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Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives

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ABSTRACT

Ultrasonic-assisted extraction (UAE) and maceration extraction (ME) were optimized using response surface methodology (RSM) for total phenolic compounds (TPC) from fresh olives. The main phenolic compounds and antioxidant activity of TPC were also investigated. The optimized result for UAE was 22 mL/g of liquid-solid ratio, 47 °C of extraction temperature and 30 min of extraction time, 7.01 mg/g of yielding, and for ME was 24 mL/g of liquid-solid ratio, 50 °C of extraction temperature and 4.7 h of extraction time, 5.18 mg/g of yielding. The HPLC analysis revealed that the extracts by UAE and ME possessed 14 main phenolic compounds, and UAE exhibited more amounts of all phenols than ME. The most abundant phenolic compounds in olive extracts were hydroxytyrosol, oleuropein and rutin. Both extracts showed excellent antioxidant activity in a dose-dependent manner. Taken together, UAE could effectively increase the yield of phenolic compounds from olives. In addition these phenolic compounds could be used as a potential source of natural antioxidants.

1. Introduction

Olive (*Olea europaea* L., Oleaceae), an important woody oil crop, is widely distributed in many Mediterranean countries and southwest China [1,2]. Olive oil obtained from olive fruits has generally acknowledged as an important role in human diet because of its health-promoting value [3,4]. The fine characteristic and biological activity of olive oil are mainly due to the presence of components, such as the optimal balance among monounsaturated, polyunsaturated and saturated fatty acids, as well as to minor components, including polyphenol, phytosterol, chlorophyll and tocopherol [5]. Phenolic compounds, the secondary metabolites of plants, have shown a wide variety of biological properties, such as antioxidant, anti-inflammatory and antitumor activity [6–8].

Currently, the phenolic compounds of olives have attracted a great deal of attention due to healthy benefits to olive oil [9]. However, extract method is a limiting process as the first stage impacting the isolation of phenolic compounds from olives. Since maceration extraction (ME), a traditional method resulting in lower yield of phenolic compounds, is still a main method to extract phenolic compounds from olives. In recent years, several novel techniques, such as supercritical fluid extraction, enzymatic extraction, microwave-assisted extraction and ultrasonic-assisted extraction have been used for extraction of phenolic compounds from plants instead of conventional technique. Among these methods, UAE have become more and more popular because it is a simple and eco-friendly method. This method utilize acoustic cavitation to disrupt plant tissues and increase mass transfer, obtaining benefits like higher efficiency, shorter extraction time and less power consumption than the conventional extraction techniques [10,11].

Response surface methodology (RSM) is a widely used statistical tool in optimizing any process when the independent parameters have combined effects [12]. The number of trials to evaluate the multiple variables and their interactions are reduced by using RSM. To the best of our knowledge, no research report exists on the optimization of UAE and ME for phenolic compounds from fresh olives and no investigation exists on the effects of different extraction methods of the major phenolic compounds and on the antioxidant activity. Therefore, this study was to optimize the methods of UAE and ME according to the maximum extraction yield of TPC. The major phenolic compounds of TPC were subse-

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quently investigated using HPLC method. Moreover, the antioxidant activities of both extracts derived from olives were also estimated *in vitro*.

2. Materials and methods

2.1. Materials and reagents

The olive fruits (*Olea europaea* L.) Picual *cv.* of yellow-green skin coloration were harvested at the second maturity in Xichang, Sichuan Province, China. The maturity stage was determined using a subjective evaluation of olive fruits epadermis and mesocarp colour according to Morelló et al. [13]. The fresh olives were stored at −80 °C. Before extraction processes, the olive pulp were separated from the kernel, grounded into fine powder with liquid nitrogen and then kept at −25 °C.

Gallic acid, hydroxytyrosol, *p*-hyroxybenzoate acid, chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, salicylic acid, benzoic acid, verbascoside, rutin, oleuropein and quercetin were purchased from Shanghai YuanYe Biotechnology Co., Ltd. (Shanghai, China). DPPH (2, 2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were analytical grade or higher.

2.2. Extraction methods

2.2.1. Ultrasonic-assisted extraction (UAE)

The frozen fruit powder of 2 g was homogenised with 80% methanol extracted in an ultrasound cleaner (KQ-100DE, Kun Shan ultrasound instrument Co., Jiangsu, China) at a power of 240 W. The suspension obtained was centrifuged (10 min, 5000 rpm, 4 °C) and then concentrated by rotatory evaporator. The residue was dissolved in 4 mL of methanol to obtain phenolic compounds of olive fruits. The single factor experiment was performed in a designed liquid-solid ratio (X_1) ranged from 10 to 50 mL/g, extraction temperature (X_2) ranged from 30 to 70 °C, extraction time (X_3) ranged from 10 to 50 min. One factor was changed, while the other factors kept constant, and each single factor experiment was repeated thrice.

2.2.2. Maceration extraction (ME)

The frozen fruit powder (2 g) was homogenised with 80% methanol and extracted in a thermostatic water bath (XMTD-4000, Yong Bright Medical Instrument Factory, Beijing, China) under the designed liquid-solid ratio (X_1) from 10 to 50 mL/g, extraction temperature (X_2) from 30 to 70 °C and extraction time (X_3) from 1 to 5 h. Other processes were performed as described for UAE in Section 2.2.1.

2.3. Experimental design

On the basis of the single-factor test result, major influence factors were selected and the optimal range of each variable was determined. Then, an RSM based on BBD experiments for UAE and ME was conducted to optimize both processes. The code and the real value of each factor are presented in Table 1 and Table 2. In order to predict the optimal conditions, experimental values were analyzed using Design-Expert 8 software and fitted to an empirical second order polynomial regression model:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2$$

where Y is predicted extraction yield of TPC, β_0 is a constant, β_i, β_{ij} and β_{ii} are the coefficients of the linear, quadratic and interactive terms, respectively of the model. X_i and X_j are the code values of liquid-solid ratio, extraction temperature and extraction time, respectively. All experiments were performed with three replications. Analysis of variance (ANOVA) was performed to evaluate the significance of each response in the model. To verify the adequacy of the models, additional experiments were done according to the optimal conditions predicted with the RSM and the obtained data were compared to values predicted by the model.

Table 1
BBD matrix and response values for TPC referred to fresh olives using UAE.

Trial	X_1 (mL/g)	X_2 (°C)	X_3 (min)	Y (mg/g)
1	(0) 20	(0) 40	(0) 30	6.72
2	(−1) 10	(0) 40	(−1) 20	5.24
3	(0) 20	(−1) 30	(−1) 20	5.47
4	(1) 30	(1) 50	(0) 30	6.48
5	(1) 30	(−1) 30	(0) 30	5.64
6	(0) 20	(1) 50	(1) 40	6.56
7	(0) 20	(1) 50	(−1) 20	6.44
8	(1) 30	(0) 40	(1) 40	5.79
9	(1) 30	(0) 40	(−1) 20	5.71
10	(0) 20	(0) 40	(0) 30	6.92
11	(−1) 10	(0) 40	(1) 40	5.46
12	(0) 20	(0) 40	(0) 30	6.78
13	(0) 20	(0) 40	(0) 30	6.69
14	(0) 20	(0) 40	(0) 30	6.75
15	(−1) 10	(1) 50	(0) 30	5.38
16	(−1) 10	(−1) 30	(0) 30	5.29
17	(0) 20	(−1) 30	(1) 40	6.30

Table 2
BBD matrix and response values for TPC referred to fresh olives using ME.

Trial	X_1 (mL/g)	X_2 (°C)	X_3 (h)	Y (mg/g)
1	(0) 20	(0) 40	(0) 4	4.78
2	(1) 30	(1) 50	(0) 4	4.58
3	(0) 20	(1) 50	(1) 5	5.02
4	(−1) 10	(−1) 30	(0) 4	3.33
5	(0) 20	(−1) 30	(−1) 3	3.74
6	(1) 30	(−1) 30	(0) 4	3.97
7	(−1) 10	(0) 40	(−1) 3	3.72
8	(−1) 10	(0) 40	(1) 5	3.44
9	(0) 20	(−1) 30	(1) 5	3.96
10	(0) 20	(0) 40	(0) 4	5.09
11	(1) 30	(0) 40	(1) 5	4.78
12	(0) 20	(0) 40	(0) 4	4.86
13	(0) 20	(0) 40	(0) 4	4.46
14	(0) 20	(1) 50	(−1) 3	4.42
15	(0) 20	(0) 40	(0) 4	4.47
16	(1) 30	(0) 40	(−1) 3	3.42
17	(−1) 10	(1) 50	(0) 4	4.33

2.4. Determination of TPC

The extracted TPC was determined by the Folin-Ciocalteu method with adapted from Malik, Bradford [14]. Briefly, 0.1 mL sample was mixed with 3 mL distilled water and 0.2 mL Folin-Ciocalteu reagent. After 5 min, 0.8 mL Na_2CO_3 solution (10%, w/v) was added to the mixture and then the reaction solution was kept in dark for 30 min at room temperature. The TPC concentration was calculated from a calibration curve with pure gallic acid as a standard. Result was expressed as mg/g on fresh matter.

2.5. HPLC analysis

The chromatographic analysis were performed using a given HPLC instrument (Agilent LC 1260 series; Agilent Technologies, USA) equipped with a diode array detector (DAD). A Zorbax SB-C18 column (5 μm, 150 × 4.6 mm; Agilent Technologies, USA) was used to separate these compounds. Procedure was performed at 35 °C, flow rate of 1.0 mL/min, injection volume of 10 μL and

detection wavelength was carried out 280 nm. The mobile phase consisted of a mixture of water/acetic acid (95:5, v/v) (solvent A), methanol (solvent B) and isopropanol (solvent C): from 95% (A)-2.5% (B)-2.5% (C) to 92% (A)-4% (B)-4% (C) in 14 min and then increased to 72% (A)-9% (B)-9% (C) in 31 min, and finally raised to 70% (A)-15% (B)-15% (C) in 15 min.

2.6. Antioxidant activity assays

2.6.1. Reducing power

The reducing power was evaluated, as previously described by Re et al. [15] with slight modifications. Various concentrations (2–19 μg/mL) of olive extracts (1 mL) were mixed with 0.1 mL of 0.2 M phosphate buffer (pH = 6.6) and 0.1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction solution was incubated at 50 °C for 20 min. Subsequently, the mixture was added to 0.1 mL trichloroacetic acid and then centrifuged for 10 min at 3000 rpm. The supernatant (0.1 mL) was diluted in 0.1 mL distilled water and then added with 0.025 mL of 0.1% $FeCl_3$. Absorbance at 700 nm was recorded, using Vc (ascorbic acid) as a positive control. An increase in the absorbance of the mixture indicated an increase in the reducing power.

2.6.2. DPPH radical scavenging activity

The effect of scavenging DPPH radical was determined according to a method as described by Brand-Williams et al. [16] and changed according to the method of Yu et al. [17]. Briefly, 0.4 mL sample solutions (7–120 μg/mL) were mixed with 2 mL of 0.2 mM DPPH ethanol solution. The reaction mixture was incubated for 30 min at room temperature in darkness and the absorbance was read at 517 nm, using Vc as a positive control. The scavenging activity of DPPH radical was measured by the following equation.

$$Scavenging\ activity(\%) = (1 - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the DPPH radical solution with ethanol and A_{sample} is the absorbance of the DPPH radical solution with tested samples.

2.6.3. Iron chelating activities

Fe^{2+}-chelating activity was evaluated according to the previous report [18], with some modifications. 0.5 mL sample solutions with different concentrations (13.7–170 μg/mL) was added with 0.74 mL distilled water and 0.02 mL $FeCl_2$ (2 mM) and reacted for 10 min. The mixture was added to 0.04 mL ferrozine (5 mM) and then kept for 20 min at room temperature. The absorbance was measured at 562 nm, using $EDTANa_2$ (Ethylenedinitrilo tetraacetic acid disodium salt) as control. Iron chelating activity was calculated by the following equation:

$$Chelating\ activity(\%) = (1 - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the control reaction in which the sample was replaced by distilled water, and A_{sample} is the absorbance of tested sample solutions.

2.7. Statistical analyses

All the experiments were carried out in triplicate and all values were expressed as means ± standard deviation (SD). Statistical analyses were performed by analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Optimization of extraction process

3.1.1. Optimization of UAE process

The design matrix and real values of BBD experiments to evaluate the effects of three variables including liquid-solid ratio (X_1), extraction temperature (X_2) and extraction time (X_3) on the extraction yield of TPC are presented in Table 1. By applying multiple regression analysis on the experimental data, neglecting the non-significant terms ($p > 0.05$), the quadratic model for predicting the optimal point for UAE was:

$$Y = 6.77 + 0.28X_1 + 0.27X_2 + 0.16X_3 + 0.19X_1X_2 - 0.18X_2X_3 - 0.86X_1^2 - 0.22X_2^2 - 0.37X_3^2 \quad (1)$$

The ANOVA for the fitted quadratic model is presented in Table 3. The model was highly significant with p-value < 0.0001. P-value of the lack of fit was 0.0719, which implied that the model equation was adequate for predicting the extraction yield of TPC under any combinations of the variables. The determination coefficient (R^2) was 0.9755, which indicated that 97.55% of the variations found on the yield of TPC could be attributed to the independent variables. The low CV (2.38%) suggested a better precision and reliability of the conducted experiments.

Table 3
ANOVA for the response surface quadratic model (Eq. (1)).

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value
Model	5.85	9	0.65	30.93	<0.0001**
X_1	0.64	1	0.64	30.47	0.0009**
X_2	0.58	1	0.58	27.77	0.0012**
X_3	0.20	1	0.20	9.46	000179*
X_1X_2	0.14	1	0.14	6.81	0.0349*
X_1X_3	0.01	1	0.01	0.26	0.6280ns
X_2X_3	0.12	1	0.12	5.86	0.0462*
X_1^2	3.10	1	3.10	147.56	<0.0001**
X_2^2	0.20	1	0.20	9.42	0.0181*
X_3^2	0.56	1	0.56	26.72	0.0013**
Residual	0.15	7	0.02		
Lack of fit	0.12	3	0.04	5.23	0.0719ns
Pure Error	0.03	4	0.01		
Cor Total	6.00	16			
R^2	0.9755				
R^2_{adj}	0.9439				
C.V (%)	2.38				

ns Not significant.
** Significant at $p < 0.01$.
* Significant at $p < 0.05$.

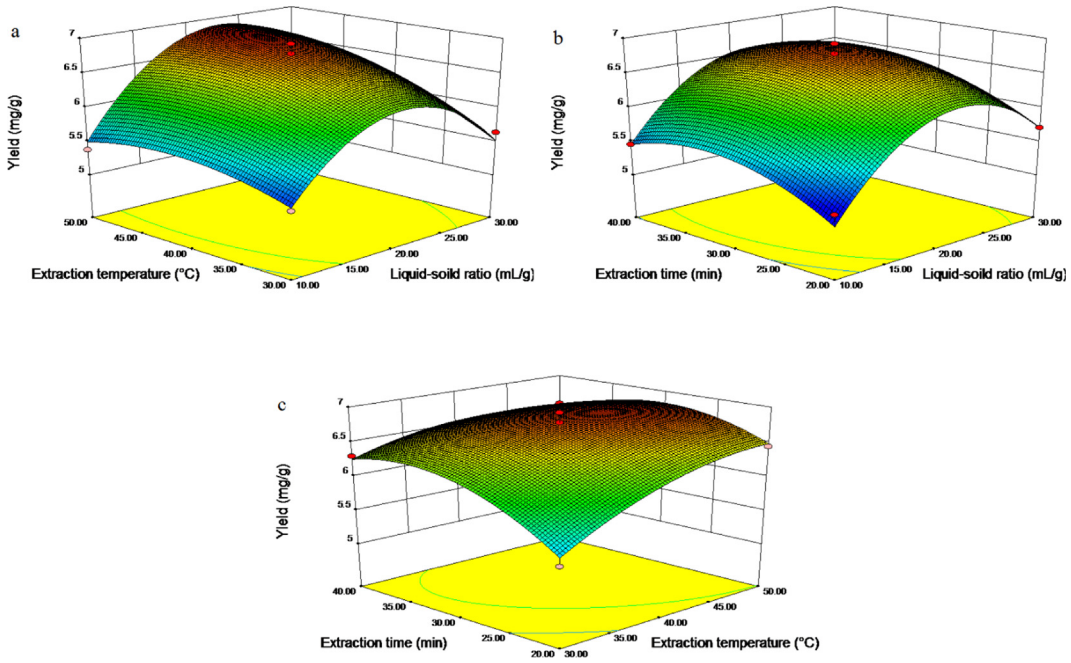

Fig. 1. Response surface plots for the TPC yield from fresh olives with UAE with respect to liquid-solid ratio and extraction temperature (a); liquid-solid ratio and extraction time (b); extraction temperature and extraction time(c).

The 3D response surface plots and two-dimensional contour plots of regression Eq. (1) were constructed using RSM based on BBD to illustrate the effects of independent variables and their interactions on the extraction yield of TPC (Fig. 1). Fig. 1a shows the effect of liquid-solid ratio and extraction temperature on the yield of TPC with extraction time fixed at 30 min. The yield increased with the increasing of temperature and ratio from 10 to 23.33 mL/g but then decreased when the ratio beyond 23.33 mL/g. Fig. 1b shows the effect of liquid-solid ratio and extraction time on the yield when the extraction temperature is fixed at 40 °C. The circular contour plot indicated the mutual interactions between temperature and time were not significant. As shown in Fig. 1c, the yield increased significantly with the increasing of time and temperature when the ratio is fixed at 20 mL/g. The effect of extraction temperature on the phenolic compounds was associated with the species of plants, which possessed different types and forms polyphenol [19].

From the model, the optimum conditions were liquid-solid ratio 22.44 mL/g, extraction temperature 47.17 °C and extraction time 30.32 min. Considering the operability in the actual test, the optimized conditions were modified as flow: ratio 22 mL/g, temperature 47 °C and time 30 min. A mean value of 7.01 mg/g was obtained and closed to the predicted value using RSM (6.91 mg/g). The results indicated that the regression model was accurate and adequate for the extraction of TPC.

3.1.2. Optimization of ME process

The extraction yield of TPC obtained in the trials of the BBD are presented in Table 2. Neglecting the non-significant terms ($p > 0.05$), the quadratic model for predicting the optimal point for ME was:

Table 4
ANOVA for the response surface quadratic model (Eq. (2)).

Source	Sum of square	Degree of freedom	Mean square	*F*-value	*p*-Value
Model	5.07	9	0.56	13.04	0.0013**
X_1	0.47	1	0.47	10.77	0.0135*
X_2	1.39	1	1.39	32.22	0.0008**
X_3	0.46	1	0.46	10.53	0.0141*
X_1X_2	0.04	1	0.04	0.88	0.3787ns
X_1X_3	0.67	1	0.67	15.45	0.0057**
X_2X_3	0.04	1	0.04	0.84	0.3886ns
X_1^2	1.34	1	1.34	31.04	0.0008**
X_2^2	0.06	1	0.06	1.34	0.2852ns
X_3^2	0.47	1	0.47	10.80	0.0134*
Residual	0.15	7	0.04		
Lack of fit	0.01	3	0.00	0.06	0.98ns
Pure Error	0.03	4	0.07		
Cor Total	6.00	16			
R^2	0.9437				
R^2_{adj}	0.8713				
C.V (%)	4.88				

ns Not significant.
** Significant at $p < 0.01$.
* Significant at $p < 0.05$.

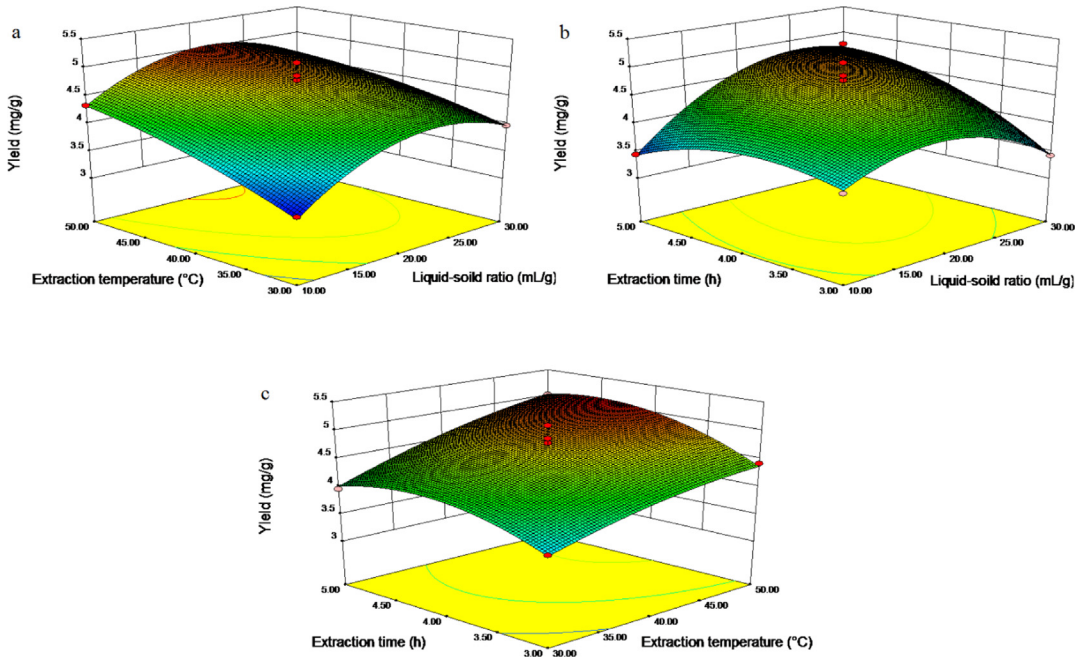

Fig. 2. Response surface plots for the TPC yield from fresh olives with ME with respect to liquid-solid ratio and extraction temperature (a); liquid-solid ratio and extraction time (b); extraction temperature and extraction time(c).

$$Y = 4.73 + 0.24X_1 + 0.42X_2 + 0.24X_3 + 0.41X_1X_3 - 0.56X_1^2 - 0.33X_3^2 \quad (2)$$

The ANOVA for the fitted model are presented in Table 4. The proposed quadratic model was highly significant with low p-value < 0.001. P-value of the lack of fit was 0.98, which implied that the model equation was adequate for predicting the extraction yield of TPC under any combinations of the variables. R^2 was 0.9437, which indicated that 94.37% of the variations was attributed to the independent variables. The low CV (4.88%) suggested that the model was reproducible and reliable.

The 3D response surface plots of regression Eq. (2) are presented in Fig. 2. Fig. 2a shows the effect of liquid-solid ratio and extraction temperature on the extraction yield of TPC with extraction time fixed at 4 h. The circular contour plot indicated the mutual interactions between ratio and temperature were not significant. As shown in Fig. 2b, ratio and time had a similar effect on the yield when the temperature is fixed at 40 °C. The yield increased slowly with the increasing of ratio and time. But with further increasing of time and ratio, the yield had a slight decrease. Fig. 2c shows the effect of time and temperature on the yield when ratio is fixed at 20 mL/g. The yield increased slightly with the increasing of time and temperature. The interactive effects of time and temperature were not significant. The extraction temperature may play an important role in the surface tension, viscosity of the liquid medium and vapor pressure [20].

From the model, the optimum conditions were liquid-solid ratio 23.926 mL/g, extraction temperature 49.72 °C and extraction time 4.67 h. Considering the operability in the actual test, the optimized conditions were modified as flows: ratio 24 mL/g, temperature 50 °C and time 4.7 h. ME was carried out under these conditions giving a real recovery of 5.09 mg/g, which was agreed closely with the predicted yield (5.18 mg/g).

3.1.3. Comparison of the optimized extraction conditions and yields

The optimal extraction conditions and the yields of TPC are summarized in Table 5. Compared to ME, UAE used less liquid-solid ratio, shorter extraction time and lower temperature. Furthermore, UAE offered a higher yields of TPC. These may be attributed by acoustic cavitation phenomena, which could produce a strong impact on the solid surface resulting in the increased extraction rate [21,22].

3.2. Phenolic composition

As shown in Table 6, the fourteen phenolic compounds are identified by HPLC method in UAE and ME extracts. These results revealed that ultrasonic application did not damage the 14 phenolic compounds. Similar report was also found in the study of Ahmad-Qasem et al. [19], in which they pointed out ultrasound application did not lead to the formation or degradation of phenolic compounds from olive leaves. Furthermore, it was noticeable that UAE possessed more amounts of phenolic compounds than ME. The data indicated that ultrasonic treatment was apt to enhance the extraction efficiency of phenolic compounds from fresh olives. Similar result has also been revealed by others [23,24]. Among these phenolic compounds, the higher contents of oleuropein, hydroxytyrosol and rutin presented in two samples. High contents of oleuropein, hydroxytyrosol and rutin were also reported by other literatures in olives [25,26].

Table 5
Experimental values of the responses at optimum conditions.

	Extraction time (min, h)	Extraction temperature (°C)	Liquid-solid ratio (mL/g)	Extraction power (W)	Extraction yield (mg/g)
UAE	30	47	22	240	7.01
ME	4.7	50	24	/	5.09

Table 6
Main phenolic compounds identified by HPLC method in fresh olives extracts obtained with UAE and ME. Rt, Retention time.

Phenolic compounds	Rt (min)	Calibration equation	R^2	Yield (μg/g)	
				UAE	ME
Gallic acid	2.9	$Y = 1554.9X + 34.9$	0.9996	36 ± 2.86	24.78 ± 0.17
Hydroxytyrosol	4.89	$Y = 1471.8X - 14.757$	0.9914	111.94 ± 6.31	96.56 ± 0.37
p-Hyroxybenzoate	9.3	$Y = 1053.1X - 12.669$	0.9950	35.11 ± 1.46	25.90 ± 0.14
Chorogenic	9.8	$Y = 2603.5X + 83.74$	0.9953	6.79 ± 0.85	2.41 ± 0.11
Caffeic	13	$Y = 4274.4X + 47.468$	0.9953	8.21 ± 0.55	1.09 ± 0.27
Syringic	15.8	$Y = 3055X + 5.95$	1.0000	4.11 ± 0.48	2.20 ± 0.03
p-Cumaric	21.8	$Y = 7783.3X + 5.0016$	0.9996	5.20 ± 0.19	1.96 ± 0.15
Ferulic	25.2	$Y = 4477.6X - 2.2356$	0.9994	2.71 ± 0.29	2.10 ± 0.00
Salicylic	28.9	$Y = 724.68X - 13.443$	0.9973	34.40 ± 2.19	25.66 ± 0.75
Benzoic	30.7	$Y = 510.52X + 3.6341$	0.9983	24.22 ± 6.93	10.86 ± 2.48
Verbascoside	35.5	$Y = 554.94X - 22.609$	0.9940	39.22 ± 2.19	20.29 ± 0.16
Rutin	38.9	$Y = 491.71X + 17.866$	0.9991	61.90 ± 9.46	46.70 ± 2.39
Oleuropein	48	$Y = 225.45X + 9.0174$	0.9930	486.07 ± 10.95	356.58 ± 20.50
Quercetin	58.8	$Y = 1326.9X - 6.403$	0.9988	5.51 ± 0.75	3.45 ± 0.03

3.3. Antioxidant activity

3.3.1. Reducing power

The correlation coefficient between absorbance and tested concentration of UAE, ME extracts and Vc were 0.9998, 0.9968 and 0.9994, respectively, which indicated that the reducing power of the both extracts and Vc all followed a dose-dependent manner. Other researchers also found that the positive correlation between the phenolic content to their antioxidant power [27,28]. As shown in Fig. 3a, with the increase of concentration, the reducing power of two extracts were close to that of Vc.

3.3.2. DPPH radical scavenging activity

As shown in Fig. 3b, all samples showed obvious scavenging effect on DPPH radical in a concentration dependent manner. At 120 μg/mL, the scavenging activity of UAE, ME extracts and Vc were 88.45%, 88.39% and 97.10%, respectively. The IC_{50} values (the concentration of sample to inhibition 50% of the radical) were 26.72, 27.40 and 28.22 μg/mL for UAE, ME extracts and Vc, respectively. Their IC_{50} values were not statistically different ($p > 0.05$). From the Fig. 3b, the UAE and ME extracts had a similar activity to Vc.

3.3.3. Iron chelating activity

As present in Fig. 3c, iron chelating capacities of extracts were in a dose-dependent manner. At the 170 μg/mL, the scavenging activity of UAE, ME extracts and $EDTANa_2$ were 94.43%, 96.87% and 100%, respectively. Their IC_{50} values were 15.48, 10.73 and 3.96 μg/mL for both extracts and $EDTANa_2$, respectively. Obviously, the activity of $EDTANa_2$ was significantly stronger than that of UAE and ME extracts ($p < 0.05$). It was reported that, at the studied frequency and power, ultrasound may inhibit or accelerate

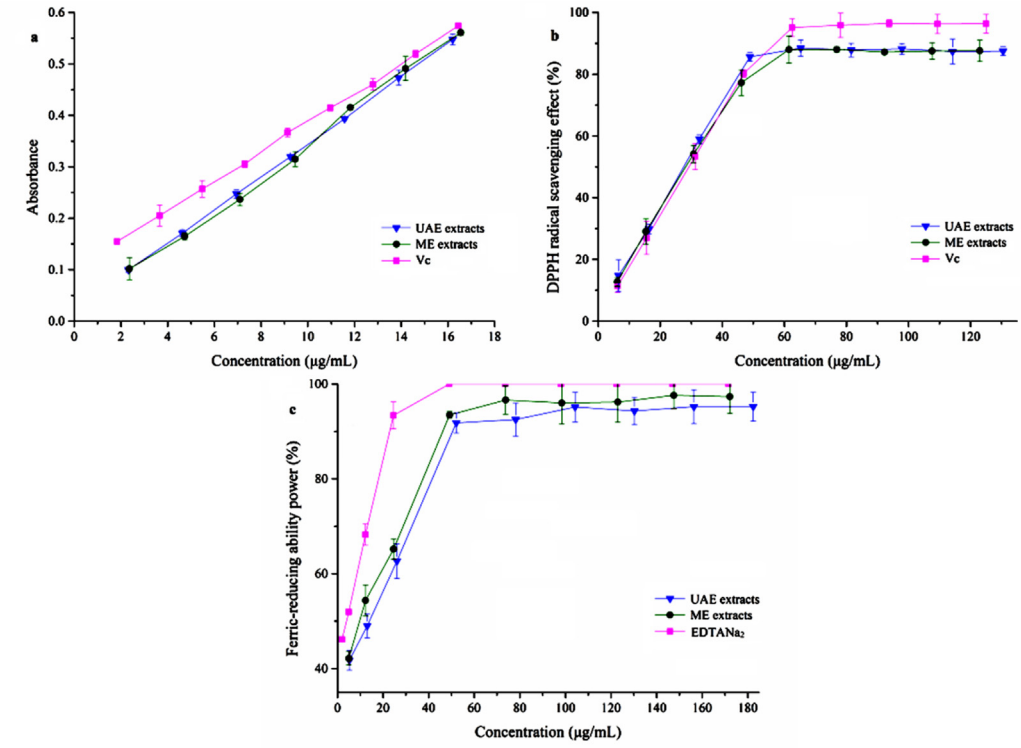

Fig. 3. Antioxidant activities of TPC from fresh olives obtained by UAE and ME method. Reducing power (a); DPPH radical scavenging (b); Iron chelating (c). Each value is the mean ± SD of triplicate measurements. UAE extracts, the TPC obtained with UAE from fresh olives. ME extracts, the TPC obtained with ME from fresh olives.

the bio-reactions for cells disruption and molecular rearrangements [29].

4. Conclusions

In this study, RSM was used to optimize the extraction process of phenolic compounds from olive fruits. The optimal conditions of UAE and ME were 22 mL/g, 47 °C and 30 min, yielding 7.01 mg/g, and 24 mL/g, 50 °C and 4.7 h, yielding 5.18 mg/g, respectively. The proposed UAE method gave the higher extraction yield with requiring less solvent, shorter time and lower temperature than ME method. Furthermore, from analysis of HPLC method, 14 phenolic compounds were found in both extracts and UAE offered higher yield than that of ME. The most abundant phenolic compounds in olives extracts were oleuropein, hydroxytyrosol and rutin. The both extracts exhibited similar specific antioxidant activities, including reducing power, DPPH radical scavenging and iron chelating capacity. Hence, UAE could be used as an effective method to extract phenolic compounds from olives. In addition, the phenolic compounds derived from olives exhibited strong antioxidant ability which might be explored as a good source of potential natural antioxidants in functional food ingredients.

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