	0	1	26±0	159±15
3	60	20	210	1	-1	0	42±1	170±13
4	60	40	210	1	1	0	37±1	163±11
5	50	40	150	0	1	-1	35±0	139±12
6	50	20	150	0	-1	-1	40±0	143±10
7	60	30	150	1	1	-1	33±1	143±12
8	40	30	150	-1	0	-1	27±0	131±8
9	50	40	270	0	1	1	25±1	120±9
10	50	30	210	0	0	0	48±0	168±10
11	40	20	210	-1	-1	0	35±1	127±11
12	40	30	270	-1	0	1	22±0	119±8
13	40	40	210	-1	1	0	30±0	132±13
14	50	30	210	0	0	0	48±2	168±16
15	50	30	210	0	0	0	48±1	168±12

*Values were expressed as the average ± standard deviation; TPC: total phenolic content; GAE: gallic acid equivalents per gram of extract; ORAC: oxygen radical absorbance capacity.

48 mg GAE/g, and ORAC values were between 119 and 170 µM TE/g. It was found that with a wave amplitude of 50%, a liquid:solid ratio of 30 mL/g, and a particle size of 210 µm, the best TPC response was achieved. Likewise, with a wave amplitude of 60%, a liquid:solid ratio of 20 mL/g, and a particle size of 210 µm, the highest antioxidant capacity was obtained.

According to the analysis of variance and multiple regressions, statistical models that described the behaviour of the results for TPC and antioxidant capacity were established, thus empirically establishing a relationship between the response variable and the study factors.

3.2.1 Optimisation of phenolic content

The analysis of variance and multiple regression showed that the linear and quadratic terms of all the study factors had a significant effect ($p \leq 0.05$) on the TPC. The empirical relationship describing the relationship between TPC and the study factors is shown in Equation 4, where X_1 is the amplitude (%), X_2 is the liquid:solid ratio (mL/g), and X_3 is the particle size (µm).

$$TPC\left(\frac{mg\ GAE}{g}\right) = 48.28 + 3.02X_1 - 2.85X_2 - 3.59X_3 - 9.24X_1^2 - 1.32X_2^2 - 12.05X_3^2 \quad (4)$$

The analyses showed that the lack of fit of the model was not significant ($p > 0.05$). The adjusted R^2 value was 98%, which showed a high degree of correlation between the experimental and predicted values. Therefore, it was established that the model had a good predictive ability. In addition, according to the values of the coefficients of the statistical model, the factor that most influenced phenolic content was the amplitude, followed by the particle size and the liquid:solid ratio. Also, the interaction between amplitude and particle size was significant ($p \leq 0.05$). This behaviour can be observed in the response surface plotted in Figure 2A, in which the liquid:solid ratio (30 mL/g) was set

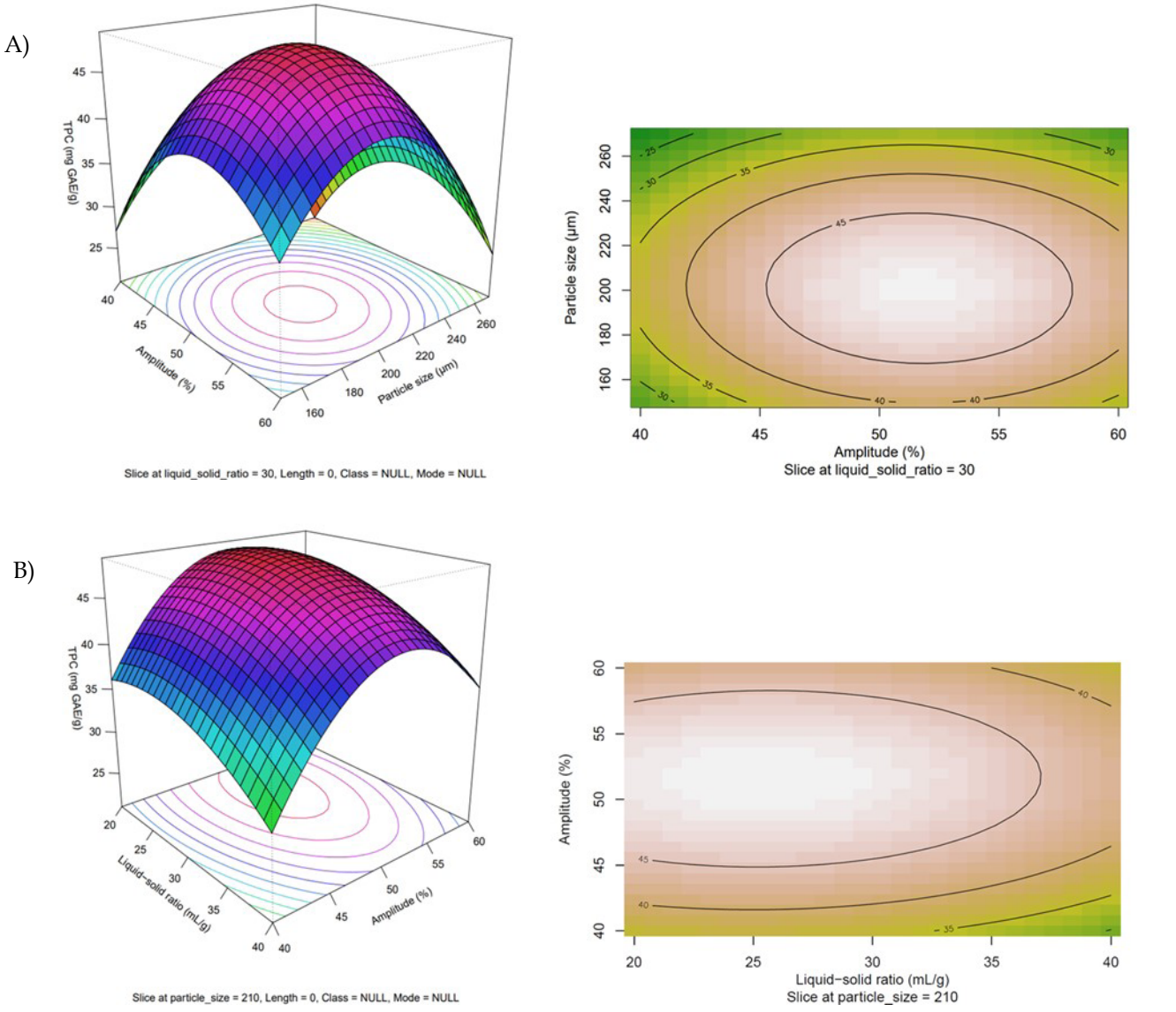

Figure 2. Response surface and contour plot for phenolic content as a function of (A) amplitude and particle size and (B) amplitude and liquid:solid ratio.

to observe the behaviour of these two factors concerning the phenolic content response.

Figure 2A indicated that the amplitude and particle size levels studied had a limiting point at which the phenolic content tended to decrease due to adverse phenomena derived from the ultrasound treatment. The degradation of phenolic compounds when a specific percentage of amplitude was exceeded has been reported in other studies, which attributed the degradation of phenolic compounds to the increase in temperature with an increase in amplitude (Bouafia *et al*., 2021; Vinatoru *et al*., 2017). Phenolic compounds tend to degrade during ultrasound treatment at temperatures close to 50°C (Setyaningsih *et al*., 2016). Although an ice bath was used during sonication in this study, as the amplitude percentage increased, temperatures close to 50°C were reached.

The interaction of amplitude and particle size (see Figure 2) further showed that an intermediate particle diameter was favourable for the process, unlike conventional solvent extraction, where the best extraction results are achieved as the particle diameter decreases (Ali *et al*., 2018). This was because the ultrasound effect promoted the size reduction of the plant matrix by breaking and fragmenting the tissues, increasing the surface area of contact with the solvent, and subsequently increasing diffusivity and mass transfer. This is of great importance and demonstrates once again the advantages of ultrasound technology since particle size reduction is an expensive operation on a commercial scale.

TPC extraction behaviour was analysed in the interaction of amplitude and liquid:solid ratio (see Figure 2B). Although the interaction of these factors was not significant (p>0.05), the TPC increased compared to that observed in Figure 2A. The combination of the factors wave amplitude and liquid:solid ratio was directly related to the cavitation phenomenon. When there is a low density of the mixture, that is, when the solvent is in a higher proportion than the solid, the diffusion process in the cellular tissues is improved, and the speed of transfer of external energy reflected in the propagation of ultrasound waves is facilitated (Dash *et al*., 2021).

Finally, Figure 2 showed that the TPC increased with the combinations of centre point levels, unlike the independent tests (see Section 3.1), where variability was observed in the levels evaluated. Such was the case for the amplitude and the liquid:solid ratio, where a trend towards an increase in TPC was found with the combinations of the maximum levels. This would represent a benefit in the operating costs generated by the energy used in the sonication processes and the amount of solvent used.

3.2.2 Optimisation of the antioxidant capacity

According to the analysis of variance and multiple regression for antioxidant capacity, the linear and quadratic terms of amplitude and particle size were significant; likewise, the quadratic term of the liquid:solid ratio factor, while the interactions of the factors were not significant. Based on these results, the statistical model described by Equation 5 was obtained, where X_1 is the amplitude (%), X_2 is the liquid-to-solid ratio (mL/g), and X_3 is the particle size (μm).

$$ORAC\left(\frac{\mu M\ TE}{g}\right) = 167.75 + 15.66X_1 - 2.24X_2 - 3.21X_3 - 7.53X_1^2 - 11.84X_2^2 - 22.11X_3^2 \quad (5)$$

The lack of fit of the model was not significant (p>0.05), which indicated that Equation 5 adequately explained the behaviour of antioxidant capacity. Likewise, the R^2 values showed a high degree of correlation between the experimental and predicted values; therefore, it was established that the model had good predictive capacity.

The antioxidant capacity assay allowed a punctual analysis to be made concerning amplitude since this factor presented a different behaviour to that observed in the phenolic content. It was observed that amplitude was highly significant (p<0.01). With an increase of up to 60%, antioxidant capacity increased (see Figure 3), while amplitude percentages higher than 50%

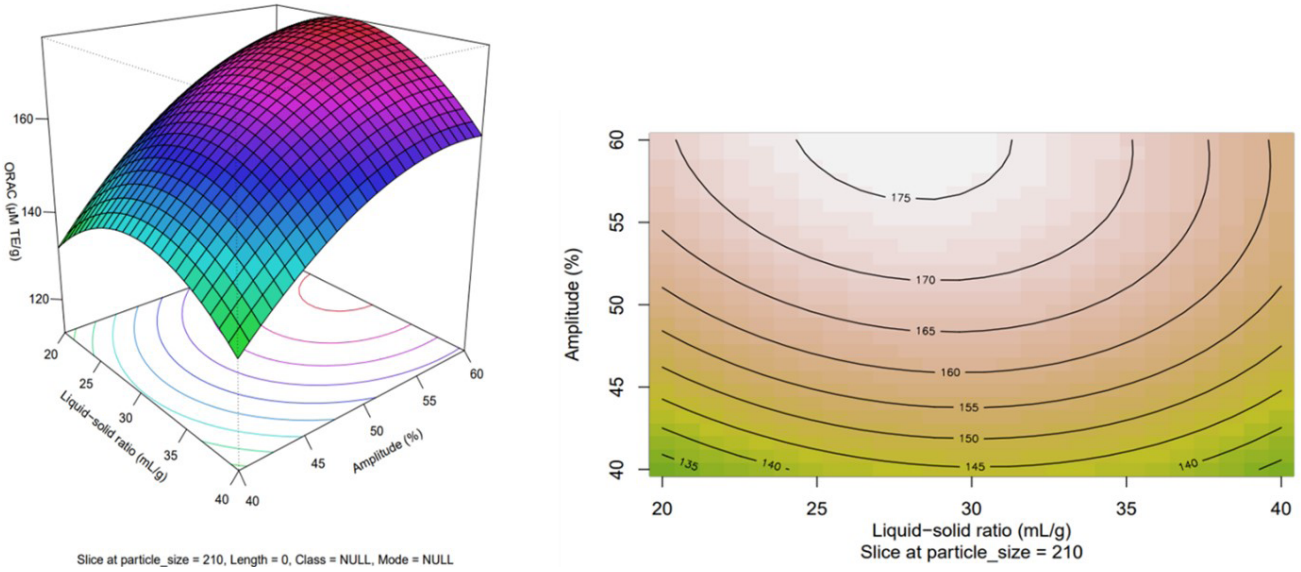

Figure 3. Response surface and contour plot for oxygen radical absorbance capacity (ORAC) as a function of amplitude and liquid:solid ratio.

decreased the TPC (see Figure 2). This could be because there were other compounds present in the plant extract, which were less susceptible to degradation at high amplitudes and which provided antioxidant capacity. These compounds could be linked to the fibrous structure that characterises the goldenberry calyx, and, therefore, a greater contribution of external energy was required to increase cavitation phenomena and allow extraction.

The ORAC analysis demonstrated the ability of the plant extract to generate reactions involving hydrogen atom transfer; likewise, the analysis suggested that the antioxidant response was mainly associated with polar compounds, among them, the compound rutin present in the calyx of goldenberry. This component has demonstrated antioxidant efficacy through the transfer of hydrogen atoms, which limits lipid peroxidation generated by peroxyl radicals in a large number of biological processes (Kalogiouri *et al*., 2021).

3.3 Experimental validation

According to the theoretical results of the optimisation of the response variables, it could be observed that some levels of the factors differed for each response variable (TPC and antioxidant capacity). Therefore, the desirability function determined by the statistical software was used, which allowed finding the optimum levels to maximise the result of the two variables: amplitude (54%), liquid:solid ratio (33 mL/g), and particle size (213 µm). The results of the experimental validation can be seen in Table 3.

3.4 Complementary analysis of antioxidant capacity

The antioxidant capacity of goldenberry calyx extract obtained under optimal conditions was evaluated by *in vitro* tests with the addition of synthetic radicals, and the results are shown in Figure 4.

The DPPH analysis was related to the ability of the extract to stabilise free radicals involving electron transfer. A value of roughly 54 µM TE/g was found for the DPPH analysis, which corresponded to the minimum concentration of goldenberry calyx extract necessary to reduce the initial concentration of the DPPH radical. Therefore, lower values corresponded to higher antioxidant activity. Likewise, the value obtained for ABTS analysis was close to 235 µM TE/g, which confirmed the aptitude of the extract to donate electrons and stabilise oxidation reactions. The ABTS radical capture capacity generated by the goldenberry extract was compared with that of Trolox®, where the highest values corresponded to a higher antioxidant capacity, while the FRAP analysis indicated the minimum value of 88 µM Fe^{2+}/g, demonstrating the reducing antioxidant power.

The ABTS assay suggested the action of hydrophilic compounds, while the DPPH and FRAP assays were related to the antioxidant action of lipophilic compounds, which evidenced the presence of different types of compounds in the extract. In some studies, it has been reported that glycosylated or cell wall-bound phenolic compounds have a higher capacity to reduce the ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) in FRAP analysis (Alara *et al*. 2021; Belwal *et al*., 2018). Therefore, it was possible to infer that the optimised conditions in the present study allowed the extraction of glycosylated phenolic compounds, which have lipophilic characteristics and promote the antioxidant capacity to reduce metal ions.

The antioxidant capacity of goldenberry calyx extract, evaluated by different *in vitro* methodologies, has been scarcely reported in the literature. In the work of Gironés-Vilaplana *et al*. (2014), in which Latin American fruits rich in phytochemicals with biological effects were evaluated, antioxidant capacities were reported for goldenberry calyx extract of 49 and 86 µM TE/g for DPPH and ABTS methodologies, respectively, which were lower values than those reported in this study.

3.5 Identification of rutin

The chromatographic conditions used allowed for obtaining a good resolution for the rutin signal from goldenberry calyx, as shown in Figure 5. The method yielded results directly proportional to the concentration of the compound, with a high correlation between the concentration of the analyte and the response of the chromatograph, and a rutin concentration of 18.93 mg rutin/g extract was found in the extract. The retention time was 15 min, and peaks were observed that indicated

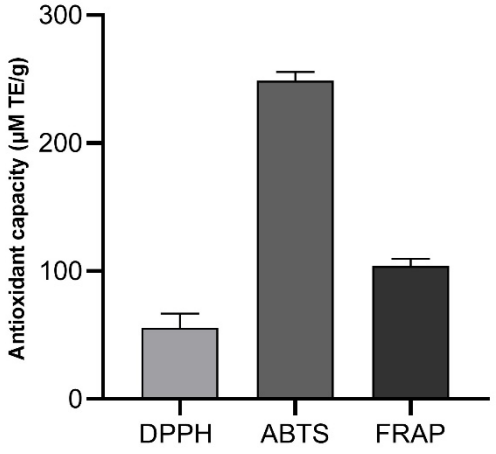

Figure 4. Antioxidant capacity of goldenberry calyx extract. Conditions: time = 10 min; amplitude = 54%; liquid:solid ratio = 33 mL/g; and particle size = 213 µm.

Table 3. Predicted and experimental results of total phenolic compounds (TPC) extraction and antioxidant capacity of goldenberry calyx extract.

Analysis	Predicted value	Experimental value (n=3)	Error (%)
TPC (mg GAE/g)	55.5	53.3	4.1
Antioxidant capacity (µmol ET/g)	168.3	167.2	0.6

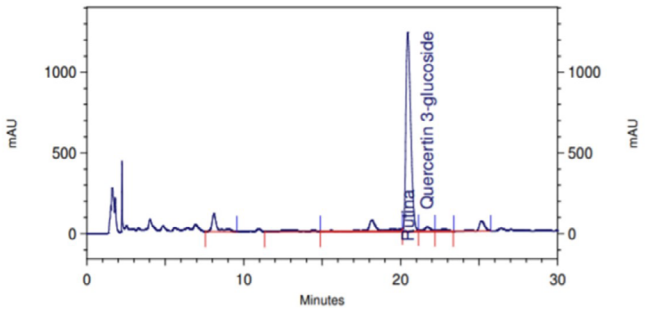

Figure 5. Chromatogram for calyx extract goldenberry: elution of the compound rutin.

the presence of other compounds in the extract, which had a lipophilic condition and could be of interest for future research.

The rutin content of goldenberry calyx from this study was higher than that reported in other studies. Carbone *et al.* (2020) performed extractions from hop cones (*Humulus lupulus*) using solid-liquid extraction with toluene (0.87 mg rutin/g extract), ultrasound-assisted extraction with water (1.09 mg rutin/g extract), ultrasound-assisted extraction with ethanol (0.30 mg rutin/g extract), ultrasound-assisted extraction with a water/ethanol (1:1) mixture (0.63 mg rutin/g extract), microwave-assisted extraction with water (0.49 mg rutin/g extract), microwave-assisted extraction with ethanol (0.44 mg rutin/g extract), and microwave-assisted extraction with a water:ethanol (1:1) mixture (0.53 mg rutin/g extract). Likewise, Nguyen *et al.* (2021) reached 5.04 mg rutin/g extract from *Physalis angulata* L. leaves. Finally, the rutin contents in this work were higher than those found in other calyx extracts from goldenberry (Cardona *et al.*, 2017; Gironés-Vilaplana *et al.*, 2014). The higher routine content of this study may have been due to the optimisation of the operating variables and solid conditions used.

4 CONCLUSION

This study demonstrated the qualities of ultrasound-assisted extraction technology, as high extraction yields of phenolic compounds were found using short extraction times, intermediate particle diameters, low amount of solvent, low energy consumption, and water and ethanol, solvents generally recognised as safe (GRAS). This favours technology transfer or industrial scale-up. Additionally, the goldenberry calyx could be a sustainable source for the extraction of metabolites that provide health benefits, such as rutin, a compound of great relevance in the pharmaceutical, cosmetic, and food industries. The high content of this bioactive compound was much higher than the values found in the literature, which could be due to the optimisation of the operating variables and solid conditions of this study. However, the high sensitivity of rutin to extrinsic factors makes it necessary to evaluate encapsulation mechanisms to limit degradation phenomena and allow the application of the extract in chemical matrices.

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