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Optimisation of the microwave-assisted extraction process of bioactive compounds with antioxidant activity from cocoa pod husk (*Theobroma cacao* L.)

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Cocoa pod husk (CPH), the main by-product of cocoa production, represents up to 80% of the fresh weight of the fruit and is often discarded, despite being a rich source of bioactive compounds such as polyphenols, flavonoids and saponins. The objective of this study was to optimise the microwave-assisted extraction (MAE) of bioactive compounds with the highest antioxidant activity from CPH using the Box–Behnken design (BBD) and Response Surface Methodology (RSM). The effects of microwave power (285, 570, and 855 W), extraction time (2, 3, and 4 min) and liquid/solid ratio (30, 40, and 50 mL g^{-1}) on the Total Phenol Content (TPC), Total Saponin Content (TSC) and antioxidant activity—DPPH Radical Scavenging Capacity (DRSC), ABTS Radical Scavenging Capacity (ARSC) and Ferric Reducing Antioxidant Power (FRAP)—of bioactive compounds were evaluated. The globally optimised conditions (855 W, 3.06 min, and 50 mL g^{-1}) achieved high extraction efficiencies, and yielded a TPC of 2.724 mg_{GAE} g^{-1}, TSC of 0.241 mg_{EE} g^{-1}, and DRSC, ARSC, and FRAP of 81.966, 99.680, and 90.890 μmol_{TE} g^{-1}, respectively, with a desirability index of 0.84. Microwave power emerged as the main factor influencing bioactive recovery, while extraction time and liquid/solid ratio modulated diffusion and prevented thermal degradation. Notably, the protocol was implemented using a modified domestic microwave oven, offering a cost-effective and sustainable alternative to conventional MAE systems. Comprehensive UHPLC-Q-Orbitrap-MS/MS profiling revealed a diverse metabolomic signature, including malic, citric, tartaric, and gluconic acids, and the phenolic compound clovamide, confirming the chemical complexity and functional potential of the extract. The integrated optimisation and profiling framework reinforces the sustainable nature and methodological novelty of MAE in enhancing the value of CPH, supporting its role as a clean-label candidate for functional nutrition, health-promoting products, and circular bioeconomy initiatives.

Sustainability spotlight

Cocoa pod husk (CPH), an abundant agro-industrial by-product, poses environmental and economic challenges due to its improper disposal, while conventional extraction techniques demand substantial energy and solvent input. This study optimises microwave-assisted extraction (MAE) to sustainably recover antioxidant-rich bioactive compounds—including phenolics and saponins—while significantly reducing resource consumption. The process integrates statistical design methodologies to enhance efficiency and scalability, offering a robust and adaptable valorisation strategy. These findings exemplify circular economy principles by converting agricultural waste into functional ingredients suitable for food, cosmetic, and pharmaceutical applications. The work directly supports SDGs 9 (Industry, Innovation and Infrastructure), 12 (Responsible Consumption and Production), and 13 (Climate Action), reinforcing the contribution of green technologies to agro-industrial sustainability.

1 Introduction

The cocoa bean is one of the most significant crops worldwide. It is estimated that world production will reach approximately 4.84 million tonnes in the period 2024/25, according to the International Cocoa Organization (ICCO).[1] This is evidence of the high global demand and economic impact of cocoa, which is not only limited to the chocolate industry, but also involves its by-products.

Cocoa pod husk (CPH) is the main by-product, representing 52–80% of the fresh weight of the fruit.[2–8] For every tonne of dry

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cocoa beans, ten tonnes of wet CPH are generated,[9] generating approximately 48.4 million tonnes globally, which could pose environmental problems, but also reflects a critical underutilization of a plant material that, until now, has been considered an unwanted waste by the cocoa and chocolate industries. Therefore, effective management of these wastes is fundamental in attempts to prevent food waste and develop circular economy concepts, within the food–energy–water (FEW) nexus, as their management significantly affects the availability of these three resources.[10] CPH is an essential source of bioactive compounds, particularly polyphenols, flavonoids, methylxanthines, and fibres, which have been associated with antioxidant, anti-inflammatory, and cardioprotective properties.[9,11–17] Thus, the valorisation of CPH would not only reduce the environmental impact of waste, but also generate integral solutions such as the production of biomass energy, water recovery, development of functional materials, recovery of bioactive compounds with antioxidant activity and enriched composting, with promising applications in the food, cosmetics, and pharmaceutical industries.[10,18] Within this framework, agro-industrial by-products, such as CPH, should not be considered as final waste, but as secondary raw materials with economic, environmental and social value. Transforming organic wastes into reusable inputs within the same or other production systems, promotes closed-material cycles.[17] Waste management approaches generally include the reuse and valorisation of by-products through innovative processes, their use as functional ingredients rich in bioactive compounds, and the extraction of valuable active ingredients.

Consequently, Microwave-Assisted Extraction (MAE) is positioned as an innovative non-conventional solvent alternative green technology for the recovery of bioactive compounds from CPH presenting significant advantages over conventional solvent extraction.[19–23] Its main benefits include a reduction in the volume of solvents used, greater time efficiency, more precise control of operating variables, a decrease in energy consumption and an increase in extraction yield.[19] However, its effectiveness does not depend solely on the use of microwaves as an energy source, but is strongly influenced by a series of operating parameters. The most decisive factors are irradiation power, extraction time and solid/liquid ratio.[24] Power controls the rate of dielectric heating and, therefore, cell disruption and metabolite release, which must be carefully optimised to avoid thermal degradation of sensitive compounds; and the liquid/solid ratio directly affects the mass transfer efficiency and concentration of the extract.[24–26] Therefore, this study aims to optimise the operating parameters of MAE—microwave power, extraction time and liquid/solid ratio—in order to maximise the recovery of bioactive compounds from CPH. In particular, it seeks to improve the extraction efficiency of total phenolic content (TPC) and total saponin content (TSC), as well as antioxidant activity, evaluated using the DPPH radical scavenging capacity (DRSC), ABTS radical scavenging capacity (ARSC) and ferric reducing antioxidant power (FRAP) methods, which was assessed as part of this optimisation process.

As a major novel contribution, unlike previous studies, this work proposes a balanced global optimisation that simultaneously considers all response parameters, using a modified domestic microwave oven as a low-cost, high-efficiency alternative. It also incorporates the identification of the metabolomic profile of the optimised extract, which broadens the understanding of the compounds responsible for bioactivity and establishes a basis for future biotechnological and nutraceutical applications.

2 Materials and methods

2.1 Chemical and analytical instruments

All chemicals used in this study were of analytical grade, including: gallic acid, trolox, acetic acid (glacial) 100%, aluminium chloride hexahydrate, aluminium chloride trihydrate, iron(III) chloride hexahydrate, 2,20-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin–Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), and 2,2-diphenyl-1-picryl-hydrazil (DPPH) were purchased from Merck KGaA (Darmstadt, Germany). Anhydrous sodium carbonate, sodium acetate trihydrate and potassium persulfate were purchased from J.T.Baker (USA). NaOH was purchased from Macron Fine Chemicals (USA). UHPLC-Q-Orbitrap-MS/MS solvents were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained using a Millipore water purification system (Milli-Q Merck Millipore, Darmstadt, Germany). Absorbance was measured using a Genesys 10S UV-VIS spectrophotometer. UHPLC-Q-Orbitrap-MS/MS was measured using a UHPLC Dionex UltiMate 3000 system coupled to a Q-Exactive orbitrap (Thermo Fisher Scientific, Waltham, MA, USA).

2.2 Sample preparation

CPH was obtained from fine aroma cocoa of Amazon origin (*Theobroma cacao* L.) of the Forastero variety, provided by Kuyay chocolates, located in the Jahuanga zone, Bagua Grande district, Utcubamba province, Amazonas region, Peru (5° 46′19.6′ ″S 78°32′57.4′ ″W, 605 m altitude).

2.3 Microwave-assisted drying (MAD)

Microwave-assisted drying (MAD) was carried out following the methodology described by Sianoun *et al.*,[27] with slight modifications. Fresh CPH was manually sliced using a stainless-steel knife to produce sheets with a thickness of 5 ± 0.3 mm. The samples were arranged in a single layer on a glass turntable to ensure uniform microwave exposure and prevent overlapping. Drying was performed using a microwave oven (Samsung MG40J5133AT; 95–950 W, 2450 MHz). The drying process was fixed at 95 W (10% power of the microwave oven).

The key experimental parameter was the sample-to-power ratio, where the loading density for MAD was maintained at 0.52 g of CPH per watt of microwave power (g W^{-1}). This ratio was achieved by placing 50.10 ± 0.02 g of sample under a microwave power of 95 W. The selected ratio was based on preliminary trials, which indicated that drying under these conditions yielded the highest TPC, increasing from 1.50 mg$_{GAE}$ g^{-1} in the fresh sample to 2.40 mg$_{GAE}$ g^{-1} after drying. These values were obtained using ultrasound-assisted extraction (UAE) as the

analytical method during preliminary experiments. It should be noted that these TPC values were obtained during preliminary experiments and are not included in the main dataset presented in this article.

All measurements were performed in triplicate. The dried samples were milled using a blade mill (MKM6003, Bosch, Slovenia) and sieved through an ASTM E11 No. 80–60 mesh (Retsch, Germany). Hence, the particle sizes were between 251 and 426 μm. The resulting flour was packaged in trilaminate vacuum bags and stored at −80 °C until further phytochemical and antioxidant activity analysis.

2.4 Microwave-assisted extraction (MAE) optimisation

The MAE process for CPH was conducted using a modified microwave oven (Samsung MG40J5133AT), as illustrated in Fig. 1. Bioactive compounds were extracted following the protocol described by Rahayu et al.[21] The experimental design included three levels for each variable: microwave power (285, 570, and 855 W), extraction time (2, 3, and 4 min), and liquid/solid ratio (30, 40, and 50 mL g^{-1}). Extractions were performed in a boiling flask using particles whose sizes were in the range between 251 and 426 μm, and an ethanol–water solution (50% v/v) as the solvent. The resulting extracts were centrifuged at 5000 rpm for 10 min, and the residues were washed with the same solvent and centrifuged again at 5000 rpm for 3 min. The final extract volume was adjusted to match the initial extraction volume using the same solvent, and then cooled for subsequent analysis.

It is important to note that the microwave system does not have active temperature control. Therefore, the extractions were performed in a boiling flask placed inside a water bath (Fig. 1). In this way, the water bath acted as a thermal buffer, preventing excessive temperature increases. The MAE protocol aimed to maintain the extraction at 80 ± 2 °C. This temperature was defined because preliminary tests showed that extraction temperatures above 90 °C caused condenser saturation, increased margins of error, and were incompatible with the proposed modification model.
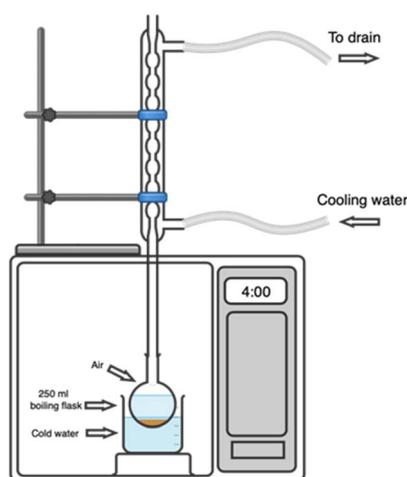

Fig. 1 Modified microwave oven for microwave-assisted extraction (MAE).

2.5 Experimental design

A Box–Behnken design (BBD) with Response Surface Methodology (RSM) was used to optimise and determine the impact of the process variables (A: microwave power, B: extraction time, C: liquid/solid ratio) on the responses: TPC, TSC, ARSC, DPPH and FRAP. These factors and their levels are listed in Table 1. The relationship between the process variables and responses is expressed using an empirical second-order polynomial equation as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

where Y is the response, β_0 the ordinate at the origin, β_i a linear coefficient, β_{ij} an interaction coefficient, β_{ii} a quadratic coefficient, and ε random error.

2.6 Determination of bioactive compounds

Bioactive compounds of CPH extract were evaluated in terms of TPC and TSC. The TPC was determined according to the method of Singleton et al.[28] and was expressed as milligrams of gallic acid equivalents per gram (mg$_{GAE}$ g^{-1}) of dried extract sample (DS extract). The TSC was determined according to the method of Nguyen et al.[12] and was expressed as milligrams of escin equivalents per gram (mg$_{EE}$ g^{-1}) of DS extract.

The identification of secondary metabolites was carried out by UHPLC-Q-Orbitrap-MS/MS following the methodology adapted from Vargas-Arana et al.[11] Extracts were prepared by dissolving 10 μL of sample (optimised CPH extract) in 90 μL of acetonitrile (UHPLC grade). Samples were centrifuged at 4200 rpm for 5 min and transferred to a 2 mL glass vial.

An ultra-high-performance liquid chromatograph (UHPLC Dionex UltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with an Acclaim™ C18 reversed-phase HPLC column (5 μm, 4.6 × 150 mm) was used, with a flow rate of 0.7 mL min^{-1}, and the injection volume was 10 μL. The mobile phases were 0.1% formic acid in water (A) and 90% acetonitrile in water (B). The gradient used was (0.00 min, 5% B); (1.00 min, 5% B); (25.00 min, 95% B); (26.00 min, 100% B); (27.00 min, 100% B); (30.00, 5% B); and 2.5 min for column equilibration before each injection.

The Q-Exactive orbitrap (HESI II, Thermo Fisher Scientific, Waltham, MA, USA) was operated in negative ion mode (ESI-), using data-dependent acquisition (DDA). Orbitrap parameters were: AGC target: 5×10^{-6}, resolution: 120 000, maximum, IT:

Table 1 Factors and levels of the Box–Behnken design for optimisation[a]

Factors	Unit	Levels		
		−1	0	+1
(A) Microwave power	W	285	570	855
(B) Extraction time	min	2	3	4
(C) Liquid/solid ratio	mL g^{-1}	30	40	50

[a] Abbreviations: W, watts; min, minutes; mL g^{-1}, millilitres per gram.

150 ms, range: m/z 100–600, HESI source (1 kV, negative mode), gas heater temp: 140 °C, capillary temperature: 300 °C, carrier gas: N_2 (sheath gas flow rate: 10, sweep gas flow rate: 1, S-lens RF level: 80). Parameters for MS^2 were: maximum IT: 50 ms, AGC target: 2×10^{-4}, resolution: 60 000.

2.7 Determination of antioxidant activity

CPH extract was tested for antioxidant activity utilizing ARSC, DRSC and FRAP. The ARSC assay was performed according to the method described by Re et al.[29] The DRSC assay was performed according to the method described by Brand-Williams et al.[30] The FRAP assay was performed according to the method described by Benzie et al.[31] All antioxidant activity assays were expressed as μmol of Trolox equivalents per gram (μmol_{TE} g^{-1}) of DS extract.

2.8 Statistical analysis

Analysis of variance (ANOVA) was carried out to evaluate the statistical significance ($p < 0.05$) of the factors. Mean comparisons were made using the Tukey HDS test ($p < 0.05$). All data are presented as mean $\pm$ standard deviation (SD). The analyses were repeated thrice unless indicated. The BBD and RSM were performed using Design-Expert version 13 (Stat-Ease Inc., Minneapolis, MN, USA).

3 Results and discussion

3.1 Microwave-assisted extraction (MAE) process

The BBD was employed to optimise the independent variables of MAE—microwave power (W), extraction time (min), and liquid/solid ratio (mL g^{-1})—in relation to the response variables TPC, TSC, DRSC, ARSC, and FRAP. The experiment was

conducted using CPH flour with a particle size range of 251–426 μm, and the results are summarised in Table 2.

The analysis showed that each response reached its highest individual value under different extraction conditions. The highest TPC (2.775 mg_{GAE} g^{-1} DS extract) and the greatest DRSC activity (90.536 μmol_{TE} g^{-1} DS extract) were both obtained at 855 W, 2 min, and 40 mL g^{-1}. In contrast, the maximum TSC (0.246 mg_{EE} g^{-1} DS extract) occurred at 855 W, 3 min, and 50 mL g^{-1}; the highest ARSC activity (102.188 μmol_{TE} g^{-1} DS extract) at 285 W, 3 min, and 50 mL g^{-1}; and the maximum FRAP value (94.795 μmol_{TE} g^{-1} DS extract) at 855 W, 4 min, and 40 mL g^{-1}.

While the individual maxima highlight the distinct sensitivity of each response to specific MAE parameters, these values are not intended to define separate operational targets. Rather, they inform the response surface behaviour that supports the subsequent global optimisation. The focus of this work lies in establishing integrated extraction conditions that ensure overall efficiency across all responses, rather than optimising them independently.

3.2 Optimisation and validation of the extraction of bioactive compounds and antioxidant activity of CPH by MAE

3.2.1 Total phenolic content (TPC). The ANOVA results for TPC indicated a highly significant model ($p < 0.0001$), accounting for 93.23% of the variance ($R^2 = 0.9323$; adjusted $R^2 = 0.9174$). Among the evaluated variables, microwave power (A) emerged as the most influential factor ($F = 396.44$; $p < 0.0001$), followed by the liquid/solid ratio (C) and extraction time (B), both of which were statistically significant. A notable interaction was observed between AB, while AC and BC showed no significance. Curvilinear effects were detected for power (A^2, $p < 0.0001$) and liquid/solid ratio (C^2, $p = 0.0187$), but not for time

Table 2 Experimental values of response variables (TPC, TSC, DRSC, ARSC and FRAP) obtained by MAE[a]

Run	Microwave power (W)	Extraction time (min)	L/S ratio (mL g^{-1})	TPC (mg$_{GAE}$ g^{-1})	TSC (mg$_{EE}$ g^{-1})	DRSC (μmol_{TE} g^{-1})	ARSC (μmol_{TE} g^{-1})	FRAP (μmol_{TE} g^{-1})
1	570	2	50	2.623 ± 0.013	0.239 ± 0.002	75.126 ± 1.608	94.749 ± 2.165	86.333 ± 1.196
2	855	2	40	2.775 ± 0.061	0.219 ± 0.002	90.536 ± 2.539	91.888 ± 3.120	87.708 ± 0.778
3	855	3	50	2.712 ± 0.023	0.246 ± 0.004	81.983 ± 1.439	101.433 ± 0.549	89.382 ± 1.164
4	570	4	30	2.409 ± 0.036	0.218 ± 0.002	76.316 ± 1.192	92.743 ± 2.220	88.621 ± 0.848
5	570	3	40	2.571 ± 0.026	0.223 ± 0.006	82.257 ± 1.158	98.557 ± 1.572	89.493 ± 0.905
6	570	3	40	2.551 ± 0.025	0.223 ± 0.005	82.819 ± 1.083	97.212 ± 1.166	87.966 ± 1.671
7	570	3	40	2.560 ± 0.020	0.226 ± 0.004	83.929 ± 2.214	98.159 ± 1.584	90.024 ± 0.867
8	855	4	40	2.699 ± 0.040	0.231 ± 0.003	87.841 ± 0.508	98.647 ± 2.551	94.795 ± 1.011
9	285	3	30	2.193 ± 0.024	0.190 ± 0.005	78.296 ± 3.422	94.716 ± 0.970	89.099 ± 1.347
10	855	3	30	2.498 ± 0.032	0.197 ± 0.003	77.890 ± 1.086	97.991 ± 1.515	82.067 ± 1.327
11	285	3	50	2.284 ± 0.019	0.194 ± 0.004	75.199 ± 0.799	102.188 ± 1.442	85.493 ± 1.046
12	285	2	40	1.985 ± 0.032	0.185 ± 0.004	76.299 ± 1.243	84.762 ± 1.267	75.335 ± 0.751
13	285	4	40	2.359 ± 0.028	0.201 ± 0.003	74.496 ± 1.890	89.214 ± 1.205	92.578 ± 1.173
14	570	2	30	2.424 ± 0.026	0.187 ± 0.002	76.128 ± 0.964	92.822 ± 0.409	71.798 ± 1.676
15	570	4	50	2.616 ± 0.020	0.218 ± 0.003	85.199 ± 2.767	95.843 ± 0.766	83.174 ± 1.170
16	570	3	40	2.554 ± 0.023	0.225 ± 0.005	83.408 ± 1.270	99.373 ± 0.628	87.600 ± 2.270
17	570	3	40	2.591 ± 0.043	0.226 ± 0.003	82.080 ± 0.726	99.036 ± 1.010	87.334 ± 0.678

[a] The results were expressed as the sample dry extract. Data are presented as mean $\pm$ SD from triplicate experiments. Abbreviations: TPC, total phenol content; TSC, total saponin content; DRSC, DPPH radical scavenging capacity; ARSC, ABTS radical scavenging capacity; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; EE, escin equivalent; TE, Trolox equivalent.

Table 3 Adequacy of the models obtained for the response variables TPC, TSC, DRSC, ARSC and FRAP[a]

Responses	R^2	Adj. R^2	p-value (model)	Significance terms	Lack-of-fit p	CV (%)
TPC	0.932	0.917	<0.0001*	A, C, AB, A^2	<0.0001*	2.29
TSC	0.967	0.960	<0.0001*	A, B, C, AC, BC, A^2, C^2	0.0619	1.14
DRSC	0.689	0.621	<0.0001*	A, C^2	<0.0001*	3.75
ARSC	0.792	0.746	<0.0001*	A, B^2	<0.0001*	2.42
FRAP	0.852	0.820	<0.0001*	B, BC, C^2	<0.0001*	2.80

[a] The p-value results were expressed as follows: $p < 0.05$, *. Abbreviations: A, microwave power; B, extraction time; C, liquid/solid ratio; CV, coefficient of variation; TPC, total phenol content; TSC, total saponin content; DRSC, DPPH radical scavenging capacity; ARSC, ABTS radical scavenging capacity; FRAP, ferric reducing antioxidant power.

$(B^2, p = 0.6728)$. Although the pure error was low, the model showed a significant lack-of-fit ($p < 0.0001$), possibly reflecting the influence of additional factors not captured by the current design (Tables 3 and S1). Nevertheless, the high coefficients of determination ($R^2 = 0.9323$; adjusted $R^2 = 0.9174$), together with the satisfactory validation performance (Table 4), support the robustness and predictive reliability of the model within the experimental scope evaluated.

Using BBD and RSM methodologies, the response surface graphs revealed that TPC increased with microwave power and liquid/solid ratio, while prolonged extraction time negatively affected yield (Fig. 2). Under specific experimental conditions, the highest individual TPC value (2.775 mg$_{GAE}$ g^{-1} DS extract) was obtained at 855 W, 40 mL g^{-1}, and 2 min (Table 2). This local maximum reflects the combined curvilinear effects of power and liquid/solid ratio identified in the model, illustrating how each parameter influences TPC recovery.

However, this does not correspond to the overall optimum conditions. When all responses were simultaneously considered, the global optimisation (855 W, 50 mL g^{-1}, 3.06 min) provided a balanced extraction performance, yielding a slightly lower TPC value (2.724 mg$_{GAE}$ g^{-1} DS extract) (Table 4), consistent with the negative effect of longer extraction times suggested by the AB interaction.

Several studies have supported the influence of extraction variables on TPC during MAE. Rincón et al.[20] applied a BBD to optimise MAE parameters for TPC extraction from CPH (100 µm, variety TCS01) using 63% ethanol. Under optimal conditions (400 W, 240 s, and 67 mL g^{-1}), they achieved a TPC of 43.94 mg$_{GAE}$ g^{-1} DS, identifying microwave power as the most significant factor, followed by the extraction time and liquid/solid ratio. Similarly, Nguyen et al.[12] optimised MAE parameters for saponin recovery from CPH (≤1400 µm) using a Central Composite design (CCD). While saponins were the primary target, a TPC of 12.40 mg$_{GAE}$ g^{-1} DS was reported under optimal conditions (600 W, 6 s min^{-1} pulsed irradiation, 40 min, 50 mL g^{-1}), identifying methanol concentration and extraction time as key variables influencing phenolic recovery. In a study by Rahayu et al.,[21] MAE was conducted on CPH (177 µm, Indonesia) using pure ethanol for varying times (4–10 min) at 800 W and 70 °C. The highest TPC (8.65 mg$_{GAE}$ mL^{-1} DS) was obtained after 10 min, demonstrating the importance of time, although prolonged exposure may differ in effect depending on the solvent and particle size. Mashuni et al.[19] reported a TPC of 853.67 mg$_{GAE}$ L^{-1} equivalent to 0.853 mg$_{GAE}$ mL^{-1} DS for CPH (250–600 µm, Forastero variety) extracted with 85% ethanol at 200 W for 20 min, showing low phenolic recovery under relatively low power and long extraction time.

Other studies without formal optimisation designs also reported diverse TPC values depending on variables such as cocoa variety, solvent extraction, particle size, and extraction method. For instance, Nguyen et al.[32] reported TPC values between 1.44 and 12.22 mg$_{GAE}$ g^{-1} DS for CPH (≤1400 µm, Trinitario variety) using water and water bath extraction. Valadez-Carmona et al.[14] obtained 1893 mg$_{GAE}$ per 100 g DS equivalent to 18.93 mg$_{GAE}$ g^{-1} DS for CPH (≤425 µm) extracted by agitation with acetone : water : acetic acid (70 : 29.5 : 0.5), mainly associated with drying treatments prior to extraction. Delgado-Ospina et al.[5] found TPC values ranging from 5.4 to 16.6 mg$_{GAE}$ g^{-1} DS for CPH (500 µm) using sonication with methanol : water and acetone : water mixtures, following heat pre-treatments. Vargas-Arana et al.[11] reported remarkably high TPC (111 mg$_{GAE}$ g^{-1} extract) from convectively dried CPH (≤425 µm, CCN-51 variety) extracted with methanol + 1% formic acid via sonication. Martínez et al.[33] found in CPH (220–640 µm) TPC values of 352.67–365.33 mg$_{GAE}$ per 100 g DS equivalent to 3.52–3.65 mg$_{GAE}$ g^{-1} DS extracted by

Table 4 Optimised model prediction for the extraction of bioactive compounds and antioxidant activity from CPH by MAE[a]

Responses	Predicted mean	Std dev	n	SE pred	95% PI low	Data mean	95% PI high	Relative error %
TPC	2.770	0.057	5	0.038	2.692	2.724	2.847	1.67%
TSC	0.246	0.004	5	N/A	0.240	0.241	0.252	1.99%
DRSC	85.683	3.021	5	2.026	81.591	81.966	89.775	4.54%
ARSC	102.580	2.319	5	1.555	99.439	99.680	105.720	2.91%
FRAP	92.075	2.417	5	1.621	88.802	90.890	95.348	1.30%

[a] The results were expressed as the sample dry extract. Abbreviations: TPC, total phenol content; TSC, total saponin content; DRSC, DPPH radical scavenging capacity; ARSC, ABTS radical scavenging capacity; FRAP, ferric reducing antioxidant power.
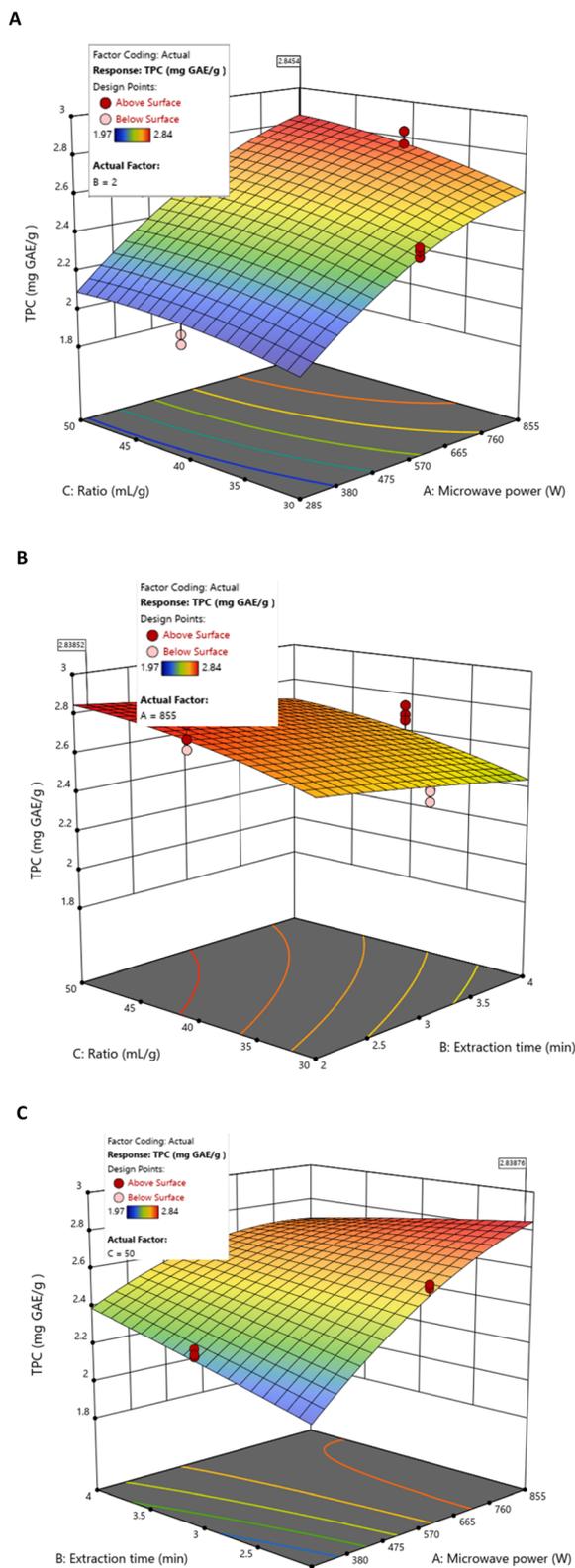
A

B

C

Fig. 2 Response surfaces of TPC by MAE as a function of fixed values for (A) extraction time (2 min), (B) microwave power (855 W), and (C) liquid/solid ratio (50 mL g^{-1}).

agitation with methanol-acetone and 206.67–227 mg$_{GAE}$ per 100 g DS with ethanol equivalent to 2.06–2.27 mg$_{GAE}$ g^{-1} DS. Comparative analysis with previous studies highlights that TPC extraction from CPH is governed by a complex interaction of factors. This multidimensional dependence highlights the variable but promising nature of MAE in various optimisation strategies.

Although our high-power, short-duration MAE approach yielded lower TPC values than those recorded with longer extraction times or higher solvent volumes, it offers an efficient compromise between processing speed and phenolic recovery. It should be noted that the intermediate particle size adopted in this study (251–426 μm) favoured effective mass transfer without negatively affecting operational performance, reaffirming its suitability for the sustainable extraction of phenolic compounds from agro-industrial waste such as CPH.[34]

On the other hand, comparatively higher TPC values can be attributed to the widespread use of intermediate polarity solvents, such as ethanol and methanol, which not only facilitate deeper penetration into lignocellulosic matrices but also exhibit superior absorption of microwave energy.[35] These physicochemical properties favour the solubilisation of bioactive compounds with intermediate polarity, particularly polyphenols.[35]

3.2.2 Total saponin content (TSC). The ANOVA results for TSC revealed a highly significant model ($p < 0.0001$), explaining 96.73% of the data variability ($R^2 = 0.9673$; adjusted $R^2 = 0.9601$). Microwave power (A) was the dominant factor ($F = 419.64$; $p < 0.0001$), followed by the liquid/solid ratio (C) ($F = 286.62$; $p < 0.0001$) and extraction time (B) ($F = 49.08$; $p < 0.0001$). Strong interactions were observed between time and ratio (BC, $F = 149.56$; $p < 0.0001$) and between microwave power and liquid/solid ratio (AC, $F = 94.56$; $p < 0.0001$), whereas the microwave power-extraction time interaction (AB) was not significant ($p = 0.1473$). All second-order terms were statistically significant, indicating curvilinear behaviour for microwave power (A^2, $F = 146.37$), extraction time (B^2, $F = 10.99$), and liquid/solid ratio (C^2, $F = 33.52$). The model showed good fit, with a non-significant lack-of-fit ($p = 0.0619$) confirming its robustness and predictive reliability within the experimental range evaluated (Tables 3 and S2).

BBD and RSM analyses indicated that TSC increased with microwave power and liquid/solid ratio, while extraction time exerted only a minor positive influence (Fig. 3). Under specific experimental conditions, the highest individual TSC value (0.246 mg$_{EE}$ g^{-1} DS extract) was observed at 855 W, 50 mL g^{-1}, and 3 min (Table 2). This local maximum illustrates the stronger influence of microwave power and liquid/solid ratio on TSC recovery, as well as the relatively limited contribution of extraction time, consistent with the model results.

However, this point does not represent the overall optimal conditions. When all responses were simultaneously considered, the global optimisation (855 W, 50 mL g^{-1}, 3.06 min) yielded a slightly lower TSC value (0.241 mg$_{EE}$ g^{-1} DS extract) (Table 4), in agreement with the model predictions and the observed curvilinear effects.

The significantly higher yields reported in other studies are strongly influenced by the extraction time, solvent polarity, pre-
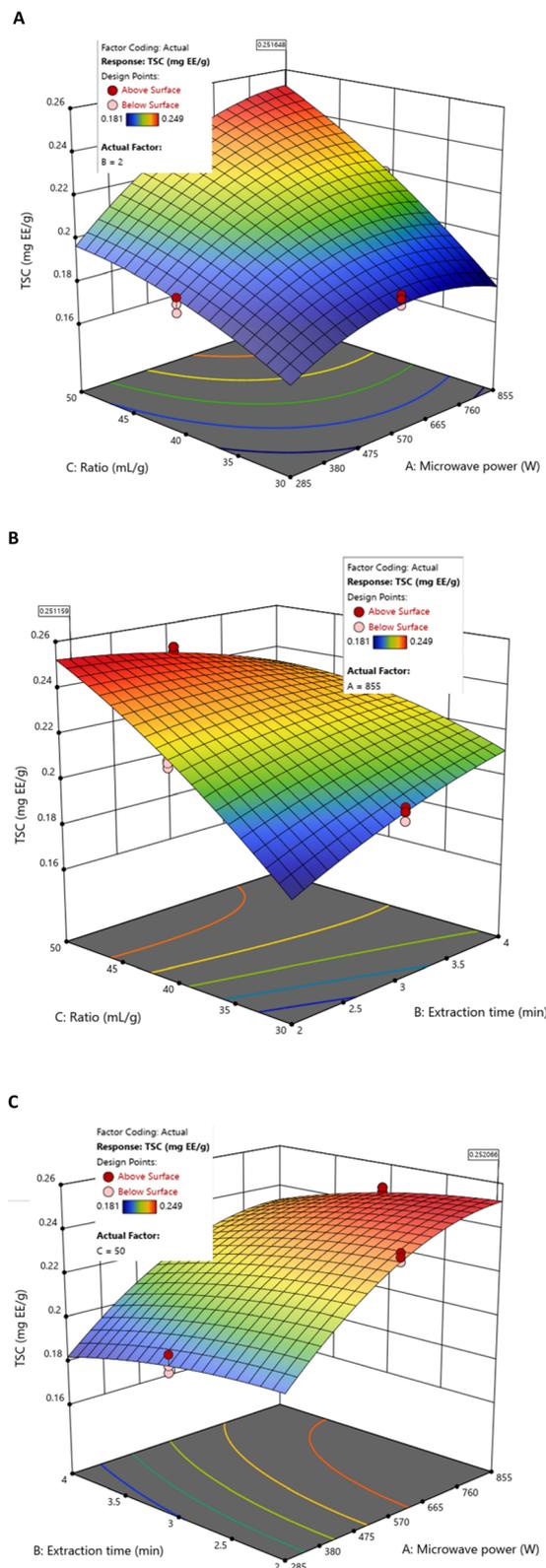
Fig. 3 Response surfaces of TSC by MAE as a function of fixed values for (A) extraction time (2 min), (B) microwave power (855 W), and (C) liquid/solid ratio (50 mL g^{-1}).

treatment, and irradiation protocols, which should be considered depending on application priorities-yield *vs.* efficiency. In this regard, Nguyen *et al.*[12] optimised the MAE parameters for TSC extraction from CPH ($\leq$1400 μm) using a CCD and obtained a significantly higher TSC of 402 mg$_{EE}$ g^{-1} DS, under conditions of 600 W, 6 s min^{-1} irradiation, 40 min, and a ratio of 50 mL g^{-1} ($R^2 = 0.71$, $p = 0.038$). The substantial difference in yield can be attributed to the use of methanol as a polar solvent, a pre-maceration stage, and prolonged extraction time, all of which improve cell wall rupture and solute diffusion. On the other hand, Nguyen *et al.*[13] reported moderate values (370 mg$_{EE}$ g^{-1} DS) for CPH ($\leq$1000 μm, Trinitario variety) under MAE conditions of 600 W, 30 min and 5 s min^{-1} irradiation, with a triple preheating stage (20 s at 600 W), using water as the solvent. These are significantly higher than those obtained in the present study, highlighting how prolonged exposure and thermal cycles can significantly increase extraction efficiency, albeit with greater energy input. On the other hand, from a chemical perspective, water favours the extraction of saponins due to its ability to interact with the hydrophilic sugar chains that characterise these compounds. Saponins are amphiphilic glycosides, with a lipophilic aglycone (sapogenin) and one or more hydrophilic sugar chains.[36] This structural duality allows water, under conditions of heating and pressure such as those generated in MAE, to facilitate the solubilisation of the polar fractions and promote the breaking of hydrogen bonds between the saponins and the plant matrix.[36]

Similarly, conventional extraction methods yielded medium to high saponin yields. In this regard, values of 3.98–118.70 mg$_{EE}$ g^{-1} DS were reported for CPH using soaking in distilled water for 30 min and bathing in water at 50 °C for 60 min, and UAE (100 W, 60 °C, 50 min) with water as the solvent, after a pre-maceration stage at 31 °C for 30 min.[32,37] The high saponin yield could be attributed to the synergistic effect of prolonged sonication and pre-treatment, which alter cell integrity and improve solute diffusion. In addition, ultrasound-induced acoustic cavitation generates microjets and shock waves that increase mass transfer and reduce solvent use.[38,39]

3.2.3 DPPH radical scavenging assay (DRSC). The ANOVA for DRSC confirmed a significant model ($p < 0.0001$), accounting for approximately 69% of the total variance ($R^2 = 0.6896$; adjusted $R^2 = 0.6215$). Microwave power (A) was the principal factor influencing DRSC ($F = 47.36$; $p < 0.0001$), while the liquid/solid ratio (C) had a moderate but statistically non-significant effect ($F = 3.19$; $p = 0.0816$). Extraction time (B) did not contribute significantly ($F = 1.37$; $p = 0.2492$). Significant interactions were observed between time and ratio (BC, $F = 8.02$; $p = 0.0071$) and between power and ratio (AC, $F = 4.28$; $p = 0.0449$), whereas the power–time interaction (AB) was negligible ($p = 0.8136$). Among the quadratic terms, only the liquid/solid ratio (C^2) demonstrated curvilinear behaviour ($F = 25.94$; $p < 0.0001$).

The model's lack-of-fit was significant ($p < 0.0001$), indicating room for further refinement (Tables 2 and S3), suggesting that additional variables or nonlinear interactions could further improve its predictive power. However, the acceptable coefficients of determination ($R^2 = 0.6896$; adjusted $R^2 = 0.6215$) and
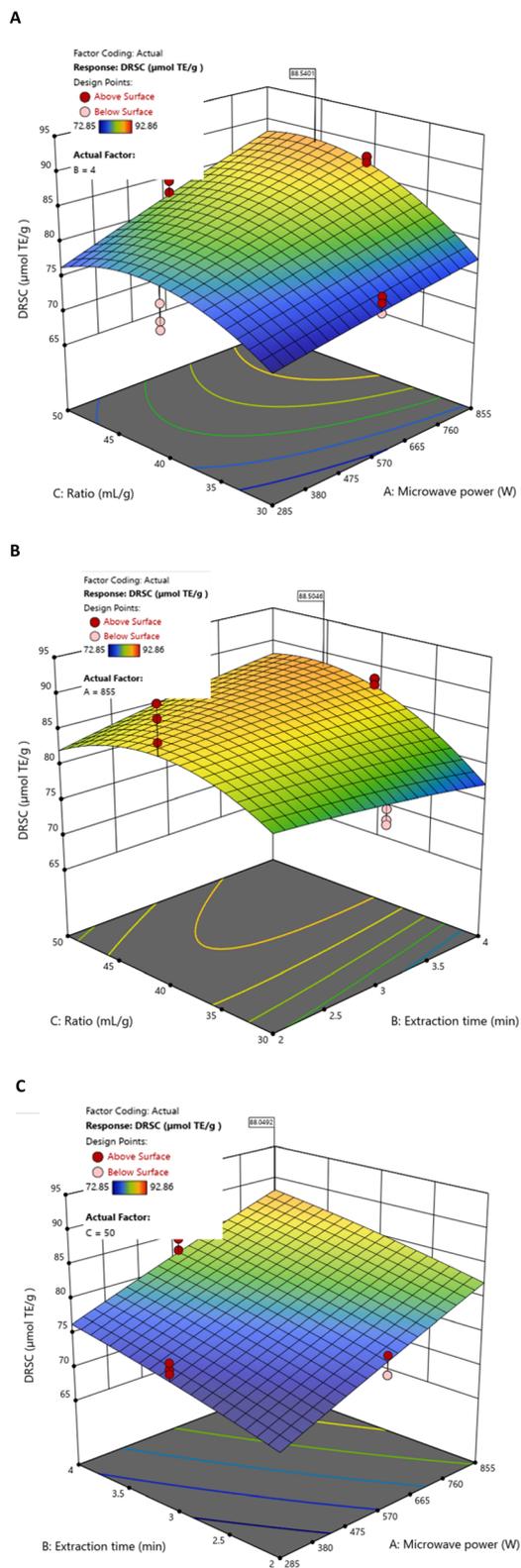
Fig. 4   Response surfaces of DRSC by MAE as a function of fixed values for (A) extraction time (4 min), (B) microwave power (855 W), and (C) liquid/solid ratio (50 mL g^{-1}).

consistent validation results (Table 4) support the reliability of the model for predicting DPPH radical scavenging activity under the MAE conditions studied.

Response surface analyses using BBD and RSM revealed that DRSC increased with microwave power and liquid/solid ratio, while extraction time exerted only a minor positive influence (Fig. 4). Under specific experimental conditions, the highest individual DRSC value (90.536 μmol_{TE} g^{-1} DS extract) was observed at 855 W, 40 mL g^{-1}, and 2 min (Table 2). This local maximum reflects the strong contribution of microwave power and liquid/solid ratio to DPPH radical scavenging capacity, and is consistent with the significant AC and BC interactions identified in the model.

However, this individual maximum does not represent the overall optimal conditions. When all responses were jointly considered, the global optimisation (855 W, 50 mL g^{-1}, 3.06 min) resulted in a lower DRSC value (81.966 μmol_{TE} g^{-1} DS extract) (Table 4), in line with the negative linear trend associated with extraction time and the modest influence of this factor predicted by the model.

In contrast, Rincón et al.[20] optimised the MAE parameters in CPH (100 μm, variety TCS01) using BBD and RSM, and reported significantly higher DRSC activity of 430.8 μmol_{TE} g^{-1} DS under the final optimised conditions with lower microwave power (400 W, 240 s, 67 mL g^{-1}), where microwave power was also the most influential factor. Furthermore, this higher antioxidant activity observed may be due to the finer particle size, solvent composition, and amount of TPC recovered. On the other hand, various non-optimised studies have reported DRSC values ranging from 87.42 to 133 μmol_{TE} g^{-1} DS,[11,40] with substantial variability attributed to differences in extraction method, particle size, solvent type, cacao variety, and pre-treatment strategies. This variability underscores that antioxidant activity is not solely governed by the parameters optimised in this study, but is also shaped by a complex interplay of physical (thermal degradation and Maillard reactions), chemical (enzymatic transformations of polyphenols), and biological (inherent profile of bioactive compounds in different cacao genotypes) factors.[41] These multidimensional influences highlight the need for broader integrative approaches when evaluating antioxidant capacity in plant matrices.

3.2.4   ABTS radical scavenging assay (ARSC). The ANOVA results for ARSC confirmed a significant model ($p < 0.0001$; $F = 17.39$), explaining 79.24% of the data variability ($R^2 = 0.7924$; adjusted $R^2 = 0.7468$). Microwave power (A) was the most influential factor ($F = 25.45$; $p < 0.0001$), followed by the liquid/solid ratio (C) ($F = 17.94$; $p = 0.001$) and extraction time (B) ($F = 10.29$; $p = 0.0026$). None of the interaction terms (AB, AC, BC) were statistically significant ($p > 0.05$), indicating limited synergistic effects among variables. Quadratic effects revealed notable curvilinear behaviour for extraction time (B^2, $F = 90.17$; $p = 0.0001$) and moderate curvature for the liquid/solid ratio (C^2, $F = 7.30$; $p = 0.0100$), while microwave power (A^2) did not demonstrate significance ($p = 0.0924$). A significant lack-of-fit ($F = 16.75$; $p < 0.0001$) suggested that the current model may not fully capture the system's complexity (Tables 3 and S4). However, the solid adjustment indicators ($R^2 = 0.7924$; adjusted
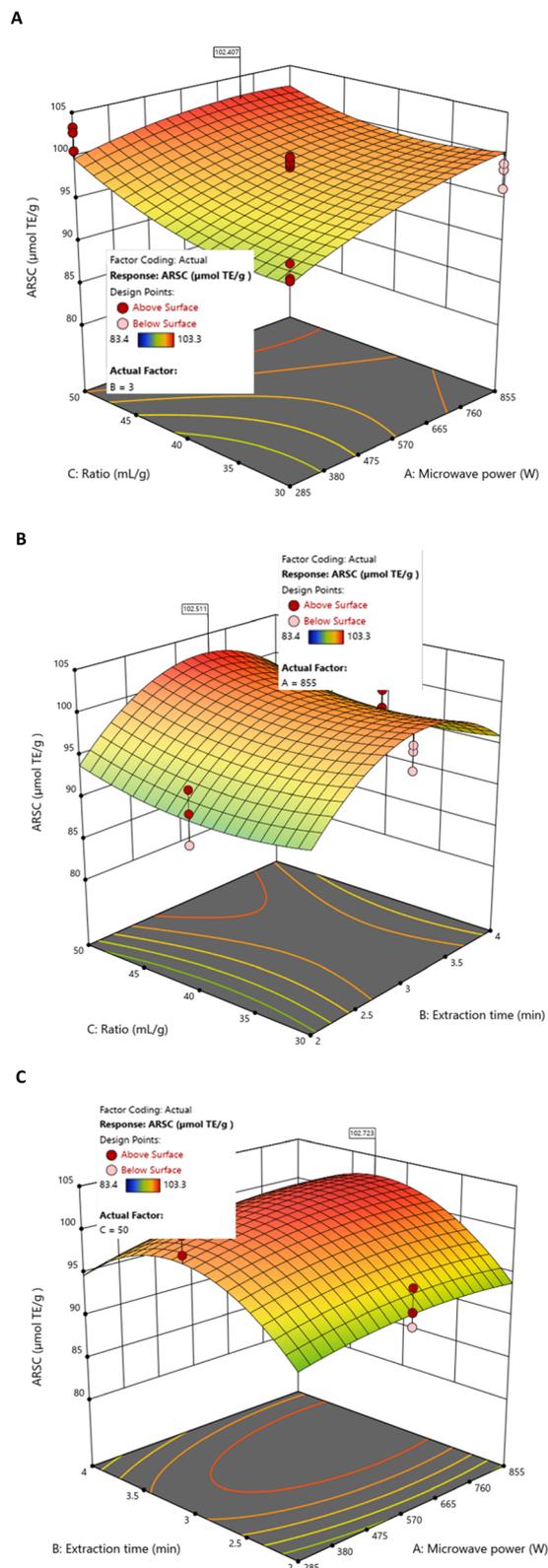
Fig. 5 Response surfaces of ARSC by MAE as a function of fixed values for (A) extraction time (3 min), (B) microwave power (855 W), and (C) liquid/solid ratio (50 mL g^{-1}).

$R^2 = 0.7468$) and the consistency of the validation (Table 4) support its predictive utility within the experimental scope studied, confirming that the model effectively captures the influence of microwave power and liquid/solid ratio on ARSC.

BBD and RSM analyses indicated that antioxidant activity increased with microwave power and liquid/solid ratio. Non-linear behaviour was also evident: moderate extraction times enhanced activity, whereas prolonged exposure led to slight reductions, likely due to degradation of thermo-sensitive compounds. Under specific experimental conditions, the individual optimum (855 W, 50 mL g^{-1}, and 3 min) predicted an antioxidant capacity between 102.407 and 102.723 μmol$_{TE}$ g^{-1} DS extract (Fig. 5). Within the experimental matrix, a similar individual maximum (102.188 μmol$_{TE}$ g^{-1} DS extract) was obtained at 285 W, 50 mL g^{-1}, and 3 min (Table 2).

This behaviour reflects the dependence of ARSC on the liquid/solid ratio and the existence of an energy saturation zone suggested by the model, where additional increases in microwave power do not proportionally enhance the response.

However, neither of these individual maxima represents the global optimal conditions. When all responses were jointly considered, the overall optimisation (855 W, 50 mL g^{-1}, 3.06 min) yielded an ARSC value of 99.680 μmol TE g^{-1} DS extract (Table 4), with a relative prediction error of 2.91%, confirming the model's reliability.

Studies using conventional methods and non-optimised protocols show considerable variability in ARSC. For example, Yapo et al.[16] reported 51.87 μmol$_{TE}$ g^{-1} DS in CPH (250 μm) extracted by boiling with 80% ethanol for 20 min. The freeze-dried CPH extract examined by Delgado-Ospina et al.[5] yielded a wider range (32.9–140 μmol$_{TE}$ g^{-1} DS), depending on the polarity and ratio of methanol : water and acetone : water mixtures under sonication-stirring. Vargas-Arana et al.[11] reported 155.38 μmol$_{TE}$ g^{-1} DS in convection-dried CPH, citing the role of formic acid (1%) in improving phenolic solubilisation. In units not comparable with our studies, it was found in CPH that MAE is approximately three times higher than UAE in terms of antioxidant activity, which could be attributed to prolonged extraction time, repeated heating cycles, and increased solute diffusion due to the polarity and thermal conductivity of the solvent.[13,37] This trend is consistent with other studies comparing UAE and MAE in polyphenol-rich matrices, which reported that MAE produced up to 23% more ARSC compared to UAE, while reducing extraction time sixfold.[13,37,42] These findings suggest that MAE typically results in more efficient recovery and better ARSC values due to rapid energy transfer and thermal stimulation of solute diffusion.[24]

What emerges from this comparative analysis is a consistent pattern: ARSC is highly sensitive to extraction kinetics and solvent physicochemistry. More importantly, antioxidant potential appears intricately linked to the phenolic fingerprint including the abundance and accessibility of low-molecular-weight flavonoids and phenolic acids. Factors such as drying intensity, matrix disruption, and solvent polarity not only affect mass transfer but also modulate the liberation of structurally bound polyphenols.[43–45]
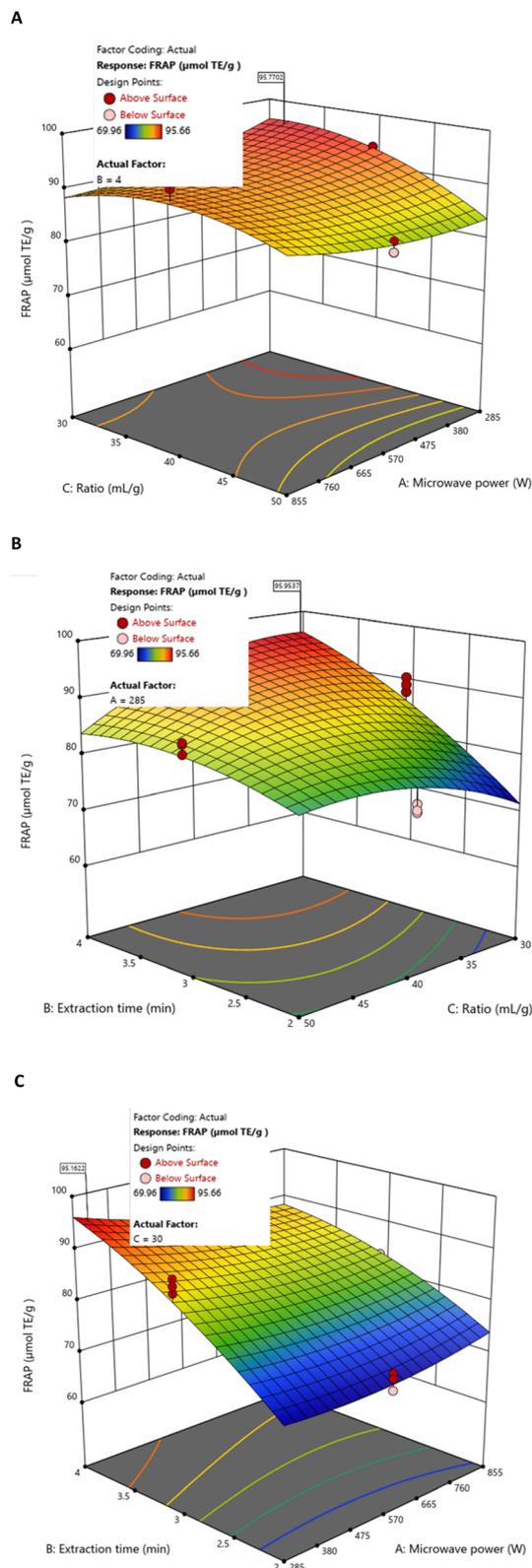
A

B

C

Fig. 6 Response surfaces of FRAP by MAE as a function of fixed values for (A) extraction time (4 min), (B) microwave power (285 W), and (C) liquid/solid ratio (30 mL g^{-1}).

3.2.5 Ferric reducing antioxidant power (FRAP). The ANOVA results for ferric reducing antioxidant power (FRAP) confirmed a significant model ($p < 0.0001$; $F = 26.38$), explaining 85.27% of data variability ($R^2 = 0.8527$; adjusted $R^2 = 0.8204$). Extraction time (B) was the most influential factor ($F = 93.12$; $p < 0.0001$), followed by the interaction between extraction time and liquid/solid ratio (BC, $F = 51.28$; $p < 0.0001$), the liquid/solid ratio (C, $F = 10.34$; $p = 0.0025$), and microwave power (A, $F = 8.43$; $p = 0.0059$). Additional interactions (AB and AC) were significant ($p = 0.0007$ and $p = 0.0003$, respectively), highlighting combined effects among variables. Quadratic terms revealed notable curvilinear behaviour, with C^2 being highly significant ($F = 27.23$; $p < 0.0001$), followed by B^2 ($F = 13.04$; $p = 0.0008$) and a moderate contribution from A^2 ($F = 5.47$; $p = 0.0244$). However, the model presented a significant lack-of-fit ($F = 31.24$; $p = 0.0001$), suggesting it may not fully capture the underlying variability (Tables 3 and S5). As in the case of DRSC and ARSC, the FRAP model showed a significant mismatch, possibly due to the multi-step redox mechanisms that govern antioxidant responses. However, the strong coefficients of determination ($R^2 = 0.8527$; adjusted $R^2 = 0.8204$) and the close agreement between experimental and predicted values (Table 4) validate the predictive reliability of the model under the MAE conditions studied.

BBD and RSM analyses indicated that microwave power and liquid/solid ratio exerted positive effects on FRAP values. The model also revealed non-linear behaviour, identifying an individual optimum at 285 W, 30 mL g^{-1}, and 4 min, with predicted FRAP values between 95.162 and 95.953 μmol$_{TE}$ g^{-1} DS extract (Fig. 6).

Within the experimental domain, the highest individual FRAP value measured (94.795 μmol$_{TE}$ g^{-1} DS extract) was obtained at 855 W, 40 mL g^{-1}, and 4 min (Table 2). This observation illustrates the strong contribution of extraction time and liquid/solid ratio, as well as the curvilinear effects suggested by the model, particularly the negative quadratic behaviour of the liquid/solid ratio.

Importantly, these individual maxima do not correspond to the global optimal conditions. When all responses were simultaneously considered, the overall optimisation (855 W, 50 mL g^{-1}, 3.06 min) produced a slightly lower FRAP value (90.890 μmol$_{TE}$ g^{-1} DS extract) (Table 4), consistent with the negative interaction between extraction time and liquid/solid ratio and the curvilinear trend predicted by the model.

In comparison, studies such as that by Rincón *et al.*[20] reported higher values (144.32 μmol$_{TE}$ g^{-1} DS) under longer conditions (400 W, 240 s, 67 mL g^{-1}), identifying microwave power as the main factor influencing antioxidant recovery, which is consistent with the present findings, and confirming the influence of the energy supplied and the availability of solvent.

Conventional methods, such as water bath or stirring with organic solvents, showed lower values and greater dispersion (53 – 181 μmol$_{TE}$ g^{-1} DS)[5,11,16] demonstrating limited efficiency without energy assistance and the significant influence of drying type, solvent, and plant variety on FRAP activity, likely

Table 5 Predictive equations in real units for the optimised MAE responses of CPH[a]

Term	TPC β	TSC β	DRSC β	ARSC β	FRAP β
Intercept	1.314×10^{-1}	-3.204×10^{0}	3.721×10^{1}	4.757×10^{1}	-6.083×10^{1}
A	3.034×10^{-3}*	4.930×10^{-4}*	-5.616×10^{-3}*	3.294×10^{-2}*	-2.919×10^{-2}
B	2.936×10^{-1}	3.876×10^{-1}*	-6.475×10^{0}	3.642×10^{1}	4.460×10^{1}*
C	3.341×10^{-2}*	3.778×10^{-2}*	2.470×10^{0}	-1.090×10^{0}	3.946×10^{0}
Interactions					
AB	-4.020×10^{-4}*	-2.700×10^{-5}	-7.330×10^{-4}	1.996×10^{-3}	-9.030×10^{-3}
AC	1.100×10^{-5}	1.800×10^{-5}*	6.390×10^{-4}	-3.620×10^{-4}	9.680×10^{-4}
BC	2.500×10^{-4}	-6.218×10^{-3}*	2.470×10^{-1}	2.833×10^{-2}	-4.996×10^{-1}*
Quadratics					
A^2	-1.270×10^{-6}*	-7.510×10^{-7}*	-2.380×10^{-6}	-1.400×10^{-5}	2.000×10^{-5}
B^2	-6.833×10^{-3}	-1.643×10^{-2}	-3.780×10^{-1}	-6.195×10^{0}*	-2.456×10^{0}
C^2	-3.930×10^{-4}	-2.870×10^{-4}*	-4.330×10^{-2}*	1.763×10^{-2}	-3.548×10^{-2}*

[a] The p-value results were expressed as follows: $p < 0.05$, *. Abbreviations: A, microwave power; B, extraction time; C, liquid/solid ratio; TPC, total phenol content; TSC, total saponin content; DRSC, DPPH radical scavenging capacity; ARSC, ABTS radical scavenging capacity; FRAP, ferric reducing antioxidant power.

due to their effect on the preservation and accessibility of phenolic compounds.[46] Together, these findings suggest that FRAP activity depends on the interaction between extraction kinetics, the chemical composition of the matrix, and the thermal conditions applied, requiring a multivariable approach for future optimisation, including analysis of the specific phenolic profile and thermal stability of the active compounds.

Overall, the correlation analysis showed significant associations between TPC, TSC, and antioxidant responses (DRSC, ARSC, and FRAP). A strong positive correlation was observed between TPC and DRSC ($r = 0.69$), indicating that phenolic compounds were primarily responsible for free radical scavenging capacity. Similarly, TPC showed a moderate correlation with ARSC ($r = 0.52$) and FRAP ($r = 0.44$), confirming the involvement of phenols in hydrogen transfer (HAT) and single electron transfer (SET) mechanisms.[47] In contrast, TSC showed a stronger correlation with FRAP ($r = 0.56$) and a moderate correlation with ARSC ($r = 0.46$), suggesting that saponins also contribute to the overall antioxidant potential, especially through electron donation reactions. These results show that the antioxidant activity of CPH extracts obtained by MAE is due to the combined and complementary effect of phenolic compounds and saponins, whose recovery depends largely on microwave power and the liquid/solid ratio used.

3.3 Validation of the models and regression equations

Global multi-response optimisation was carried out considering six responses: bioactive compounds (TPC and TSC) and antioxidant activity (DRSC, ARSC and FRAP). The composite desirability reached 0.840 and an algorithm was applied to identify the combination of factors that maximized the overall response, found to be 855 W, 3.06 min and 50 mL g^{-1}.

To verify the predictive accuracy of the MAE model, a confirmation test was conducted in which the predicted values were compared with the newly measured data. Table 4 reports the predicted mean, the experimentally obtained mean, and the 95% prediction intervals for each response. In every case the experimental values lie inside the corresponding interval, demonstrating that the model reliably forecasts future observations within the specified limits. All models exhibited high accuracy, with relative errors below 5% for most responses (TPC: 1.67%; TSC: 1.99%; ARSC: 2.91%; FRAP: 1.30%), except for DRSC (4.54%), which remained within acceptable limits for complex biological matrices (Table 4).

Table 5 summarises the final second-order models, expressed in real (uncoded) units, that describe the influence of microwave power (A), extraction time (B) and liquid/solid ratio (C) on each response CPH extract during MAE.

For TPC, microwave power and liquid/solid ratio significantly enhanced recovery, although curvilinear trends suggested potential thermal degradation or saturation effects at elevated levels. Notably, TPC showed a strong negative interaction between power and time, underlining the importance of controlled energy input. In contrast, TSC displayed a complex response profile, with all primary factors, interactions (AC and BC), and quadratic effects contributing significantly. This underscores the intricacy of saponin release mechanisms and suggests a narrower operating window for optimal yield. Antioxidant assays presented divergent behaviours. DRSC and ARSC confirmed microwave power as the primary enhancer of radical scavenging capacity, while ARSC exhibited nonlinear effects of time, possibly due to compound instability. FRAP, conversely, was most responsive to extraction time, with significant interaction and curvature effects, indicating a unique sensitivity among assays to thermal and solvent dynamics.

Overall, the findings indicate that selective optimisation is crucial: while high power favours polyphenols and radical scavenging activities, prolonged exposure may compromise compound integrity. The integration of curvilinear and interaction terms in the models enhances predictive reliability and

supports tailored parameter adjustment to maximise functional compound recovery.

3.4 Characterisation of the phytochemical profile in CPH extract obtained by MAE using UHPLC-Q-Orbitrap-MS/MS

Chromatography by UHPLC-Q-Orbitrap-MS/MS in negative ionisation mode allowed the identification of phytochemical compounds from the CPH extract, obtained by MAD and 50% hydroalcoholic extraction based on optimised MAE parameters (855 W, 3.06 min and 50 mL g^{-1}). Several compounds were detected and tentatively identified due to the lack of proper standards. These include organic acids, such as tartaric acid ($C_4H_6O_6$), $[M-H]^-$ m/z 149.0084, citric acid ($C_6H_8O_7$), $[M-H]^-$ m/z 191.0193, gluconic acid ($C_6H_{12}O_7$), $[M-H]^-$ m/z 195.0506 and malic acid ($C_4H_6O_5$), $[M-H]^-$ m/z 133.0136. Malic and citric acids have previously been reported in CPH, coexisting with other weak acids, which underscores its potential as a source of food-grade pectin.[48]

In agreement with our findings, Ramos-Escudero *et al.*[49] identified malic, tartaric, citric and gluconic acids with molecular ions $[M-H]^-$ m/z 133.0163, 149.0108, 191.0214, and 195.053, respectively, using HPLC-ESI-qTOF-MS in hydroalcoholic CPH extracts. Similarly, Abdul Karim *et al.*[50] reported the same acids with molecular ions $[M-H]^-$ m/z 133, 149, 191 and 195 by LC-MS/MS in the hydroalcoholic CPH extracts, while Vargas-Arana *et al.*[11] detected tartaric and gluconic acids $[M-H]^-$ m/z 149.0086 and 195.0507, respectively, in methanolic CPH extracts analysed by UHPLC-MS.

Additionally, the phenolic compound clovamide ($C_{18}H_{17}NO_7$) with a molecular ion $[M-H]^-$ m/z 358.093 was detected and provisionally identified. This compound has also been reported by Gomez *et al.*[51] with $[M-H]^-$ m/z 358.2 using HPLC-DAD-ESI-IT-MS/MS in hydroalcoholic CPH extracts.

In summary, UHPLC-Q-Orbitrap-MS/MS analyses confirmed the presence of key organic acids (malic, tartaric, citric, and gluconic) and phenolic compounds such as clovamide in CPH extracts by MAE, in line with previous reports. These results reinforce the potential of CPH as a valuable source of bioactive compounds for applications in the food and nutraceutical industries.

4 Conclusion

Overall, the globally optimised multi-response MAE protocol (855 W, 3.06 min, and 50 mL g^{-1}), with a desirability index of 0.84, demonstrated a rapid, energy-efficient, and scalable approach for recovering a chemically diverse range of bioactive compounds from CPH. The method yielded 2.724 mg$_{GAE}$ g^{-1} TPC, 0.241 mg$_{EE}$ g^{-1} TSC, and antioxidant capacities of 81.966, 99.680, and 90.890 μmol$_{TE}$ g^{-1} for DRSC, ARSC, and FRAP assays, respectively, surpassing conventional extractions in both efficiency and antioxidant recovery.

Microwave power was identified as the key driver of extraction efficiency, particularly for phenolic and antioxidant indices, whereas the liquid/solid ratio and extraction time influenced compound recovery by enhancing diffusion before

thermal degradation occurred. Importantly, the use of a modified domestic microwave provides an accessible and sustainable alternative to laboratory-scale MAE systems, supporting broader technological adoption in resource-limited contexts.

Beyond its quantitative performance, UHPLC-Q-Orbitrap-MS/MS profiling revealed a complex metabolomic signature, including malic, tartaric, citric, and gluconic acids, as well as clovamide, further substantiating the chemical richness of CPH. Collectively, these findings highlight the methodological novelty and practical feasibility of the optimised MAE protocol, positioning CPH as a valuable clean-label source of bioactives for functional foods and nutraceuticals, while contributing to the circular economy and sustainable revalorisation of agro-industrial by-products.

Author contributions

Esteban Jurado-Beizaga (E. J. B.) and Erick Alvarez-Yanamango (E. A. Y.) conceived and designed the experimentation; E. J. B. performed the experiments and wrote the original manuscript; Oscar Herrera-Calderon (O. H. C.), Alfredo Ibañez (A. I.), and E. A. Y. validated and reviewed the final manuscript.

Conflicts of interest

The authors declare no conflicts.

Data availability

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