Enhancing bio-recovery of bioactive compounds extracted from *Citrus medica* L. Var. *sarcodactylis*: optimization performance of integrated of pulsed-ultrasonic/microwave technique
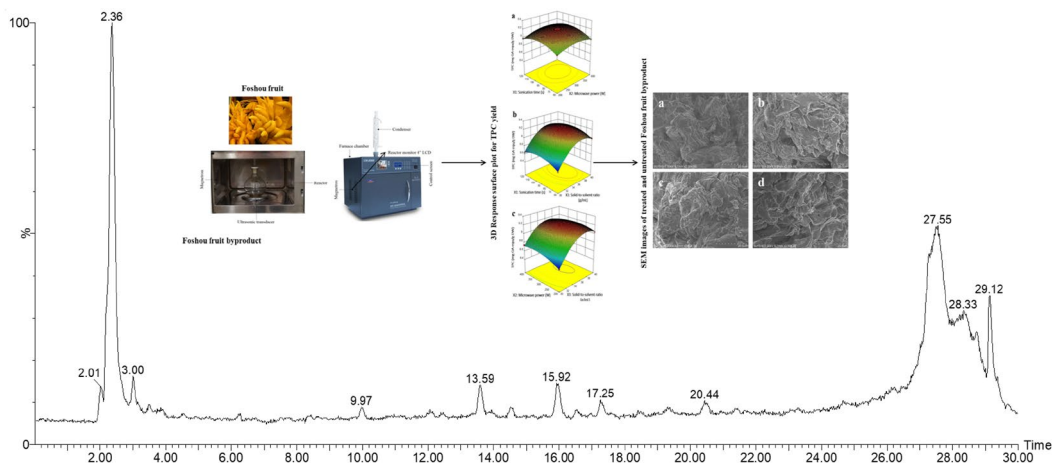
Amer Ali Mahdi[1,2,3] · Marwan M. A. Rashed[1,4] · Waleed Al-Ansi[1,3] · Mohamed Ismael Ahmed[1,5] · Mohammed Obadi[1] · Qi Jiang[1,2] · Husnain Raza[1] · Hongxin Wang[1,2]

Abstract

This study mainly aimed to optimize a sustainable and green process for extracting bioactive compounds from Foshou fruit by using an integrated technique based on ultrasonic-microwave assisted extraction (UMAE). Response surface methodology (RSM) based on a Box–Behnken design was applied to determine optimal conditions. The following optimized UMAE processing parameters were obtained: sonication time (96.13 s), microwave power (305.28 W), and solid/solvent ratio (1:37). Based on a total phenolic compound extraction yield of 9.21 mg gallic acid (GA) equ/g dry weight (DW), a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity with a half maximal inhibitory concentration (IC50) of 27.52 μg GA equ, and an antioxidant capacity detected by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay of 8.79 mg trolox equ/g DW. The optimized UMAE extract was superior to those obtained using microwave-assisted extraction (MAE) or conventional solvent extraction (CSE) methods. Scanning electron microscopy (SEM) analysis showed that the three extraction methods affected the sample tissue microstructure. Among them, UMAE caused the most marked structural disruption. UPLC-PDA-Q-TOF-MS analysis identified 67 phenolic compounds in the optimized UMAE extract of the Foshou fruit extract. This study indicated that the integrated UMAE technique is a suitable and safe technique to enhance the qualitative and quantitative extraction of phenolic compounds from Foshou fruit. These phenolic compounds can be used as a functional food ingredient in industrial production.

Graphical abstract

Extended author information available on the last page of the article

Introduction

Citron (*Citrus medica* L.) is one of the three ancestral *Citrus* species, together with mandarin orange (*C. reticulata* Blanco) and pummelo (*C. maxima* (Burm.) Merr.) [1]. Foshou fruit (*C. medica* L. var. *sarcodactylis* Swingle) is classified in Rutaceae family and broadly cultivated in Asian countries. It is usually consumed as a functional food and raw material for some traditional Chinese medicines used to treat chronic diseases because of its content of bioactive compounds, especially phenolic compounds [2].

Phenolic compounds are bioactive, aromatic, and secondary plant metabolites that are widespread in the plant kingdom, have great potential for applications that reduce the risks of several chronic diseases [3, 4], and are closely associated with the effective management of chronic diseases [5]. These compounds have become important because of their associated health benefits, including protection against oxidative stress damage and lifestyle diseases [6]. Luna-Guevara et al. [7] emphasized that phenolic compounds elicit effects against several lifestyle diseases, such as obesity, types of cancers, diabetes mellitus, hypertension, and atherosclerosis. The use of phenolic compounds as bioactive substances has prompted researchers to optimize the efficacy of eco-friendly extraction procedures for green extraction strategies to save time, energy, and total production costs [8]. Green extraction involves innovative extraction technologies with advantages of using safe extraction techniques and clean solvents that do not include toxic chemicals, thereby avoiding the use of organic solvents [6–10].

Phenolic compound extraction via green extraction methods requires the optimization of various extraction parameters, such as raw material amount, and volume of solvent needed [6]. Innovative efforts devoted to improving extraction methods and ensuring compliance with optimization strategies include new extraction processes and alternatives to classical solvents. Alternative extraction processes should be developed on the basis of the modification of current technologies and their protocols [11, 12]. In contrast to classical extraction techniques, ultrasonic-assisted extraction (UAE) has emerged as a promising green extraction technology that fulfills various criteria, including low cost, eco-friendliness, safety, speed, and ease of use [13]. As an important improvement of UAE, ultrasonic/microwave-assisted extraction (UMAE) is an integration of UAE with microwave-assisted extraction (MAE) via simultaneous irradiation [14].

Green extraction should be associated with the use of safe solvents, considering that the exacerbation of environmental pollution caused by the excessive use of volatile and dangerous organic solvents has motivated researchers to develop alternative green solvents. Green solvents are mainly utilized to reduce the use of toxic solvents with their associated negative environmental impacts [8–16]. In this sense, water is considered to be a safe, environmentally sustainable, and green solvent [17].

Green extraction operations should be linked to effective statistical methods to determine the efficiency of extraction techniques, such as response surface methodology (RSM), which includes several arithmetical and statistical methods that can be used to identify the correlations between independent variables and responses. As an experimental methodology, RSM is used to define the effects of independent variables by analyzing the influences of independent variables on responses and to generate a mathematical model [18, 19]. RSM has been used to optimize extraction procedures for various natural products. Moreover, the resultant models generated by RSM correspond well with actual experimental outcomes [20].

The quality of extracts should be determined by special techniques, such as liquid chromatography coupled with mass spectrometry (LC-MS); LC-MS plays an important role in the determination, characterization, and identification of phenolic compounds in food and the classification and authentication of natural extracts [21]. As well the time-of-flight (TOF) is one of high-resolution mass spectrometry (HRMS) instrumentation and is considered the most generally used analyzer for the LC analysis of phenolic compounds in food matrices [22].

This study focused on finding the optimal conditions for the extraction of total phenolic compounds (TPCs) from Foshou fruit through UMAE as one of the most promising extraction technologies to obtain a safe extract applicable to the food industry. Consequently, the goals of this study were to:

- Investigate the effects of various process parameters on the efficiency of TPC extraction from Foshou fruit using the UMAE process in preliminary single-factor experiments;
- Optimize the UMAE conditions using RSM;
- Compare optimized UMAE extract with those obtained by MAE and CSE in terms of TPC yield, antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging activity (DPPH•-SA) assay, and antioxidant capacity determined by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation scavenging effect (ABTS•+-SE) assay;
- Compare the effects of the three extraction technologies on the Foshou microstructure;
- Identification the phenolic compounds in the optimized UMAE extract of Foshou fruit using the UPLC-PDA-Q-TOF-MS.

Materials and methods

Chemicals

Gallic acid (CAS:149-91-7) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. China. Folin phenol reagent (Catalog Number: 73104861), Sodium Carbonate (CAS:497-19-8), Potassium persulfate (CAS:7727-21-1), Methanol (CAS: 67-56-1) and Ethanol (CAS:64-17-5) were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCR), China. DPPH (CAS:1898-66-4) and Trolox (CAS:53188-07-1) were purchased from Sigma–Aldrich (USA). ABTS (CAS:30931-67-0) was purchased from TCI Europe N.V. (Belgium). All other chemical substances and solvents used were of analytical grade and stored in ideal conditions according to the manufacturer's specifications.

Fruit preparation

Foshou fruit (*C. medica* L. *var. sarcodactylis*) was provided by Zhejiang Golden Hand Biological Technology Co., Ltd. in Jinhua, Zhejiang Province, China in November 2016. The whole Foshou fruits (peels and pulps) were washed with distilled water and cut into small slices with a thickness about 1 mm. The slices were dried to constant weight at 55 °C in an air oven and then milled using an electric grinder to obtain a fine powder. The fine powder was passed through a 40-mesh sieve to obtain Foshou fruit powder, which was packaged in polyethylene bags under vacuum and stored at 4 °C until analysis.

Ultrasonic/microwave equipment

The integrated techno-extraction procedures were performed using a CW-2000 Ultrasonic/microwave Cooperative Extractor/Reactor (Shanxi, Xi'an, China) with an adjustable microwave power range of 10–800 W, a microwave frequency of 2,450 MHz, the ultrasonic power level at a constant 50 W, and an ultrasonic frequency of 40 kHz. The ultrasonic transducer was directly linked to a reactor flask with a volume of 250 mL. The reactor flask was linked to a reflux condenser. Extractions were performed under a constant ultrasonic power/frequency ratio of 50 W/40 kHz.

TPC extraction procedures

Ultrasonic/microwave-assisted extraction (UMAE)

To obtain the ultrasonic/microwave-assisted Foshou fruit extract (UMAE), 2 g of Foshou fruit powder was extracted with distilled water at solid/solvent ratios ranging from 1:20 to 1:45 (g/mL), microwave power ranged from 200 to 700 W, and sonication time from 30 to 180 s in a single-factor experiment. In the RSM experiment, 2 g of Foshou fruit powder was extracted with distilled water (solid/solvent ratios of 1:30, 1:35, and 1:40; g/mL) at microwave power levels of 200, 300 and 400 W, and sonication times of 60, 90 and 180 s. The extraction temperature was controlled using a discontinuous model that did not exceed 70 °C during the sonication time, while the ultrasonic-microwave extractor was operated discontinuously, operated in running-cooling-running stages. The running time did not exceed 60 s and the extract was cooled for 120 s between the two running stages. After the extraction process, the Foshou fruit extract was allowed to cool to room temperature. The obtained volume of extract was adjusted to 100 mL in a standard flask and centrifuged at 4,000 rpm for 20 min. The supernatant solution was filtered under vacuum through the No.1 Whatman filter paper in a Buchner funnel and stored at 4 °C until further use.

Microwave-assisted extraction (MAE)

To obtain microwave-assisted Foshou fruit extract (MAE), the CW-2000 Ultrasonic/microwave Cooperative Extractor/Reactor was used in the item of microwave mode (ultrasonic mode turned off). The extraction parameters were used according to Dahmoune et al. [23] with slight modification. Briefly, 2 g of Foshou fruit powder was extracted by 60 mL distilled water (solid/solvent ratio 1:30, g/mL) with a microwave power setting at 400 W and an extraction time of 120 s. The subsequent steps, including filtration and centrifugation, were performed as described above in UMAE.

Conventional solvent extraction (CSE)

With a slight modification, the conventional solvent extract (CSE) was prepared according to Spigno et al. [24]. In brief, 2 g of Foshou fruit powder was extracted with 100 mL of distilled water (solid/solvent ratio 1:50, g/mL). The mixture was incubated in a shaking water bath for 2 h at 60 °C with shaking speed at 110 rpm. The subsequent steps including filtration and centrifugation were performed as described in UMAE.

Experimental design

Single-factor experiments

The effects of various extraction parameters (sonication time, microwave power, and solid/solvent ratio) were studied using a single-factor experimental design to make a

preliminary determination of the extraction parameter values to be used in the RSM experiment. The other parameters were maintained at constant values as shown in Table 1.

Orthogonal experiment design

To optimize the effects of sonication time (X1), microwave power (X2), and solid/solvent ratio (X3), RSM was coupled with a quadratic model based on the Box–Behnken design (BBD) using Design Expert 10.0.0 (Stat-Ease, Inc., USA) software to determine the optimal parameter values required for maximum TPC yield. The BBD output was 17 runs, including 5 replicates as a center point. The data were subjected to regression analysis to ensure that the obtained responses between the independent variables (X1, X2, and X3) were well fitted with the general second-order polynomial equation (quadratic model) in Eq. (1):

$$\text{TPC yield} = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \leqslant i \leqslant j}^{k} \beta_{ij} x_i x_j \qquad (1)$$

where β_0 is the regression coefficient for the intercept (a constant value); k is the number of variables (3); β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and interactive terms, respectively; and x_i, x_{ii}, and x_{ij} represent the coded independent variables. The actual values of factor levels (z_i) were converted to coded values (x_i) in a range without dimensions (-1 to $+1$) using the following formula:

$$x_i = (z_i - z_i^0 / \Delta_{zi})\beta_d \qquad (2)$$

where Δ_{zi} indicates the difference between the actual value in the central point (z_i^0) and the actual value of the lowest or highest level of a variable, and β_d is the main coded limit value in the model for each variable [25].

Analysis of variance (ANOVA) was used to evaluate the adequacy of the model by determining the lack of fit, F test value, p value $Prob > F$, and the coefficient of determination (R^2). Based on the fitted polynomial equation, 3D surface plots were generated using regression coefficients to visualize the relationship between the values of each factor and the response, and to predict the optimal conditions. To validate the model, supplementary extraction trials were performed under the optimal conditions predicted by RSM and the results were compared with the predicted values generated by the regression model.

Analytical methods

Determination of TPC

The TPC contents of Foshou fruit extracts were determined using the Folin–Ciocalteu assay according to Chen et al. [26] with some modification. Briefly, 200 µL of Foshou fruit

Table 1 Results of single-factor experiments for Ultrasonic/Microwave-assisted extraction (UMAE) from Foshou fruit

Experiment	No.	Sonication time (s)	Microwave power (W)	Solid/Solvent ratio (g:ml)	TPC yield mg GA equ/g DW
Effect of sonication time	1	30	500	1:30	8.21 ± 0.20^{ab}
	2	60	500	1:30	8.55 ± 0.29^{ab}
	3	90	500	1:30	8.67 ± 0.27^{b}
	4	120	500	1:30	8.43 ± 0.20^{ab}
	5	150	500	1:30	8.38 ± 0.38^{ab}
	6	180	500	1:30	8.13 ± 0.04^{a}
Effect of microwave power	1	90	200	1:30	8.59 ± 0.10^{a}
	2	90	300	1:30	9.10 ± 0.15^{b}
	3	90	400	1:30	8.76 ± 0.29^{ab}
	4	90	500	1:30	8.68 ± 0.23^{a}
	5	90	600	1:30	8.61 ± 0.23^{a}
	6	90	700	1:30	8.48 ± 0.13^{a}
Effect of solid/solvent ratio	1	90	500	1:20	8.19 ± 0.14^{a}
	2	90	500	1:25	8.72 ± 0.17^{b}
	3	90	500	1:30	8.99 ± 0.11^{c}
	4	90	500	1:35	9.18 ± 0.06^{d}
	5	90	500	1:40	8.95 ± 0.17^{c}
	6	90	500	1:45	8.71 ± 0.06^{b}

Values represent the mean $\pm$ SD (n = 3)

s seconds, *W* watts, *g:ml* gram per milliliters, *TPC* total phenolic compounds, *GA equ/g DW* gallic acid equivalents/gram dry weight

extract was added to 5 mL (0.2 N) of Folin phenol reagent. After 5 min, 4 mL of 7.5% sodium carbonate solution was added to the solution. The optical density of the mixture was measured at 765 nm using a UNICO UV-2100 spectrophotometer (UNICO SCIENTIFIC, HongKong) after incubation at room temperature in the dark for 105 min. The results were expressed as mg of gallic acid equivalents per gram dry weight (mg GA equ/g DW) according to the linear equation for gallic acid $y = 0.0093x + 0.0214$ at an R^2 value of 0.99.

Determination of antioxidants activity

Determination of antioxidant activity by DPPH·-SA assay

The scavenging activity of Foshou fruit extract against DPPH• was determined according Rashed et al. [27] with a slight modification according DPPH• methanolic solution (3.5 mL, 60 μM) was added to 300 μL of Foshou fruit extract. After shaking, the reaction was carried out in the dark at room temperature. Absorbance was recorded at 517 nm after 40 min of incubation and converted to %DPPH•SA using the following equation:

$$\% \text{ DPPH}^{\bullet}\text{-SA } = [(A_0 - A_1)/A_0] \times 100 \qquad (3)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Trolox solution (0.375 μg/mL) was used as a standard for comparing the results. The IC_{50} was expressed as μg GA equ.

Determination of antioxidant capacity by ABTS·+-SE assay

To evaluate the antioxidant capacity of Foshou fruit, ABTS•+SE assay according to the method of Rashed et al. [28] was used with slight modification. The ABTS stock solution was prepared by dissolving 38.41 mg of the ABTS in 10 mL of deionized water (7 mM). Potassium persulfate solution (2.45 mM) was prepared by dissolving 6.62 mg of potassium persulfate in 10 mL of deionized water. Then, 0.5 mL of the ABTS stock solution was mixed with 0.5 mL of potassium persulfate solution (1:1, v/v) to prepare the ABTS•+ solution. The ABTS•+ solution was kept for 12 h at room temperature in the dark to obtain a stable blue-green cation radical solution. The stable ABTS•+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm before use. Then, 50 μL of Foshou fruit extract was added to 3.5 mL of the ABTS•+ solution and the mixture was allowed to react at room temperature in the dark. The absorbance at 734 nm was measured relative to a blank (distilled water instead of the extract) after reaction for 10 min. Trolox was used as a standard to plot a Trolox calibration curve at concentrations of 2.25, 4.50, 6.75, 9.00, 11.25, 13.5, and 16.5 μg/ml Trolox. Results were expressed as mg Trolox equivalents per g dry weight (mg Trolox equ/g

DW) according to Zhang et al. [29] using the linear equation $y = -0.033x + 0.6531$ and an R^2 value of 0.99.

UPLC-PDA-Q-TOF-MS analysis

The UPLC-PDA-Q-TOF-MS analysis were done according to the method described by He et al. [30], with some modifications. The analysis were carried out on a WATERS ACQUITY UPLC device, a WATERS ACQUITY PDA detector (set at 200 and 600 nm.), and a WATERS UPLC BEH C18 column (1.7 μm, 2.1 mm, I.D., 100 mm) at 45 °C (temperature of column oven) (Waters Corp., Milford, USA). The injection volume of the sample was 1 μl. The mobile phase comprised of acetonitrile (A) and water with 0.1% formic acid (B) with a flow rate of 0.3 ml/min. Mass spectrometry executed on a WATERS MALDI SYNAPT Q-TOF MS device with electrospray ionization mass spectrometry (ESI-MS) (Waters Corp., Milford, USA).

The ESI-MS spectra were obtained in negative ion mode in the mass range of 20 to 1500 m/z. The voltage of capillary was 3.0 kV, while 20 V was the voltage of sample cone and collision energy was 6.0 eV. The desolvation gas temperature was 400 °C with 700 L/h flow, and the temperature of source block was 100 °C and the flow of cone gas was 50 L/h. All processes controlled and data analysis by MarkerLynx software (Waters, Milford, MA, USA). The widely accepted accuracy for confirmation of compositions was set at less than 10 ppm. The phenolic compounds were identified by comparing the molecular weight according to MS data which extracted by MarkerLynx software with the databases of the European Bioinformatics Institute and ChemSpider [31, 32].

Scanning electron microscopy (SEM) observation

Foshou fruit powder specimens (extracted and unextracted samples) were visually observed using a scanning electron microscope (SU 1510, Hitachi Corp., Tokyo, Japan) to investigate the effects of the different extraction methods on the Foshou fruit surface microstructure. After extraction, powder residues were dried in an oven at 50 °C until a constant weight was obtained before SEM analysis. The SEM observation were done at an accelerating beam voltage of 5.00 kV under high vacuum conditions, and a working distance of 9.5–9.8 mm.

Statistical analysis

Each extraction trial and all analyses were conducted in triplicate and all the data in this paper are reported as the mean ± SD. Microsoft Office Excel 2016 software was used to generate the linear equations and the IC_{50} values for the DPPH•SA assay. IBM SPSS Statistics software (Version 20.0. Armonk, NY: IBM Corp.) was used to determine the

significance of differences between means at the 95% confidence level ($P \leq 0.05$) using one-way analysis and Duncan's test.

Results and discussions

Single-factor experiments

The results of single factor experiments are summarized in Table 1. The TPC yield from Foshou fruit extract was the highest (8.67 mg GA equ/g DW) at a sonication time of 90 s, constant microwave power of 500 W, and a solid/solvent ratio of 1:30 (g/mL). With the sonication time increased to 180 s, the yield decreased to 8.13 mg GA equ/g DW due to thermal degradation of the phenolic compounds. Based on these outcomes, we chose a sonication time of 90 s for the subsequent single-factor experiments, while sonication times of 60, 90, and 120 s were used for RSM experiments. The microwave power level had a significant effect on the TPC yield. With an increasing in microwave power from 200 W to 300 W, the TPC yield increased from 8.59 mg GA equ/g DW to 9.10 mg GA equ/g DW (the highest TPC yield), respectively. With higher microwave power levels of 400, 500, 600, and 700 W, the TPC yields decreased slightly to 8.76, 8.68, 8.61, and 8.48 mg GA equ/g DW, respectively. Statistically, there were no significant differences between the TPC yields at microwave power levels of 200, 500, 600, and 700 W, probably due to the degradation of phenolic compounds when using high levels of microwave power during the extraction process [33]. We chose a microwave power level of 300 W for use in subsequent single-factor experiments, while microwave power levels of 200, 300, and 400 W were used in RSM experiments. With increasingly wide solid/solvent ratios of 1:20, 1:25, 1:30, and 1:35, the TPC yields increased (8.19 8.72, 8.99, and 9.18 mg GA equ/g DW, respectively). A further widening of the solid/solvent ratio caused a decrease in the TPC yield. The highest TPC yield of 9.18 mg GA equ/g DW was obtained with a solid/solvent ratio of 1:35. Based on these results, we chose solid/solvent ratios of 1:30, 1:35, and 1:40 for the RSM experiments.

Modeling of UMAE

Optimization and prediction of extraction parameter values using RSM

To enhance the TPC yield, we used RSM based BBD to determine the optimal combination of the independent variables. The parameters included sonication time (X1), microwave power (X2), and solid/solvent ratio (X3) at a fixed sonication power/frequency ratio of 50 W/40 kHz. The coded and uncoded levels of the independent variables shown in Table 2. As well actual and predicted values of the dependent variables (response) are shown in Table 3. The actual values of TPC yield ranged from 8.56 to 9.20 mg GA equ/g DW, while the predicted values ranged from 8.57 to 9.18 mg GA equ/g DW. Based on multiple regression analysis, a final second-order polynomial equation in terms of coded factors with disregard the non-significant terms ($P > 0.01$) was created to obtain the maximum TPC yield from Foshou fruit (Eq. 4).

$$TPC = 9.18 + 0.039A + 0.036B + 0.20C + 0.017AB$$
$$+ 0.000AC - 0.040BC - 0.12A^2 - 0.083B^2 \qquad (4)$$
$$- 0.25C^2$$

The optimal predicted extraction conditions to achieve the highest TPC yield (9.21 mg GA equ/g DW) were as follows: X1 = 96.1285 s, X2 = 305.278 W, and X3 = 1:37.The regression coefficients of the mathematical model describing the TPC yield as a function of A, B, and C are shown in Table 4. F test and p values were used to analyze the significance of each coefficient. Bezerra et al. [25] confirmed that a model will be well fitted to the experimental data if it shows a significant regression and a non-significant lack of fit. In this study, the model F value (163.44) was significant, and the p-value $Prob > F$ was < 0.0001 (< 0.05). Furthermore, the lack of fit F-value of < 0.001 was not significant, and the p-value $Prob > F$ was 0.51 (> 0.05). The value of R^2 was (0.9953), indicating that the model satisfactorily described the real relationships between the chosen variables. The Adjusted R-squared (R^2_{Adj}) value in the model (0.9892) was close to R^2. Moreover, the Predicted R-squared (R^2_{pred}) value (0.9648) was in reasonable agreement with R^2_{adj}. This confirms that the model was highly significant, where the difference was < 0.2. The regression coefficient from the experimental data and the adjusted data were reasonably close to 1, which indicated a high degree of correlation between the observed and predicted values.

The 3-D response surface plots of the interactions between the factors X1, X2, and X3 are shown in Fig. 1. All maximum points as critical points were found within the experimental region, indicating that they represented the optimal values. The overlap between X1 and X2 is shown in Fig. 1a. These two factors had a positive effect on the TPC yield with the same degree of proximity. X3 produced a curve that was broader than the X1 and X2 curves, indicating that it had the strongest effect.

Validation of the predictive model

We obtained the optimal values of the selected independent factors by solving the regression equation and analyzing the contour and response surface plots. To evaluate the

validity of the model in Eq. (4), the extraction of TPC from of Foshou fruit was performed in triplicate under the optimal conditions, which included; X1 (96 s), X2 (305 W), and X3 (1:37). There was no significant difference between the experimental (9.21 mg GA equ/g DW) and predicted (9.22 mg GA equ/g DW) mean TPC yield values; hence, this model can be used to optimize TPC extraction from Foshou fruit.

Investigation of the UMAE, MAE, and CSE effects on the yield of TPC

Results of TPC that obtained with the optimized UMAE conditions were compared with those obtained using the MAE and CSE techniques. The highest TPC yield of 9.21 mg GA equ/g DW was obtained using UMAE, followed by the yields from MAE (9.09 mg GA equ/g DW) and CSE (8.72 mg GA equ/g DW), with significant differences between both UMAE and MAE, UMAE and CSE. Overall,

the TPC yields obtained using water as a solvent were higher than those obtained by Liu et al. [34] and Jayaprakasha, Patil [35] who obtained TPC yields of 5.86 mg GA equ/g DW using 60% ethanol and 7.18 mg GA equ/g DW using 80% methanol, respectively.

Antioxidants activity

Antioxidant activity determination by DPPH•-SA assay

The IC_{50} values calculated based DPPH$^\bullet$-SA assay differed significantly between the UMAE, MAE, and CSE. UMAE reached the lower IC_{50} (27.52 μg GA equ) compared to the MAE (30.36 μg GA equ) and CSE (31.17 μg GA equ). Castillo et al. [36] studied the antioxidant activity of the *C. medica* and found that the IC_{50} (DPPH) was approximately 29.45 μg GA equ, which represented a lower antioxidant activity than was obtained with UMAE in this study, but higher than the activities we obtained with MAE and CSE.

Table 2 Experimental Factor levels (coded and uncoded)

Factors	Coded			Uncoded		
	− 1	0	+ 1			
(X1) Sonication time (s)	− 1	0	+ 1	60	90	120
(X2) Microwave power (W)	− 1	0	+ 1	200	300	400
(X3) Solid/Solvent ratio (g:ml)	− 1	0	+ 1	1:30	1:35	1:40

s seconds, *W* watts, *g:ml* gram per milliliters

Table 3 Box–Behnken design matrix, with the actual and predicted values of TPC yield from Foshou fruit

	Factor 1	Factor 2	Factor 3	TPC (mg GA equ/g DW)	
Run	Sonication time (s)	Microwave power (W)	Solid/solvent ratio (g:ml)	Actual	Predicted
1	60	200	1:35	8.93 ± 0.22	8.92
2	90	400	1:40	9.05 ± 0.23	9.05
3	60	300	1:40	8.99 ± 0.31	8.98
4	90	200	1:40	9.04 ± 0.36	9.06
5	90	400	1:30	8.74 ± 0.23	8.72
6	120	300	1:40	9.07 ± 0.20	9.06
7*	90	300	1:35	9.20 ± 0.18	9.18
8*	90	300	1:35	9.16 ± 0.19	9.18
9	60	300	1:30	8.56 ± 0.25	8.57
10	90	200	1:30	8.57 ± 0.19	8.57
11*	90	300	1:35	9.15 ± 0.38	9.18
12	120	400	1:35	9.06 ± 0.13	9.07
13	120	200	1:35	8.97 ± 0.14	8.96
14	60	400	1:35	8.95 ± 0.32	8.96
15*	90	300	1:35	9.18 ± 0.26	9.18
16*	90	300	1:35	9.20 ± 0.31	9.18
17	120	300	1:30	8.64 ± 0.19	8.65

s seconds, *W* watts, *g:ml* gram/milliliters, *TPC* total phenolic compounds, *GA equ/g DW* gallic acid equivalents/gram dry weight

*Central point; values represent the mean ± SD (n = 3)

Table 4 Analysis of variance (ANOVA) for the experimental results obtained by using ultrasonic/microwave-assisted extraction (UMAE)

Source	Sum of squares	df	Mean square	F value	p value Prob. > F	
Model	0.74	9	0.082	163.44	< 0.0001	Significant
A—*Sonication time*	0.012	1	0.012	23.99	0.0018	
B—*Microwave power*	0.011	1	0.011	21.00	0.0025	
C—*Solid/Solvent ratio*	0.34	1	0.34	671.44	< 0.0001	
AB	1.225E−003	1	1.225E−003	2.45	0.1618	
AC	0.000	1	0.000	0.000	1.0000	
BC	6.400E−003	1	6.400E−003	12.78	0.0090	
A^2	0.058	1	0.058	116.59	< 0.0001	
B^2	0.029	1	0.029	57.58	0.0001	
C^2	0.25	1	0.25	505.78	< 0.0001	
Residual	3.505E−003	7	5.007E−004			
Lack of fit	1.425E−003	3	4.750E−004	0.91	0.5100	Not significant
Pure error	2.080E−003	4	5.200E−004			
Cor total	0.74	16				
Mean 8.97	Std. Dev. 0.022	C.V. % 0.25	R^2 0.9953	R^2_{Adj} 0.9892		R^2_{Pred} 0.9648

Antioxidant capacity determination by ABTS$^{•+}$-SE assay

The antioxidant capacity values of the UMAE, MAE, and CSE extracts determined in term of ABTS$^{•+}$-SE assay were significantly different. The UMAE achieved the highest activity (8.79 mg Trolox equ/g DW), followed by MAE (8.39 mg Trolox equ/g DW), and CSE (8.17 mg Trolox equ/g DW). Sun et al. [37] determined the antioxidant capacities of the main citrus species and the results ranged from 104.64 to 269.40 mg/g Trolox equivalent. In another investigation, Menichini et al. [38] reported that the IC$_{50}$ of *C. medica* L. cv *Diamante* (Rutaceae) peel extract in terms of ABTS$^{•+}$-SE (%)was 3.48 mg/mL.

Double simultaneous irradiation (ultrasound/microwave) causes synergic effects on the extraction of bioactive compounds from plant matrices. Ultrasound dramatically increases the extraction efficiency through the cavity phenomenon that induces the release of soluble compounds from plant matrices by crashing cell walls.

At the same time, microwave quickly heats whole plant matrices, thereby causing the transmigration of dissolved molecules. Furthermore, the simultaneous irradiation enhances solvent permeation into the matrix and increases the solubility of compounds. Additionally, the power levels required by UMAE are lower than those of two single energy sources alone [11–14].

Our results showed that UMAE was considered the best technique. It allowed increasing the TPC yields by 5.62% and 1.32% compared with those of CSE and MAE, respectively. UMAE also improved the antioxidant activities by 11.71% and 2.60% compared with those of CSE and MAE, respectively. By contrast, UMAE increased the antioxidant capacities by 7.56% and 4.77% compared with those of CSE

and MAE, respectively. UMAE shortened the extraction time by 98.66% and 19.89% compared with those of CSE and MAE, respectively.

UPLC-PDA-Q-TOF-MS analysis

According to the analysis of the optimized UMAE extract of Foshou fruit using the technique of chromatography, 67 phenolic compounds appeared in the extract (Table 5). The base peak intensity (BPI) chromatogram of optimized UMAE extract of Foshou fruit (negative ion mode) shown in Fig. 2.

Interestingly, the optimized UMAE extract of Foshou fruit contains many compounds that have important properties such as anti-cancer, anti-inflammatory, anti-diabetic, and antimicrobial, as an example 4′-hydroxychalcone is one of Chalcones, and has a potential effect on the prevention and treatment of the inflammatory and cancer [39]. The norathyriol found in Mango fruit and has an anti-cancer activity, where decreased the viability of MCF-7 breast cancer cells [40]. Tian et al. [41] confirmed that the bellidifolin compound significantly decreased in the fasting blood glucose sample, in addition to improving the oral glucose tolerance and decresing of the fasting serum insulin. In the study conducted by Qiu, Chen [42], ophioglonin showed slight anti-hepatitis B virus surface antigen activity at 25 µM. 2,2′,4′-trihydroxy-6′-methoxy-3′,5′-dimethylchalcone possessed an antiprotozoal activity against *Leishmania donovani* at IC$_{50}$ 7.5 µg/mL of methanolic extract of *Psorothamnus polydenius* [43]. Bauhinoxepins A found in *Bauhinia saccocalyx* roots and showed antimycobacterial activity [44]. 4-Hydroxycordoin is an isopentenyloxychalcone and has a clear ability against three major periodontal pathogen bacteria *Prevotella intermedia, Porphyromonas gingivalis*,
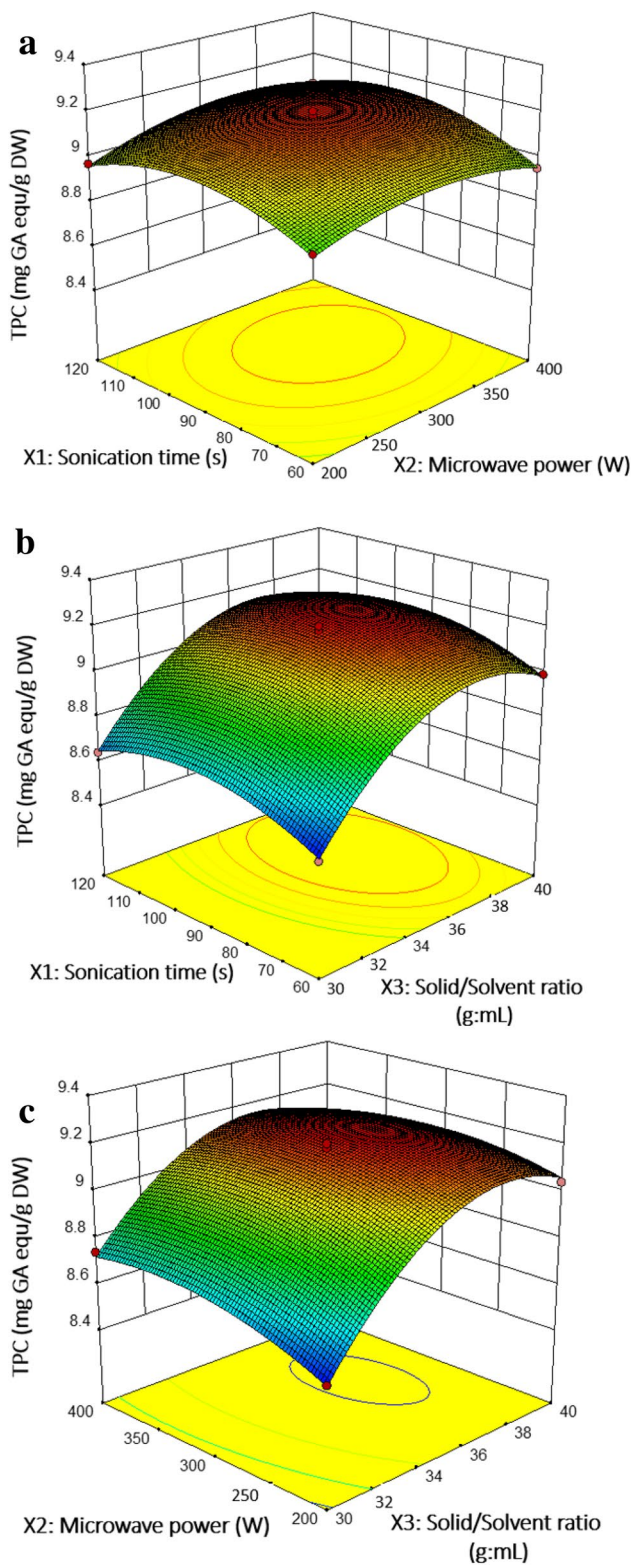
and *Fusobacterium nucleatum*, as well anti-inflammatory effect [45]. Pterolinus D is one of benzofurans, was found in extract of *Pterocarpus santalinus*, Pterolinus D showed significant inhibition as an anti-inflammatory [46]. Abyssinone C isolated from *Erythrina abyssinica* and showed moderate cytotoxic activity against the human colorectal cancer cell line at IC_{50} 15.1 μM [47]. The Blancoxanthone was isolated from *Calophyllum blancoi* and exhibited significant anti-coronavirus activity [48]. Brasixanthone B is a xanthones found for the first time in *Calophyllum brasilienses* and showed significant inhibitory activity against Epstein-Barr virus in Raji cells [49]. 4′-demethyldeoxypodophyllotoxin showed activity against the human epidermoid carcinoma and P-388 lymphocytic leukemia [50]. The kweichowenol B isolated from *Uvaria kweichowensis* leaves and showed antitumour activity based on yellow tetrazolium assay [51]. Erybraedin A had high activity against the BC and NCI-H187 cells (IC_{50} 2.9 μg/ml of *Erythrina stricta* roots [52].

SEM analysis

The SEM observation showed varying damage to the surface microstructures of the plant cellular tissues due to different extraction methods between UMAE, MAE, and CSE. The combination of disruption of plant tissues by acoustic cavitation caused by ultrasonic/microwaves and solvent flow into plant cell tissues facilitated by thermal stress resulting from microwave irradiation can improve the effectiveness of extraction [14–53].

SEM images of treated and untreated Foshou fruit powder are shown in Fig. 3. Images of untreated Foshou fruit powder and powder after extraction by UMAE, MAE, and CSE under the optimized conditions are shown in Fig. 3a–d, respectively. The SEM images showed remarkable changes in the microstructure of the Foshou fruit powder samples after extraction. Ultrasonic/microwave-assisted extraction through the cavitation phenomenon helped to decaythe sample plant tissues, while microwave power led to further disintegration of the plant tissue. By contrast, the conventional solvent extraction had only a slight effect on the tissue microstructure, with no oserve of disintegration.

Conclusions

In conclusion, this investigation has enhanced green-procedures extraction to maximize the total phenolic content (TPC) yield from Foshou fruit using water as an environment-friendly solvent and ultrasonic/microwave-assisted as integrated technology. Response surface methodology based

Fig. 1 3D Response surface plot for TPC yield **a** sonication time (s) with microwave power (W); **b** sonication time (s) with solid/solvent ratio (g:mL); **c** microwave power (W) with solid/solvent ratio (g:mL)

Table 5 The content of phenolic compounds in the Foshou fruit extraction according to the UPLC-PDA-Q-TOF-MS analysis

No.	Phenolic compounds	ChemSpider ID	Average mass (Da)	Molecular formula
1	*p*-Hydroxyphenylbut-3-ene-2-one	86341	162.185	$C_{10}H_{10}O_2$
2	3-hydroxyphenyl propanoate	213621	166.174	$C_9H_{10}O_3$
3	Isoacetovanillone	86383	166.174	$C_9H_{10}O_3$
4	3-(2-hydroxyphenyl)propanoic acid	850	166.174	$C_9H_{10}O_3$
5	Paeonol	10621	166.174	$C_9H_{10}O_3$
6	4-(4-Hydroxyphenyl)-2-butanol	88266	166.217	$C_{10}H_{14}O_2$
7	Ferulic acid	689	194.184	$C_{10}H_{10}O_4$
8	2-(4-Hydroxyphenyl)-3-methylbutyric acid	100571	194.227	$C_{11}H_{14}O_3$
9	Acetosyringone	16280	196.200	$C_{10}H_{12}O_4$
10	2′,6′-Dimethoxy-4′-hydroxyacetophenone	391208	196.200	$C_{10}H_{12}O_4$
11	Eugenin	9777	206.195	$C_{11}H_{10}O_4$
12	Leptorumol	26631404	206.195	$C_{11}H_{10}O_4$
13	Pisonin E	26631542	220.178	$C_{11}H_8O_5$
14	4′-Hydroxychalcone	4445525	224.255	$C_{15}H_{12}O_2$
15	2,3-Bis(hydroxymethyl)-7-hydroxy-6-methoxy-1-tetralone	26384422	252.263	$C_{13}H_{16}O_5$
16	Norathyriol	4444975	260.199	$C_{13}H_8O_6$
17	Norswertianin	4444977	260.199	$C_{13}H_8O_6$
18	Athyriol	4444941	274.226	$C_{14}H_{10}O_6$
19	Swertianin	4444980	274.226	$C_{14}H_{10}O_6$
20	Bellidifolin	4444942	274.226	$C_{14}H_{10}O_6$
21	Ophioglonin	9636002	314.246	$C_{16}H_{10}O_7$
22	Wedelolactone	4445124	314.246	$C_{16}H_{10}O_7$
23	2,2′,4′-Trihydroxy-6′-methoxy-3′,5′-dimethylchalcone	9946086	314.332	$C_{18}H_{18}O_5$
24	(*E*)-4,2′,4′-Trihydroxy-6′-methoxy-3′,5′-dimethylchalcone	26363219	314.332	$C_{18}H_{18}O_5$
25	Cefatrizine	4918615	462.503	$C_{18}H_{18}N_6O_5S_2$
26	Pterolinus C	26629959	316.348	$C_{18}H_{20}O_5$
27	Cajanol	26392261	316.305	$C_{17}H_{16}O_6$
28	(+)-Pisiferic acid	142446	316.435	$C_{20}H_{28}O_3$
29	Triptobenzene A	58827400	316.435	$C_{20}H_{28}O_3$
30	Pterolinus E	26630078	318.321	$C_{17}H_{18}O_6$
31	Cylindol A	8601421	318.278	$C_{16}H_{14}O_7$
32	2-Hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxoethyl]benzoic acid	26633602	318.278	$C_{16}H_{14}O_7$
33	Griffithane A	26636847	318.364	$C_{18}H_{22}O_5$
34	(−)-(4*S*,5*S*,10*R*)-10,12,18-Trihydroxy-7-oxo-20-norabieta-8,11,13-triene	26390807	318.407	$C_{19}H_{26}O_4$
35	abyssinone I	390670	322.354	$C_{20}H_{18}O_4$
36	Bauhinoxepin A	9898347	322.354	$C_{20}H_{18}O_4$
37	8-Prenyldaidzein	23550835	322.354	$C_{20}H_{18}O_4$
38	(−)-Phaseolin	58145272	324.370	$C_{20}H_{20}O_4$
39	4-Hydroxycordoin	4732316	324.370	$C_{20}H_{20}O_4$
40	*N*-feruloylserotonin	4766454	352.384	$C_{20}H_{20}N_2O_4$
41	(−)-Demethoxypinoresinol	8489888	328.359	$C_{19}H_{20}O_5$
42	(5*R*)-5-Hydroxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone	136694	328.402	$C_{20}H_{24}O_4$
43	Juglanin B	24721805	328.402	$C_{20}H_{24}O_4$
44	Pterolinus D	26630077	334.321	$C_{17}H_{18}O_7$
45	Bussealin B	26381829	334.364	$C_{18}H_{22}O_6$
46	Triptobenzene K	58170306	342.386	$C_{20}H_{22}O_5$
47	Kadsurenin K	2340650	342.386	$C_{20}H_{22}O_5$
48	Neotriptophenolide	117981	342.429	$C_{21}H_{26}O_4$
49	7-(4″-Hydroxy-3″-methoxyphenyl)-5-methoxy-1-phenyl-3-heptanone	28533010	342.429	$C_{21}H_{26}O_4$
50	Dihydrocurcumin	4522656	370.396	$C_{21}H_{22}O_6$

Table 5 (continued)

No.	Phenolic compounds	ChemSpider ID	Average mass (Da)	Molecular formula
51	Candidachalcone	26334170	370.396	$C_{21}H_{22}O_6$
52	Abyssinone C	28532919	372.369	$C_{20}H_{20}O_7$
53	Glycosmisic acid	22913536	372.369	$C_{20}H_{20}O_7$
54	Vladinol D	30786452	374.384	$C_{20}H_{22}O_7$
55	(−)-(7*R*,8*R*,8′*R*)-4,4′-Dihydroxy-3,3′,5′-trimethoxy-7,9′-epoxylignan	26630118	374.427	$C_{21}H_{26}O_6$
56	(+)-(8*S*,8′*R*)-4-Hydroxy-3,3′,4′,5′-tetramethoxylignan	26629055	374.471	$C_{22}H_{30}O_5$
57	Guaiacylglycerol β-coniferyl ether	4947720	376.400	$C_{20}H_{24}O_7$
58	(7*R*)-7-Hydroxylariciresinol	8197966	376.400	$C_{20}H_{24}O_7$
59	Blancoxanthone	9878297	378.418	$C_{23}H_{22}O_5$
60	Brasixanthone B	8537718	378.418	$C_{23}H_{22}O_5$
61	4′-demethyldeoxypodophyllotoxin	141208	384.379	$C_{21}H_{20}O_7$
62	Kweichowenol B	9524559	384.379	$C_{21}H_{20}O_7$
63	(−)-medioresinol	158029	388.411	$C_{21}H_{24}O_7$
64	Simulanol	20057219	388.411	$C_{21}H_{24}O_7$
65	(−)-5′-methoxyisolariciresinol	34448446	390.427	$C_{21}H_{26}O_7$
66	Abyssinone VI	4444631	392.487	$C_{25}H_{28}O_4$
67	Erybraedin A	20006335	392.487	$C_{25}H_{28}O_4$

Da Dalton
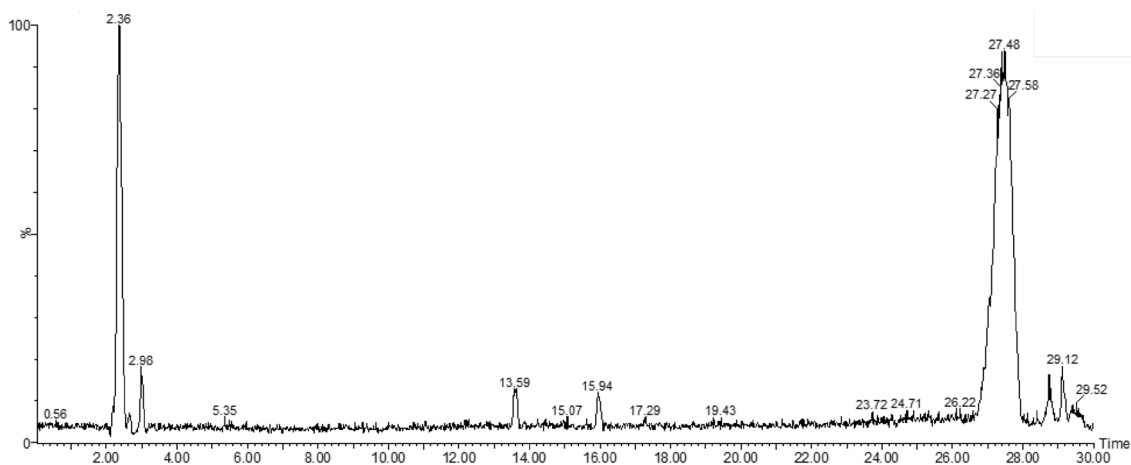
Fig. 2 The base peak intensity (BPI) chromatogram of optimized UMAE extract of Foshou fruit in the negative ion mode

a Box-Behnken design was successfully used to optimize the performance of ultrasonic/microwave-assisted technique and enhance the extraction processes. Findings of this work showed that the integrated ultrasonic/microwave-assisted extraction was more efficient than the microwave-assisted extraction or conventional solvent extraction as obtaining the highest TPC yield and antioxidant activity from Foshou fruit. In sum, Foshou fruit can be considered a good source of TPC that possess a high antioxidant activity, and thus can be used as safe food additives instead of synthetic additives. In the same context, ultrasonic/microwave-assisted extraction can be used as a safe integrated technique that does not require the use of harmful solvents.
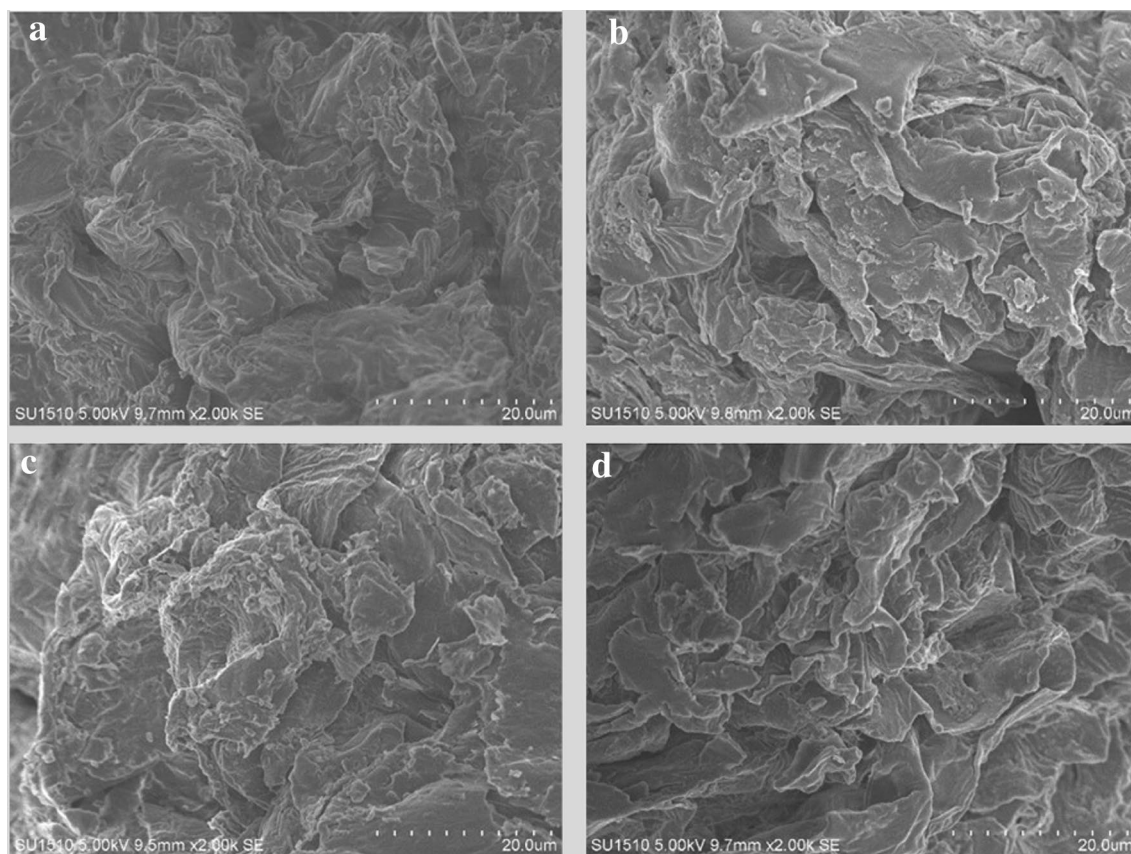
Fig. 3 Scanning electron microscope images of Foshou fruit powders untreated (**a**), after extraction under the optimized conditions of ultrasonic/microwave-assisted extraction (**b**), microwave-assisted extraction (**c**) and conventional solvent extraction (**d**)

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Affiliations

Amer Ali Mahdi[1,2,3] · Marwan M. A. Rashed[1,4] · Waleed Al-Ansi[1,3] · Mohamed Ismael Ahmed[1,5] · Mohammed Obadi[1] · Qi Jiang[1,2] · Husnain Raza[1] · Hongxin Wang[1,2]

✉ Hongxin Wang
hxwang@jiangnan.edu.cn

1 State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China

2 National Engineering Research Center for Functional Food, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China

3 Department of Food Science and Technology, Faculty of Agriculture, Sana'a University, Sana'a, Yemen

4 Solid-State Fermentation Resource Utilization Key Laboratory of Sichuan Province, College of Life Science & Food Engineering, Yibin University, 8 Jiusheng Road Wuliangye Avenue, Yibin, Sichuan 644000, China

5 Department of Food Science and Technology, Nyala Technical College South Darfur, Nyala, Sudan