Optimization of ultrasound-assisted extraction (UAE) of phenolic compounds from olive cake

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Abstract The use of ultrasound in ultrasound-assisted extraction (UAE) is one of the main applications of this technology in food industry. This study aimed to optimize UAE conditions for olive cake extract (OCE) through response surface methodology (RSM). The optimal UAE conditions were obtained with extraction temperature of 56 °C, extraction time of 3 min, duty cycle of 0.6 s, and solid to solvent ratio of 3.6%. At the optimum conditions, the total phenolic compounds (TPC) content and antioxidant activity (AA) were measured 4.04 mg/g and 68.9%, respectively. The linear term of temperature had the most effect on TPC content and AA of OCE prepared by UAE. Protocatechuic acid and cinnamic acid were characterized as the highest (19.5%) and lowest (1.6%) phenolic compound measured in OCE extracted by UAE. This research revealed that UAE is an effective method to extract phenolic compounds from olive cake. RSM successfully optimized UAE conditions for OCE.

Keywords Olive cake · Ultrasound-assisted extraction · Phenolic compounds · Optimization · Response surface methodology

Introduction

Nowadays, the awareness of food consumers about the importance of natural bioactive compounds with nutraceutical activity is increasing day by day. Hence, the

food industry is seeking these compounds from processed agricultural products and their by-products (Yolmeh and Mahoonak 2016). In addition to economic efficiency, the using by-products of food industry have environmental importance through decreasing environmental hazards (Wijngaard et al. 2012).

Olive (*Olea europaea* L.) and processed olive products are containing many bioactive compounds such as phenolic compounds. This fruit is one of the oldest oil sources, which have been used in all over the world (Azizpour et al. 2017). Black olive mill wastewater, solid waste, and olive cake are by-products of olive oil industry. Since these by-products constitute major part of olive oil industry (35 kg olive cake and 440 l wastewater per 100 kg of olive) control them is important (Alu'datt et al. 2010).

Olive cake is recognized an inexpensive biomass for phenolic compounds, which is widely available in Mediterranean countries and the northern provinces of Iran. However, these by-products of olive oil industry represent environmental problems (Lesage-Meessen et al. 2001). Thus, many researchers have aimed to decrease these environmental problems and on other hand increase economic value. So far, olive cake has been used as animal feed, fuel, and fertilizer (Keskin and Kaya 2010).

Several potentially bioactivity such as antioxidant, antiviral, anti-cancer, anti-inflammatory, hypoglycaemic and hypolipidaemic have been reported for phenolic compounds of olive. Olive mill waste is containing considerable amount (98%) of the total phenolic compounds (TPC) content of whole olive (Tripoli et al. 2005; Alu'datt et al. 2010).

The traditional extraction methods are very time-consuming and need large amount of solvents for bioactive compounds. Currently, several modern extraction methods have been developed for extraction of phenolic

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compounds, such as enzymatic extraction, ultrasonic-assisted extraction (UAE), and supercritical fluid extraction (Azmir et al. 2013). The UAE is less expensive and much easier compared to some other extraction methods such as microwave-assisted extraction (MAE) and supercritical fluid extraction (Yolmeh et al. 2014).

Recently, several studies have been reported on the application of UAE for different natural components. The acoustic cavitation is the main reason to increase extraction efficiency in UAE, which have generated by ultrasound waves. Increasing penetrating power of solvent and the contact surface between solid and solvent are the other effects of ultrasound waves (Soleimanzadeh et al. 2015b). Different parameters are involving in UAE of herbal components such as type and concentration of solvent, extraction time, extraction temperature, duty cycle, and solid to solvent ratio. Hence, the optimization of UAE process is important to maximize extraction efficiency. Mathematical models such as response surface methodology (RSM) have been successfully used to optimize UAE of herbal components (Vinatoru 2001).

There is no research available in the literature concerning on UAE of phenolic compounds from olive cake. Therefore, this study was aimed to introduce an optimal condition to UAE of olive cake extract (OCE) through RSM.

Materials and methods

Materials

Olive cake was prepared from Sina Ebtakare Samen plant (Bandar-e Gaz, Iran). All chemicals used in this study were analytical grade and prepared from Merck (Germany) and Sigma-Aldrich (UK) companies.

Ground and defatting

Olive cake was ground using a grinder (922SS Manual Feed Grinder, Marblehead, USA) and defatted using Soxhlet method and petroleum ether as solvent (1 l). The defatted olive cake was stored at -18°C until analysis.

Ultrasound-assisted extraction (UAE)

Ground olive cake (2 mm in diameter) was placed in beaker (containing 30 ml methanol), and then put in a water bath. UAE process was done using an ultrasonic device (Misonix, XL2020 model, Germany) equipped with a probe 19 mm in diameter. Frequency and power of the ultrasonic device were set at 20 kHz and 200 W, respectively. After ultrasonic extraction, the extracts were

centrifuged at 10,000g for 10 min. The harvested supernatant was filtered by cheesecloth and stored at -18°C until analysis (Rodrigues et al. 2008).

Total phenolic compound (TPC)

TPC of OCE was measured using Folin–Ciocalteu standard method (Hoff and Singleton 1977). Briefly, a standard calibration curve was plotted using gallic acid solution (50 mg/50 ml). Each OCE was diluted within distilled water (10 ml OCE in 40 ml distilled water). Then, 1 ml of the above solution, 7.5 ml of distilled water and 0.5 ml Folin–Ciocalteu reagent were mixed in test tube. After 4 min, 1 ml of 5% sodium carbonate was added to the above test tube and mixed. After 1 h, the absorbance of green color was measured spectrophotometrically by UV-160A spectrophotometer (Shimadzu, Japan) at 725 nm. TPC was expressed as milligrams of gallic acid equivalents per gram of dry olive cake (mg/g) (Azizpour et al. 2017).

Antioxidant activity

Antioxidant activity (AA) of the OCE was measured using the method described by Alu'datt et al. (2010). An emulsion containing 3 ml β -Carotene solution (5 mg β -Carotene within 50 ml chloroform), 50 μl linoleic acid, and 400 mg Tween-20 was prepared in a flask. After removing chloroform using a stream of nitrogen, 100 ml distilled water was added and followed by mixing and saturating with oxygen. An amount of OCE (100 μl) and the β -carotene/linoleic acid emulsion (3 ml) were mixed. After incubation at 50°C for 60 min, oxidation bleaching of the emulsion was evaluated through measuring the absorbance at 470 nm during 60 min. 100 μl solvent instead of OCE and distilled water were considered as control and blank, respectively. The AA was calculated according to the following formula:

$$\text{AA}\% = 100(\text{DRc} - \text{DRs})/\text{DRc}$$

$$\text{DRc} = \frac{\ln x/y}{60}$$

$$\text{DRs} = \frac{\ln x/y}{60}$$

where DRc and DRs are the degradation rate of the control the degradation rate of the sample, respectively. x and y are the initial absorbance (0) min and the absorbance after 60 min, respectively.

Phenolic compounds

OCE extracted at the optimum UAE conditions and phenolic compound standards were dissolved in methanol, and

followed by centrifuging at 5000g for 10 min, subsequently flushed by nitrogen stream and stored at 18 °C until HPLC analyses. RP-HPLC assay was done according to the method described by Alu'datt et al. (2010) using high-pressure liquid chromatography equipped with a programmable solvent module delivery system (Beckman, model, 125, Saint Paul, USA).

Experimental design

A central composite design (CCD) containing four variable at five levels was used to optimize UAE of OCE with respect to four important independent variables, namely temperature, sonication time, duty cycle (on time/off time), and seed to solvent ratio. The off time was considered for

the ultrasound device to burst of created bubbles, so the duty cycle and total time were 0.2–0.8 and 1 s, respectively (Yolmeh and Najafzadeh 2014). Minitab® version 17 was used to analyze the obtained results, namely total phenolic compounds (TPC) and antioxidant activity (AA). The applied design was composed of 31 runs, 16 cube points, 7 center points in cube, 8 axial points, 0 center point in axial.

Statistical analysis

The least square multiple regression approach was used to study the relationship between the independent variable and responses. The proper models for each response were chose based on R^2 , R^2 -adj, R^2 -pred, root-mean-square error (RMSE), and absolute average deviation (AAD). R^2 value

Table 1 The CCD matrix and experimental data for TPC and AA of OCE extracted by UAE method

Run no.	Independent variables				Responses	
	Temperature (°C)	Time (min)	Duty cycle (s)	Solid to solvent ratio (%)	TPC (mg/g)	AA (%)
1	41.25	4	0.35	8	2.92	69.5
2	35	6	0.5	6	2.61	67.2
3	47.5	6	0.5	6	3.65	75.7
4	53.75	4	0.35	4	3.60	77.1
5	41.25	8	0.65	4	2.98	72.3
6	47.5	6	0.5	6	3.69	60.9
7	47.5	6	0.5	6	3.64	60.3
8	47.5	6	0.8	6	2.23	59.9
9	53.75	4	0.35	8	3.66	76.4
10	47.5	10	0.5	6	3.25	60.1
11	53.75	8	0.35	4	4.25	74.1
12	41.25	4	0.65	8	3.26	72.6
13	47.5	6	0.2	6	2.85	63.2
14	53.75	8	0.35	8	3.71	73.8
15	41.25	4	0.65	4	3.07	73.5
16	53.75	4	0.65	4	4.31	79.3
17	41.25	8	0.65	8	3.53	70.1
18	41.25	8	0.35	8	3.17	69.4
19	60	6	0.5	6	4.22	56.1
20	41.25	4	0.35	4	3.02	70.3
21	47.5	6	0.5	10	3.00	65.4
22	53.75	8	0.65	8	4.55	75.5
23	47.5	6	0.5	6	3.55	75.8
24	47.5	6	0.5	6	3.63	75.5
25	47.5	6	0.5	6	3.66	75.9
26	47.5	2	0.5	6	3.50	65.1
27	47.5	6	0.5	6	3.70	76.0
28	47.5	6	0.5	2	2.95	65.6
29	41.25	8	0.35	4	3.13	69.6
30	53.75	8	0.65	4	5.02	78.2
31	53.75	4	0.65	8	4.90	80.0

Table 2 The statistics of the four fitted models

Models	Statistics	Responses	
		TPC	AA
Linear	R ² (%)	21.93	16.21
	R ² -adj (%)	16.60	11.31
	R ² -pred (%)	8.97	6.47
	RMSEP	0.538	0.493
	ADD (%)	5.08	4.52
Linear-squares	R ² (%)	84.97	81.89
	R ² -adj (%)	78.76	86.37
	R ² -pred (%)	66.58	59.35
	RMSEP	0.286	0.244
	ADD (%)	3.99	3.34
Linear-interactions	R ² (%)	31.77	24.95
	R ² -adj (%)	18.39	10.23
	R ² -pred (%)	13.39	4.59
	RMSEP	0.486	0.327
	ADD (%)	5.63	4.88
Full quadratic	R ² (%)	86.38	83.45
	R ² -adj (%)	79.89	76.30
	R ² -pred (%)	36.03	27.09
	RMSEP	0.348	0.311
	ADD (%)	4.40	3.11

must be closed to one. RMSE and AAD must be as low as possible, which these conditions mean selected model is accurate (Soleimanzadeh et al. 2015a). The analysis of variance (ANOVA) was used to investigate the statistical significance of the regression coefficients by conducting Duncan tests at 5% level. The obtained surface plots were used to investigate interactive effects between independent variables.

The UAE optimizing process was aimed to maximize TPC and AA at the same weights ($w = 1$) and the validity of the optimum conditions was studied through the desirability values of the responses which ranged in 0 to 1. The desirability value must be closed to 1, which shows the more desirable optimal conditions.

Results and discussion

Fitting the response surface models

According to applied design, 31 treatments were done and obtained data are shown in Table 1. TPC and AA results of OCE were ranged in 2.23–5.02 and 60.1–80.0, respectively. Based on results shown in Table 2, linear-square was suitable model for both responses compared to other

models. In fact, R² values of linear-square model for each response were the highest. As well as, RMSE and AAD values of selected model for each response were the lowest (Table 2).

The models are as follows:

$$\begin{aligned} \text{TPC (mg/g)} = & -0.39 - 0.029X_1 - 0.082X_2 + 8.53X_3 \\ & + 0.204X_4 + 0.00115(X_1)^2 \\ & + 0.0088(X_2)^2 - 7.72(X_3)^2 - 0.0162(X_4)^2. \end{aligned}$$

$$\begin{aligned} \text{AA (\%)} = & 26.2 + 1.72X_1 - 1.50X_2 + 44.7X_3 - 1.81X_4 \\ & - 0.0160(X_1)^2 \\ & + 0.056(X_2)^2 - 40.6(X_3)^2 + 0.153(X_4)^2 \end{aligned}$$

Generally in ANOVA, large F-value and small *P* value show a more significant effect on response (Ghorbannezhad et al. 2016). Therefore, as is shown in Table 3, linear term of temperature had the most effect on the both responses. Linear terms of temperature and duty cycle, and quadratic terms of duty cycle and seed to solvent ratio had significant effect ($P < 0.05$) on TPC content. Linear and quadratic terms of temperature and time significantly affected on AA ($P < 0.05$) (Table 3). *P* value for lack-of-fit of both responses were insignificant ($P > 0.05$), which is conforming the model accuracy (Table 3).

Effects of UAE conditions on the TPC

Figure 1a, b shows the interactive effects between solid to solvent ratio and duty cycle on TPC content of OCE. TPC content was initially increased by increasing the solid to solvent ratio, but subsequently decreased, may probably be due to saturation of the solvent at the high solid to solvent ratios (Fig. 1a). This was in agreement with results of Yolmeh et al. (2014). They optimize UAE of annatto pigments and reported that the extraction yield was decreased at high solid to solvent ratios. As is shown in Fig. 1a, b, TPC content was initially increased by lengthen duty cycle up to about 0.6 s, but decreased at higher from 0.6 s duty cycles. This decreasing at high duty cycles is because of absence enough time to burst the bubbles created by ultrasound waves (Yolmeh and Najafzadeh 2014).

Figure 1c, d shows the interactive effects between extraction temperature and time on TPC content of OCE. The TPC content was increased by increasing extraction temperature. This increasing is could be attributed to increase kinetic energy of solvent and its penetrating power (Vinatoru 2001). This finding is in agreement with previous results about UAE of TPC from sugar beet molasses (Chen et al. 2015), *Aronia melanocarpa* (Ramić et al. 2015), blueberry (He et al. 2016), and star fruit (Annegowda et al.

Table 3 ANOVA of the selected models for TPC and AA of OCE

Source	Degrees of freedom	Mean of squares	F	P
<i>TPC</i>				
Regression	8	0.961	35.22	0.002
Linear	4	1.638	8.87	0.000
Temperature (X_1)	1	6.141	20.31	0.000
Time (X_2)	1	0.050	0.96	0.384
Duty cycle (X_3)	1	0.355	4.35	0.045
Solid to solvent ratio (X_4)	1	0.007	0.58	0.624
Square	4	0.285	4.95	0.026
(X_1) ²	1	0.058	1.51	0.142
(X_2) ²	1	0.035	1.24	0.215
(X_3) ²	1	0.863	5.73	0.014
(X_4) ²	1	0.120	4.60	0.038
Residual error	22	0.200	–	–
Lack-of-fit	16	0.274	3.50	0.096
Pure error	6	0.002	–	–
Total	30	–	–	–
<i>AA</i>				
Regression	14	20.194	40.51	0.000
Linear	4	27.502	4.98	0.000
X_1	1	35.284	6.05	0.000
X_2	1	65.670	4.24	0.044
X_3	1	9.004	1.42	0.145
X_4	1	0.050	1.33	0.170
Square	4	12.886	5.10	0.026
(X_1) ²	1	11.218	5.15	0.012
(X_2) ²	1	1.430	5.02	0.029
(X_3) ²	1	23.880	1.85	0.094
(X_4) ²	1	10.681	1.50	0.138
Residual error	12	45.753	–	–
Lack-of-fit	10	42.316	0.67	0.686
Pure error	2	5.920	–	–
Total	26	–	–	–

2012). The TPC content was slightly increased by lengthen the extraction process, as is shown in Fig. 1c, d.

Effects of UAE conditions on the AA

AA of OCE was increased by increasing extraction temperature and duty cycle, but decreased at high extraction temperatures and duty cycles (Fig. 2a, b). This decrease at high temperatures was probably caused by damaging effects of this condition on AA of phenolic compounds (Spigno et al. 2007). Similarly, Hammi et al. (2015) and Chen et al. (2015) reported that AA activity of phenolic compounds was decreased at high temperatures of UAE of Tunisian *Zizyphus lotus* fruits and sugar beet molasses, respectively. Singh et al. (2017) studied UAE of

polyphenols and their distribution in whole mung bean, hull and cotyledon, and reported that the AA of phenolic compounds was decreased at high sonication temperatures. The AA decreasing at high duty cycles is probably due to decreasing TPC of OCE at this condition (Fig. 2a, b). As shown in Fig. 2a, the maximum AA was achieved at extraction temperature of 55 °C and duty cycle of 0.6 s.

Figure 2c, d shows the interactive effects between extraction time and seed to solvent ratio on AA of OCE, when extraction temperature and duty cycle were kept at 47.5 °C and 0.5 s, respectively. AA was initially increased by increasing extraction time, but remarkably decreased at high extraction times (Fig. 2c, d). AA decreased probably due to the formation of sound chemical components at the high extraction times. These components might produce

Fig. 1 The interactive effects of solid to solvent ratio and duty cycle (a, b), and temperature and time (c, d) on the TPC of OCE

Fig. 2 The interactive effects of duty cycle and temperature (a, b), and time and solid to solvent ratio (c, d) on the AA of OCE

Table 4 Phenolic compounds (assayed using RP-HPLC) of OCE

Phenolic compound	Content (%)
Gallic acid	6.9 ± 0.13
Protocatechuic acid	19.5 ± 0.18
Hydroxybenzoic acid	3.9 ± 0.16
Vanillic acid	9.7 ± 0.19
Caffeic acid	2.8 ± 0.06
Syringic acid	5.1 ± 0.18
Sinapic acid	16.3 ± 0.24
Ferulic acid	6.5 ± 0.18
<i>p</i> -Coumaric acid	11.1 ± 0.25
Rutin	2.9 ± 0.08
Hesperidin	7.6 ± 0.22
Quercetin	6.1 ± 0.15
Cinnamic acid	1.6 ± 0.13

free radicals and thus have oxidative effect on phenolic compounds (Ma et al. 2009). Ghafoor and Choi (2009) and Zhang et al. (2011) also reported similar results for UAE of TPC and antioxidants from grape peel and UAE of ellagic acid from *Platycarya strobilacea*, respectively.

Optimization of the UAE process

UAE of OCE was optimized through numerical optimization technique which weight and importance values for both responses were considered same (Yolmeh and Jafari 2017). The extraction temperature of 56 °C, extraction time of 3 min, duty cycle of 0.6 s, and solid to solvent ratio of 3.6% were found as the optimum conditions of UAE for OCE. At this optimum condition, the TPC content and AA were acquired 4.34 mg/g and 73.5%, respectively as predicted results, and composite desirability was 80%.

Validation of optimizing process

The ability of selected models to optimize the UAE process was validated in the lab condition and the experimental results for TPC content and AA at the optimum UAE conditions were 4.04 mg/g and 68.9%, respectively. The experimental and predicted results revealed that the RSM algorithm successfully predicted the optimum conditions for UAE of OCE. Optimization of UAE of phenolic compounds have been successfully carried out for different agricultural products such as wheat bran (Wang et al. 2008), coconut (*Cocos nucifera*) shell powder (Rodrigues et al. 2008), *Euryale ferox* seed shells (Liu et al. 2013), and *Sparganii rhizoma* (Wang et al. 2013) through RSM.

RP-HPLC assay of phenolic compounds

Phenolic compounds profile of OCE is shown in Table 4. Protocatechuic acid and cinnamic acid were characterized as the highest (19.5%) and lowest (1.6%) phenolic compounds measured in OCE, as is shown in Table 4. Suárez et al. (2009) and Alu'datt et al. (2010) also studied phenolic compounds of olive cake, and reported similar phenolic compound profiles.

Conclusion

The results of this research showed that UAE is an effective method to extract phenolic compound from olive cake. The extraction temperature of 56 °C, extraction time of 3 min, duty cycle of 0.6 s, and solid to solvent ratio of 3.6% were found as the optimum UAE conditions for UAE of TPC from olive cake. The linear term of temperature had the most effect on TPC content and AA of OCE prepared by UAE.

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