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Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel

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ABSTRACT

There is a great demand for developing efficient extraction methods in order to reduce extraction time and increase the yield and activity of functional antioxidants. The yields, activities, and extraction kinetics of antioxidants from dry peel of pomegranate marc were studied using ultrasound-assisted extraction in continuous and pulsed modes and the results were compared with conventional extraction (CE) at a temperature of 25 ± 2 °C and water/peel ratio of 50/1, w/w. The studied factors were intensity level and treatment time for continuous ultrasound-assisted extraction (CUAE), and intensity level, number of pulse repetition, and pulse duration and interval for pulsed ultrasound-assisted extraction (PUAE). The results showed that all factors significantly affected the antioxidant yield, but only treatment time had a significant effect on the antioxidant activity. Compared to CE, PUAE at intensity level of 59.2 W/cm^2, and the 5 and 5 s of pulse duration and interval increased the antioxidant yield by 22% and reduced the extraction time by 87%. Similarly, CUAE at the same intensity level increased the antioxidant yield by 24% and reduced the extraction time by 90%. Since PUAE had 50% energy saving compared to CUAE, we recommend using PUAE for the extraction with antioxidant yield of 14.5% and DPPH scavenging activity of 5.8 g/g. A second-order kinetic model was successfully developed for describing the mechanism of ultrasound-assisted extractions under PUAE and CUAE. This research clearly demonstrated the superiority of PUAE for producing antioxidants from peel of pomegranate marc.

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1. Introduction

Reported researches have shown that pomegranate juice has nutritional and medical benefits such as antioxidative, anticancer, and antimutagenic efficacy [1–5]. Because of these benefits, its production in the United States has increased rapidly in recent years. The juice processing generates about 3.3 thousand tons of by-products each year in California alone. The by-product is normally called pomegranate marc, and either used as cattle feeds or directly disposed as wastes. Our measurements had shown that pomegranate marc contained 78% peel and 22% seeds on wet basis (w.b.) [6]. The results from our previous studies also showed that the peel had higher content of antioxidants than the seeds and could be a good source for producing high-value antioxidants [6]. Thus, the peel was used in this study for further improving the extraction performance.

Antioxidants can be extracted with various solvents and supercritical fluid extraction methods [7–9]. Our previous research had demonstrated that water was an environmentally friendly and efficient extraction solvent for producing antioxidants from pomegranate marc [6]. Therefore, water was also used as the extraction solvent in this research. In order to reduce the extraction time and improve the yield and activity of antioxidants, new extraction techniques need to be developed. Among the non-conventional extraction methods, the technology of ultrasound-assisted extraction has shown high extraction efficiency and low energy and solvent consumptions and thereby its usage as an alternative method has been on the rise [10]. For instance, the applications of ultrasonic technique in the extraction of bioactive compounds for producing functional additives or nutraceuticals have been reported [11–14]. The mechanism of ultrasound-assisted extraction is attributed to mechanical, cavitation, and thermal efficacies which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes [15–19]. However, no research has been found in the literature on the ultrasound-assisted extraction of antioxidants from pomegranate marc.

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Nomenclature

T	extraction time (min)
PC_e, PC_t	equilibrium concentration of total phenolics and total phenolic concentration in the liquid extract at a given extraction time t (g/L)
k	second-order extraction rate constant (L/g min)
H	initial extraction rate (g/L min)
C_d, C_e, C_f	2,2-diphenyl-1-picrylhydrazyl (DPPH) concentration equivalents in the control solution, sample solution, and blank solution (g/L)
N	dilution factor of the liquid extract
V_t	total volume of the liquid extract at a given extraction time t (L)
W_0	dry weight of sample (g)

The determination of kinetic parameters should be very important for designing efficient ultrasound-assisted extraction process for antioxidant production from pomegranate marc. However, no relevant kinetic model of ultrasound-assisted antioxidant extraction was reported. Because the mechanism of ultrasound-assisted extraction is expected to be similar as conventional solid–liquid extraction, but with enhanced extraction, the second-order kinetic model applied in conventional extraction [20,21] could be used to model the ultrasound-assisted extraction in this research.

The objectives of this research were to (1) study the effects of processing factors of ultrasound-assisted extraction on the yield, activity, and extraction kinetics of antioxidants (total phenolics in terms of tannic acid equivalents) from pomegranate peel; (2) compare the performances of ultrasound-assisted extraction with conventional extraction and determine the optimum extraction conditions; and (3) determine the kinetic parameters that describe the mechanism of ultrasound-assisted extraction. The ultrasound-assisted extraction was conducted using two different modes, continuous mode and pulsed mode.

2. Materials and methods

2.1. Materials, reagents, and equipment

Pomegranate marc was obtained from a commercial pomegranate juice processor (Stiebs Pomegranate Products, Madera, CA, USA) after the juice processing of pomegranate fruit (Wonderful variety). It was stored at −18 °C at the Western Regional Research Center, United States Department of Agriculture–Agricultural Research Service (USDA–ARS) in Albany, California until use. Prior to the experiment, pomegranate marc was thawed at 4 °C and then dried at 40 °C using hot air in a cabinet drier (CPM Wolverine Proctor LLC, Horsham, PA, USA). The dried peel was manually separated from the seeds and then ground using a hammer mill (WBB-6, Gruendler Pulverizing Co., Saint Louis, MO, USA) to achieve the particle size less than 40-mesh. The moisture content of peel powder determined with an oven method by drying to a constant weight at 105 °C [22] was 11.7% (w.b.).

Folin–Ciocalteu reagent, tannic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich Company (Saint Louis, MO, USA). Methanol and sodium carbonate (Na_2CO_3) were obtained from Fisher scientific Inc. (Pittsburgh, PA, USA). All the reagents were of analytical grade.

The sonicator (Sonicator 3000, Misonix, Inc., Farmingdale, NY, USA) used in this study has a constant frequency of 20 kHz, a probe with area of 1.267 cm^2, and intensity levels (power per unit area of the sonicator probe) ranged from 2.4 to 59.2 W/cm^2, and can be operated in continuous and pulsed modes. During the extraction process, the sample container was held in a thermostat-controlled water bath at temperature of 25 ± 2 °C and the water/peel ratio was 50/1, w/w, unless specified otherwise. The experimental conditions were determined based on our preliminary tests. The sample container was covered with an aluminum-foil paper to prevent oxidative change from light. The liquid extracts from all samples were also separated and then the antioxidants were analyzed by using the same methods described in the Sections 2.2. and 2.6.

2.2. Extraction performance of continuous ultrasound-assisted extraction

The continuous ultrasound-assisted extraction (CUAE) of antioxidants was performed in the sonicator under continuous mode with various intensity levels of 2.4, 4.7, 7.1, 18.9, 23.7, 30.8, 37.9, 45.0, 52.1, and 59.2 W/cm^2 and the experimental design is shown in Table 1. The extraction times were 2, 10, 20, 30, 60, and 90 min. The liquid extract was separated from the residue by centrifugation (Marathon 21000R, Fisher Scientific Inc., Pittsburgh, PA, USA) at 3500 rpm for 20 min at 4 °C. The amount of antioxidants (in terms of tannic acid equivalents) in the liquid extract was analyzed to quantify the yield and DPPH scavenging activity.

2.3. Extraction performance of pulsed ultrasound-assisted extraction

The pulse duration and pulse interval refer to "on" time and "off" time of the sonicator. The total time of a pulse duration period plus a pulse interval period is the cycle time. A duty cycle (expressed as a percentage) is the proportion of the pulse duration period relative to the cycle time. The number of pulse repetition denotes the number of cycle during the entire extraction time. Thus, the total extraction time is calculated by multiplying the cycle time by the number of pulse repetition.

For the pulsed ultrasound-assisted extraction (PUAE), the processing factors, including intensity level, number of pulse repetition, and pulse duration and interval were studied using the sonicator under pulsed mode following the experimental design shown in Table 1.

2.4. Comparison of extraction performance

The performances of ultrasound-assisted extractions in continuous and pulsed modes were evaluated and compared with conventional extraction (CE) with the total extraction times of 2, 10, 20, 30, 60, and 90 min (Table 1). CE was aided by the use of a magnetic stirring device (Isotemp, Fisher Scientific Inc., Pittsburgh, PA, USA) with stirring speed of 1200 rpm.

2.5. Kinetic model of ultrasound-assisted extraction

The study determined the kinetic parameters of ultrasound-assisted extraction, such as extraction rate constant, which are important for evaluating the extraction potential of antioxidants from the pomegranate peel. In order to quantify the extraction rate (total phenolic concentration gain per unit of extraction time), the

Table 1
Experimental design of extraction performance.

Design	Extraction method	Intensity level (W/cm^2)	Pulsed duration/interval (s/s)	Number of pulse repetition	Extraction time (min)
	Continuous ultrasound-assisted extraction	2.4, 4.7, 7.1, 18.9, 23.7, 30.8, 37.9, 45.0, 52.1, 59.2			2, 10, 20, 30, 60, 90
	Pulsed ultrasound-assisted extraction	2.4, 4.7, 7.1, 18.9, 23.7, 30.8, 37.9, 45.0, 52.1, 59.2	5/5	360	
		59.2	2/1, 3/1, 4/1, 5/1, 6/1, 7/1, 9/1, 12/1, 2/5, 3/5, 4/5, 5/5, 6/5, 7/5, 9/5, 12/5, 2/15, 3/15, 4/15, 5/15, 6/15, 7/15, 9/15, 12/15,	360	
		59.2	5/1, 5/5, 5/15	30, 60, 90, 120, 180, 270, 360, 540, 720	
Comparison	Continuous ultrasound-assisted extraction	59.2			2, 10, 20, 30, 60, 90
	Pulsed ultrasound-assisted extraction	30.8	5/5		2, 10, 20, 30, 60, 90
		59.2	2/2		2, 10, 20, 30, 60, 90
		59.2	5/5		2, 10, 20, 30, 60, 90
		59.2	5/15		2, 10, 20, 30, 60, 90
	Conventional extraction				2, 10, 20, 30, 60, 90

second-order rate law applied in conventional extraction study [20,21] was used. The general second-order model can be written as:

$$\frac{d(PC_t)}{dt} = k(PC_e - PC_t)^2 \quad (1)$$

where, k is the second-order extraction rate constant (L/g min), PC_e is the equilibrium concentration of total phenolics in the liquid extract (g/L), and PC_t is the total phenolic concentration in the liquid extract at a given extraction time t(g/L).

The integrated rate law for a second-order extraction under the boundary conditions $t = 0$ to t and $PC_t = 0$ to PC_t, can be written as an Eq. (2) or a linearized Eq. (3):

$$PC_t = \frac{(PC_e)^2 kt}{1 + kt(PC_e)} \quad (2)$$

$$\frac{t}{PC_t} = \frac{1}{k(PC_e)^2} + \frac{t}{PC_e} \quad (3)$$

The initial extraction rate, h (g/L min), when t approaches 0, can be defined as:

$$h = k(PC_e)^2 \quad (4)$$

The h, PC_e, and k were determined by the Eq. (3) using Origin Pro 7.5SR1 (V 7.5776, Originlab Corporation, Northampton, MA, USA) and Eq. (4).

Table 2
Experimental and ANOVA results of total phenolic yields from pomegranate peel at different intensity levels and treatment times obtained by continuous ultrasound-assisted extraction.

Treatment time (min)	Intensity level (W/cm2)										
	2.4	4.7	7.1	18.9	23.7	30.8	37.9	45.0	52.1	59.2	Overall
2	2.7dE[a]	2.9fE	3.2eE	4.4dD	4.5dD	4.9eCD	5.5eBCD	5.7dBC	6.5 dB	8.5cA	4.9f
10	5.0cF	5.3eF	6.3dE	7.6cD	8.0cD	9.2dC	10.6 dB	10.2cB	10.2cB	13.1bA	8.6e
20	6.4bE	6.8dE	8.9cD	10.8bC	10.9bC	11.0cdC	11.2cdBC	12.6bAB	12.8bAB	13.5bA	10.5d
30	8.4aD	9.6cCD	11.1bBC	11.1bBC	11.2bBC	12.7bcAB	12.5bcAB	12.4bAB	12.4bAB	13.7bA	11.5c
60	8.1aD	12.2bC	12.0abBC	13.6aAB	14.1aA	14.2aA	14.0abA	14.0aA	14.1aA	14.8aA	13.1b
90	8.2aD	13.5aBC	13.1aC	13.8aABC	14.0aABC	14.3aABC	14.4aAB	14.3aABC	14.5aAB	14.8aA	13.5a
Overall	6.5G	8.4F	9.1E	10.2D	10.4D	11.0C	11.4CB	11.5CB	11.7B	13.1A	

Factor	Degree of freedom	Sum of squares	Mean square	F value	$Pr > F$
Model	59	3017.865	51.150	148.64	<0.0001[b]
Intensity level	9	787.014	87.446	254.11	<0.0001[b]
Treatment time	5	2087.139	417.428	1213.00	<0.0001[b]
Intensity * time	45	143.712	3.194	9.28	<0.0001[b]
Error	180	61.943	0.344		
R^2	97.989%				
Coefficient Variation	5.673				
RMSE	0.587				

[a] The different letters in lower case in the same column mean significant difference at $P < 0.05$; the different letters in upper case in the same row mean significant difference at $P < 0.05$.
[b] The $Pr > F$ value lower than 0.05 means significant difference.

2.6. Analysis assay

2.6.1. Determination of antioxidant yield

The amount of antioxidants in the extracts was determined using the total phenolics in terms of tannic acid equivalents, according to a modified Folin–Ciocalteu method [23]. An extract sample of 60 μL was mixed with 2 mL of Na_2CO_3 (7.5%), and 2.5 mL of 10-fold diluted Folin–Ciocalteu reagent thoroughly using a vortex mixer (K-550-G Vortex-Genie, Scientific Industries Inc., Bohemia, NY, USA). The mixed solution was held in a water bath for 30 min at 25 °C and then its absorbance was measured at 760 nm using a spectrophotometer (Genesys 10Bio UV–Visible spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Three measurements were conducted for each liquid sample and the test was replicated three times. The blank was prepared using the above procedure, but the extract was replaced by the same volume of DI water. The total phenolic yield%, was calculated using Eq. (5):

$$\text{Total phenolic yield} = \frac{PC_tV_t}{100W_0} \times 100\% \quad (5)$$

where, V_t is the volume of the liquid extract at a given extraction time t (L) and W_0 is the dry weight of sample (g).

2.6.2. Determination of antioxidant activity

The antioxidant activity was determined using the DPPH equivalent, according to an adapted colorimetric procedure [9]. An extract sample of 60 μL was reacted with 3 mL of DPPH solution in methanol (0.05 g/L). The sample solution was mixed thoroughly using a vortex mixer and held in a water bath for 20 min at 25 °C. The sample absorbance was measured at 517 nm using a spectrophotometer. Three measurements were conducted for each liquid sample and the test was replicated three times. The control solution included 60 μL of DI water and 3 mL of DPPH solution in methanol (0.05 g/L). The blank solution contained 60 μL of extract and 3 mL of methanol. The DPPH scavenging activity g/g, was calculated using Eq. (6):

$$\text{DPPH scavenging activity} = \frac{nV_t[C_d - (C_e - C_f)]}{PC_tV_t} \quad (6)$$

where C_d is the DPPH concentration equivalent in the control solution (g/L), C_e is the DPPH concentration equivalent in the sample solution (g/L), C_f is the DPPH concentration equivalent in the blank solution (g/L), and n is the dilution factor of the liquid extract.

All reported weights and percentages were dry basis (d.b.) unless specified otherwise. All extraction trials were carried out in triplicate and the reported results are averages.

2.7. Statistical analysis

Tukey's studentized range (HSD) test, using a SAS software package (Ver. 9.2., SAS Institute Inc., Cary, NC, USA) was performed to determine if there were significant differences in the total phenolic yields and DPPH scavenging activities of antioxidants at various intensity levels and treatment times during CUAE. The significance was determined using least significant difference (LSD) ($\alpha = 0.05$).

3. Results and discussion

3.1. Extraction performance of continuous ultrasound-assisted extraction

Table 2 shows the yields of antioxidants (total phenolics) from pomegranate peel at different intensity levels and treatment times for CUAE processing. It can be seen that the total phenolic yields significantly improved with increased intensity level (2.4–59.2 W/cm^2) and treatment time (2–90 min) ($P < 0.05$). It is believed that the increase in total phenolic yield was mainly due to the improved cavitation and mechanical effect of ultrasound which increased the contact surface area between solid and liquid phases and caused greater penetration of solvent into the peel matrix. A similar study reported that ultrasonic powers from 3.2 to 56 W significantly increased the yield of extracted phenolic compounds from Satsuma Mandarin peels by 58–82% with increased treatment times ranging from 10 to 60 min [24]. Similar results were also found for extraction of anthocyanin and ascorbic acid [25]. The ANOVA results (Table 2) further shows that intensity level, treatment time, and their interaction had significant and positive effects on the total phenolic yield because the $Pr > F$ value and coefficient of determination (R^2) of the developed model were <0.05 and 97.989%, respectively. The results indicated that the ultrasound-assisted extraction in continuous mode was effective, and higher

Table 3
Experimental and ANOVA results of DPPH scavenging activities of antioxidants from pomegranate peel at different intensity levels and treatment times obtained by continuous ultrasound-assisted extraction.

Treatment time (min)	Intensity level (W/cm2)										
	2.4	4.7	7.1	18.9	23.7	30.8	37.9	45.0	52.1	59.2	Overall
2	6.3aA[a]	6.1aA	6.6aA	7.0aA	6.8aA	6.5aA	5.9aA	6.2abA	5.9aA	6.0aA	6.3a
10	6.8aAB	6.1aABC	7.2aA	5.4abBC	5.9aABC	5.5aBC	5.3aBC	6.6aABC	5.1aC	6.6aABC	6.0ab
20	5.7aA	6.2aA	5.6aA	5.0bA	4.8aA	5.8aA	5.8aA	5.8abA	5.3aA	6.1aA	5.6bc
30	5.0aA	5.9aA	5.1aA	5.0bA	5.5aA	5.1aA	5.5aA	6.4aA	5.1aA	5.7aA	5.4c
60	5.6aA	5.6aA	5.6aA	5.5abA	5.5aA	5.6aA	5.6aA	5.3abA	5.5aA	5.6aA	5.5bc
90	4.6aB	5.2aA	4.9aA	5.1bA	5.0aA	5.0aA	5.2aA	5.0bA	4.4aA	4.3aA	4.9d
Overall	5.7A	5.8A	5.8A	5.5A	5.6A	5.6A	5.6A	5.9A	5.2A	5.7A	

Factor	Degree of freedom	Sum of squares	Mean square	F value	$Pr > F$
Model	59	47.285	0.801	2.36	0.0006[b]
Intensity level	9	4.319	0.480	1.42	0.2019
Treatment time	5	25.925	5.185	15.30	<0.0001[b]
Intensity * time	45	17.042	0.379	1.12	0.3409
Error	60	20.336	0.339		
R^2	69.926%				
Coefficient Variation	10.332				
RMSE	0.582				

[a] The different letters in lower case in the same column mean significant difference at $P < 0.05$; the different letters in upper case in the same row mean significant difference at $P < 0.05$.
[b] The $Pr > F$ value lower than 0.05 means significant difference.

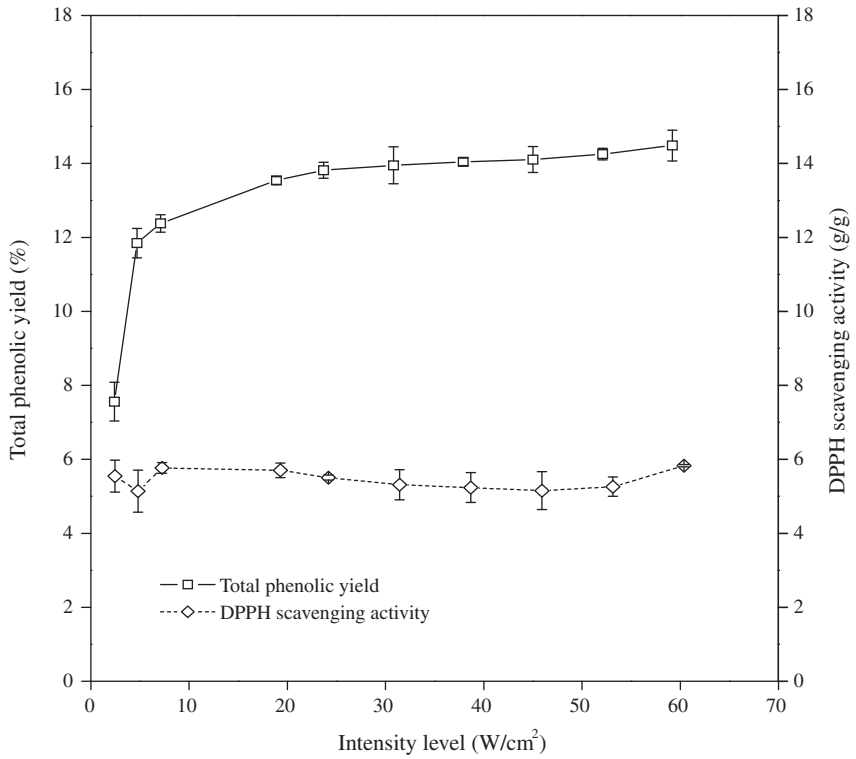

Fig. 1. Total phenolic yields and DPPH scavenging activities of antioxidants from pomegranate peel at different intensity levels by pulsed ultrasound-assisted extraction with the number of pulse repetition of 360 and 5 s of both pulse duration and interval.

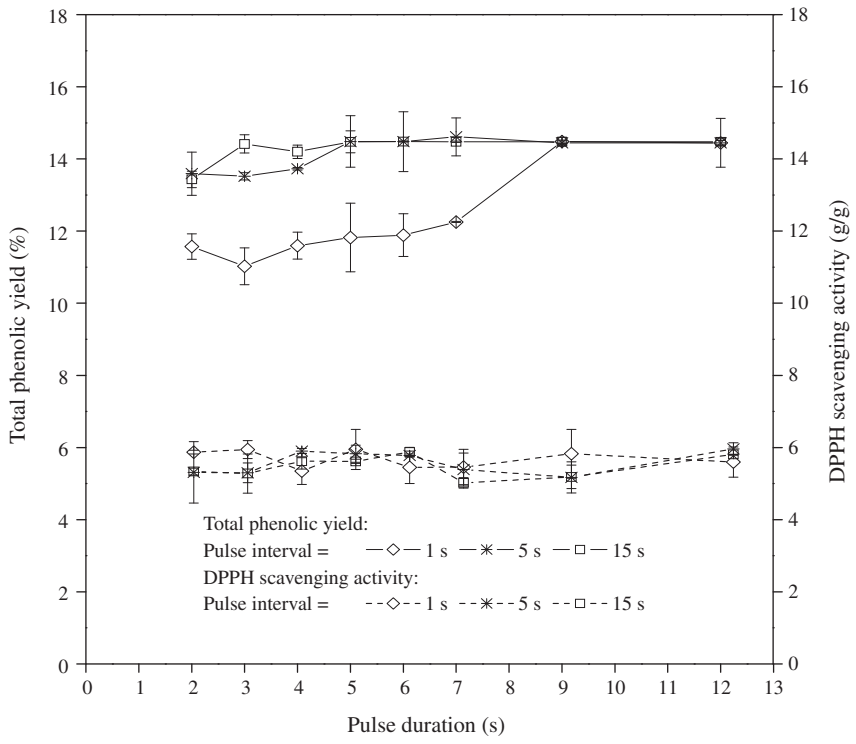

Fig. 2. Total phenolic yields and DPPH scavenging activities of antioxidants from pomegranate peel at different pulse durations and intervals by pulsed ultrasound-assisted extraction with intensity level of 59.2 W/cm^2 and number of pulse repetition of 360.

intensity level and longer treatment time were beneficial to the extraction process of antioxidants from pomegranate peel. The highest yield of antioxidants was 14.8% and obtained at the intensity level of 59.2 W/cm^2 and treatment time of 60 min for CUAE.

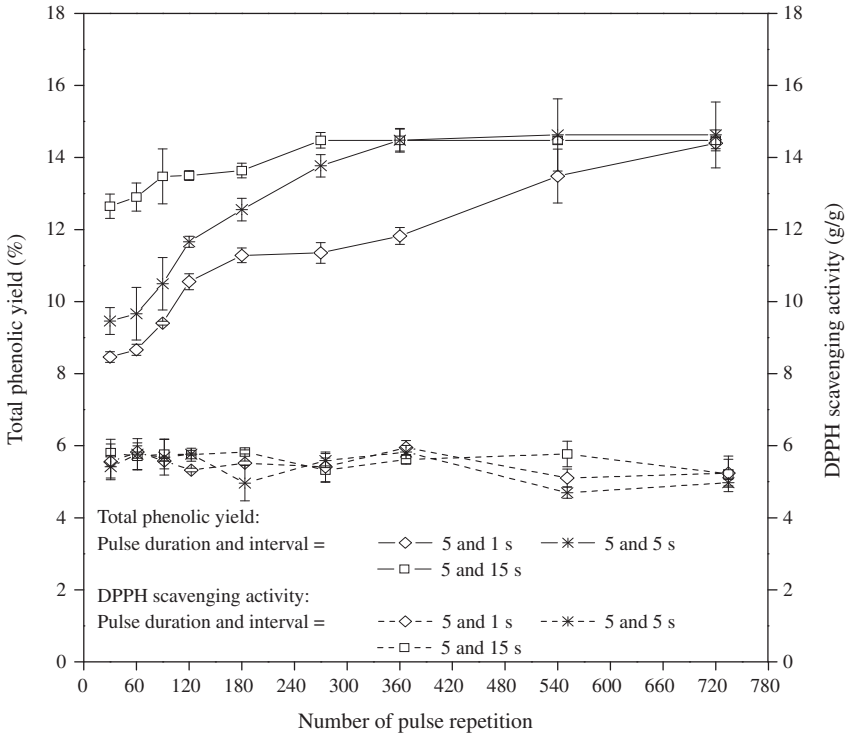

Fig. 3. Total phenolic yields and DPPH scavenging activities of antioxidants from pomegranate peel at different numbers of pulse repetition by pulsed ultrasound-assisted extraction with intensity level of 59.2 W/cm^2 and three combinations of pulse durations and intervals, 5 and 1, 5 and 5, and 5 and 15 s.

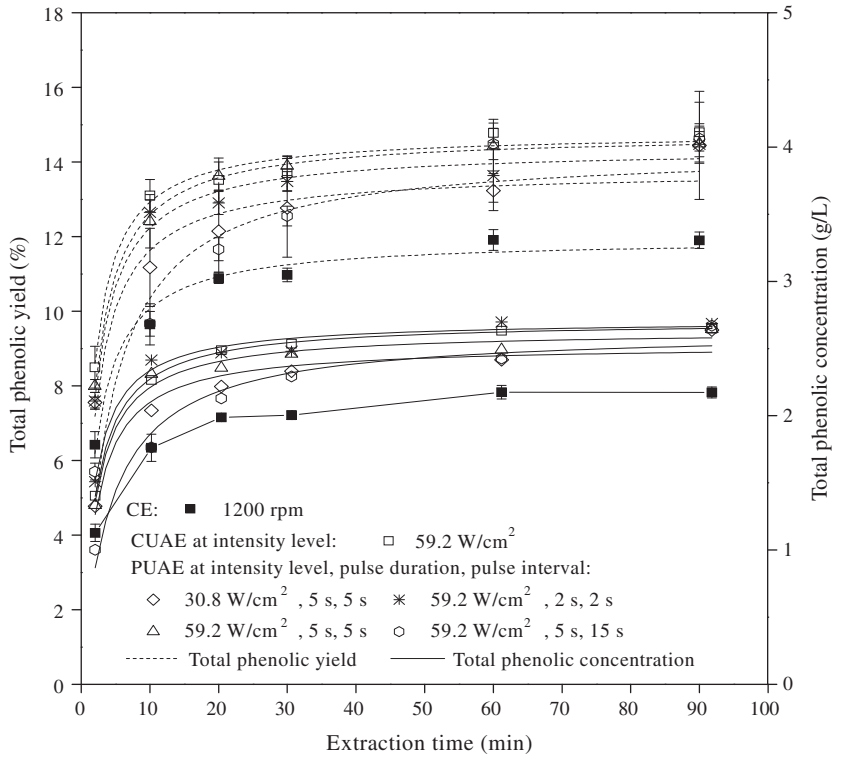

Fig. 4. Comparison of total phenolic yields and concentrations of antioxidants from pomegranate peel at different extraction times for continuous ultrasound-assisted extraction (CUAE), pulsed ultrasound-assisted extraction (PUAE), and conventional extraction (CE) (Dash lines are the non-linear fitting curves).

Table 3 lists the DPPH scavenging activities of antioxidants from pomegranate peel at different intensity levels and treatment times by CUAE. Statistical analysis showed that the overall antioxidant activities significantly decreased from 6.3 to 4.9 g/g with increased treatment times from 2 to 90 min, but did not significantly change with increased intensity levels ($P < 0.05$). A similar trend had been

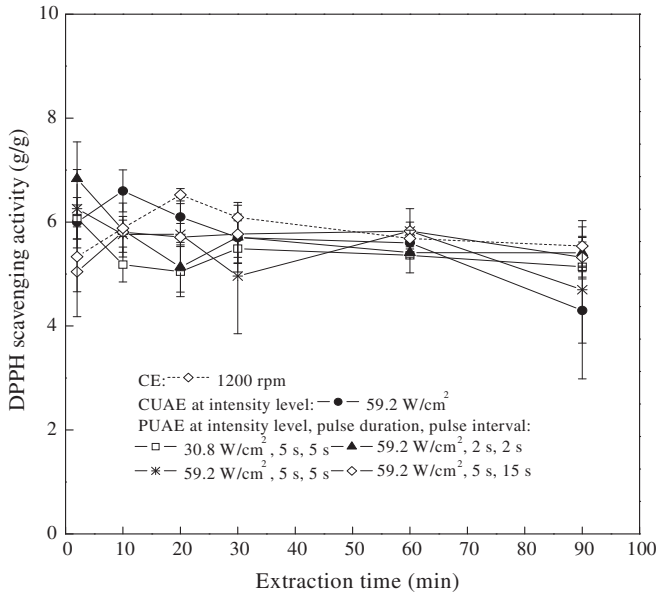

Fig. 5. Comparison of DPPH scavenging activities of antioxidants from pomegranate peel at different extraction times for continuous ultrasound-assisted extraction (CUAE), pulsed ultrasound-assisted extraction (PUAE), and conventional extraction (CE).

reported on the minimal degradation of anthocyanin content, color and ascorbic acid in orange juice caused by increased ultrasonic power [25,26]. The ANOVA results further verified that antioxidant activity was only sensitive to the treatment time. The observed decrease of antioxidant activity with treatment time could be due to cavitation, which involves the formation, growth, and collapse of microscopic bubbles.

3.2. Extraction performance of pulsed ultrasound-assisted extraction

Figs. 1–3 respectively show the effects of intensity levels, pulse durations and intervals, and numbers of pulse repetition on the yields and activities of antioxidants for PUAE. It can be seen that antioxidant activities fluctuated with all these factors and ranged from 5.0 to 6.0 g/g. However, the total phenolic yields varied with different extraction conditions.

The total phenolic yields rapidly increased from 7.6% to 12.4% when the intensity level changed from 2.4 to 7.1 W/cm^2 and then the highest yield of 14.5% was achieved at 59.2 W/cm^2 (Fig. 1). A positive correlation between the total phenolic yield and intensity level was observed during PUAE. A similar trend was also observed in the case of CUAE. This is consistent with previous finding from Ma et al. [27] who reported that the yield of phenolic compounds from citrus peel significantly depended on ultrasonic intensity. Based on the results of the present study, high intensity level benefited the extraction process and therefore the intensity level of 59.2 W/cm^2 was used in the study on the processing performance of other factors.

The data of total phenolic yields clearly showed that the equilibrium was not reached with the combination of short pulse duration and interval (Fig. 2). Because the repetitions were the same for the different combinations of pulse duration and interval, a short interval indicates a short total processing time which did not allow sufficient time for completing the mass transfer. The results indicated that an optimal combination of pulse duration and interval was critical for reducing the overall processing time and energy. Based on the obtained present results, when the intensity level was 59.2 W/cm^2 and the number of pulse repetition was 360, three

Table 4
Comparison of extraction times and total phenolic yields from pomegranate peel for continuous ultrasound-assisted extraction (CUAE), pulsed ultrasound-assisted extraction (PUAE), and conventional extraction (CE).

Mode	Condition (intensity level, pulse duration and interval)	Extraction time[a]	Total phenolic yield[c]
CUAE	59.2 W/cm^2	6 min (90%)[b]	14.8% (24%)[d]
	52.1 W/cm^2	15 min (75%)	14.1% (18%)
	45.0 W/cm2	16 min (73%)	14.0% (18%)
	37.9 W/cm^2	18 min (70%)	14.0% (18%)
	30.8 W/cm^2	22 min (63%)	14.2% (19%)
	23.7 W/cm^2	29 min (52%)	14.1% (18%)
	18.9 W/cm^2	32 min (47%)	13.6% (14%)
	7.1 W/cm^2	50 min (17%)	12.0% (1%)
	4.7 W/cm^2	56 min (7%)	12.2% (3%)
	2.4 W/cm^2	>60 min	8.1%
PUAE	30.8 W/cm^2, 5 s, 5 s	12 min (80%)	13.2% (11%)
	59.2 W/cm^2, 2 s, 2 s	9 min (85%)	13.6% (14%)
	59.2 W/cm^2, 5 s, 5 s	8 min (87%)	14.5% (22%)
	59.2 W/cm^2, 5 s, 15 s	19 min (68%)	14.5% (22%)
CE		60 min	11.9%

[a] Extraction time denotes the extraction time at total phenolic yield of 11.9%, predicted by the second-order kinetic model.
[b] Values in the parenthesis are the extraction time reduction compared to the extraction time of 60 min from conventional extraction.
[c] Total phenolic yield denotes the total phenolic yield at extraction time of 60 min.
[d] Values in the parenthesis are the antioxidant yield increase compared to total phenolic yield of 11.9% from conventional extraction.

recommended combinations of pulse duration and interval were 9 and 1 s, 5 and 5 s, and 3 and 15 s. The three combinations corresponded to the cycle times of 10, 10, and 18 s, and duty cycles of 90%, 50%, and 16.7%. Similar total phenolic yields (14.4–14.5%) were achieved with the corresponding total extraction times of 60, 60, and 108 min. Thus, the first two combinations gave higher extraction rates than the third one. To obtain high extraction rate with low energy consumption, the second combination was considered as the best for producing antioxidants from pomegranate peel.

Regarding the effects of the numbers of pulse repetition (Fig. 3), we observed that total phenolic yields increased initially and then reached equilibrium with increased numbers of pulse repetition. It needed more numbers of repetition to reach equilibrium total phenolic yield with the combination of pulse duration and interval of 5 and 1 s compared to the combination of 5 and 5 s, and 5 and 15 s. The required repetitions to reach equilibrium total phenolic yields were 720, 360, and 270 for the three combinations of pulse duration and interval, 5 and 1 s, 5 and 5 s, and 5 and 15 s, respectively, when the intensity level was 59.2 W/cm^2. The three combinations had corresponding cycle times of 6, 10, and 20 s, duty cycles of 83.3%, 50%, and 25%, and total extraction times of 72, 60, and 90 min, and achieved similar total phenolic yields (14.4–14.6%). The second combination had low energy consumption and the highest extraction rate, which was also in agreement with the finding regarding the effects of pulse duration and interval.

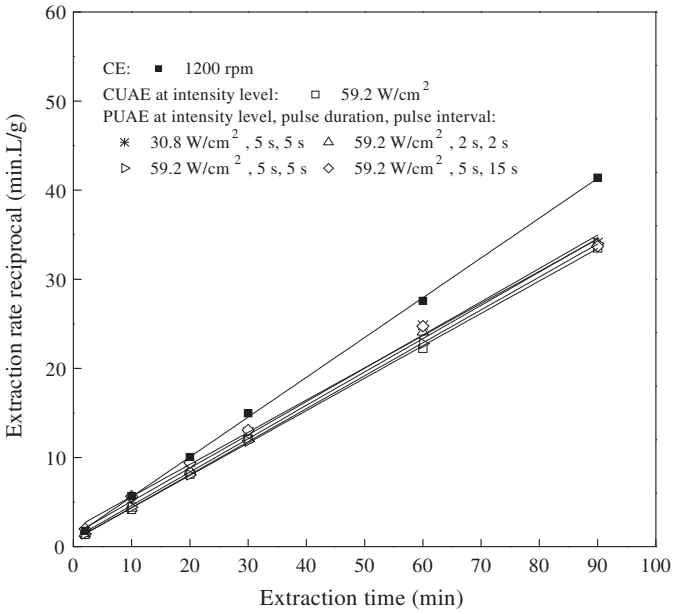

Fig. 6. Comparison of extraction rate reciprocal (t/C_t) of antioxidants from pomegranate peel at different extraction times (t) for continuous ultrasound-assisted extraction (CUAE), pulsed ultrasound-assisted extraction (PUAE), and conventional extraction (CE).

Table 5
Comparison of kinetic parameters of antioxidants from pomegranate peel for continuous ultrasound-assisted extraction (CUAE), pulsed ultrasound-assisted extraction (PUAE) and conventional extraction (CE).

Mode	Condition (intensity level, pulse duration and interval)	Initial extraction rate, h (g/L min)	Extraction rate constant, k (L/g min)	Equilibrium concentration of total phenolics, PC_e (g/L)	R^2
CUAE	59.2 W/cm^2	1.398	0.185	2.7	0.999
	52.1 W/cm^2	0.645	0.085	2.8	0.995
	45.0 W/cm^2	0.621	0.083	2.7	0.971
	37.9 W/cm^2	0.539	0.070	2.8	0.995
	30.8 W/cm^2	0.455	0.058	2.8	0.996
	23.7 W/cm^2	0.353	0.045	2.8	0.995
	18.9 W/cm^2	0.346	0.046	2.7	0.999
	7.1 W/cm^2	0.246	0.035	2.7	0.999
	4.7 W/cm^2	0.124	0.018	2.6	0.999
	2.4 W/cm^2	0.031	0.012	1.6	0.999
PUAE	30.8 W/cm^2, 5 s, 5 s	0.746	0.104	2.7	0.997
	59.2 W/cm^2, 2 s, 2 s	1.128	0.158	2.7	0.999
	59.2 W/cm^2, 5 s, 5 s	1.456	0.199	2.7	0.999
	59.2 W/cm^2, 5 s, 15 s	0.498	0.065	2.8	0.957
CE		0.882	0.176	2.2	0.999

In general, the results indicated that all factors, including intensity level, combination of pulse duration and interval, and number of pulse repetition had prominent effect on antioxidant yields, but not much effect on antioxidant activities. Antioxidants obtained from PUAE at the intensity level of 59.2 W/cm^2, number of pulse repetition of 360, and pulse duration and interval of 5 and 5 s had the highest yield of 14.5% and DPPH scavenging activity of 5.8 g/g at extraction time of 60 min.

3.3. Performance comparison of different extraction methods

Fig. 4 shows the yields and concentrations of antioxidants from pomegranate peel at different extraction times under CUAE, PUAE, and CE. Because the result from extraction performance of CUAE showed that high intensity level performed the best, the only intensity level of 59.2 W/cm^2 was used for CUAE in this part of study. The curves of total phenolic yields with extraction times were nonlinearly fitted by transforming Eq. (2), using Origin software. All of the extraction methods show similar characteristics of two stage extraction: the first stage involves the dissolution of soluble constituents near particle surfaces into the water and is characterized by a rapid extraction rate; the second stage involves mass transfer of soluble constituents from the internal material into the solvent by diffusion process and is characterized by a slow extraction rate [28]. However, ultrasound-assisted extractions in both modes had high extraction rates (total phenolic concentration gain per unit of extraction time), yields, and concentrations of total phenolics compared to CE. Similarly, it was reported that the application of ultrasound assisted extraction improved the extraction yield of bioactive compounds by 6–35% compared to conventional processing [29]. When the intensity level was 59.2 W/cm^2 and extraction time was 60 min, the highest yields were 14.5% and 14.8% for PUAE with a combination of pulse duration and interval of 5 and 5 s and CUAE, respectively. PUAE with other combinations or conditions had lower total phenolic yield due to the reason described in Section 3.2.

Fig. 5 shows the DPPH scavenging activities of antioxidants produced with the three extraction methods at different extraction times. All DPPH scavenging activities of antioxidants fluctuated with the increase of extraction times but did not have much difference among the three extraction methods.

Table 4 shows the extraction times needed for all three methods to achieve total phenolic yield of 11.9% which was achieved by CE at extraction time of 60 min. Compared to CE, the reductions of extraction time and yield increases of ultrasound-assisted extractions are also shown in Table 4. It clearly shows that CUAE with high intensity level is preferred with minimum extraction time and high total phenolic yield. In addition, we observed that the temperature of extraction sample can be easily controlled under PUAE than CUAE due to less heat generation and accumulation.

3.4. Extraction kinetics of different extraction methods

Fig. 6 presents the linearized forms of the second-order model for the three different extraction methods. The kinetic parameters were determined from the slope and intercept by plotting t/PC_t against t and listed in Table 5. The results showed that the h, k, and PC_e were affected by processing factors for CUAE and PUAE. At intensity level of 59.2 W/cm^2, PUAE with pulse duration and interval of 5 and 5 s and CUAE had higher values of h and k than CE. This verified that the ultrasound-assisted extraction in either continuous mode or pulsed mode could greatly improve the extraction rates of antioxidants from pomegranate peel. The second-order model fitted well the experimental results because of the obtained high coefficient of determination (R^2 = 0.957–0.999). Thus, the second-order model applied in conventional extraction can be used in describing the mechanism of ultrasound-assisted extraction in either continuous mode or pulsed mode.

4. Conclusions

This research studied the yields, activities, and extraction kinetics of antioxidants from pomegranate peel using ultrasound-assisted extractions in continuous and pulsed modes and the results were compared with conventional extraction. The results showed that high intensity level and long extraction time in CUAE

significantly benefited the antioxidant yields, but treatment time had negative effect on antioxidant activity when extraction was too long. For PUAE, intensity level, number of pulse repetition, and pulse duration and interval greatly affected the antioxidant yields, but not the antioxidant activities. At intensity level of 59.2 W/cm^2, PUAE with the pulse duration and interval of 5 and 5 s and CUAE had similar antioxidant yields (14.5% and 14.8%) and DPPH scavenging activities (5.8 and 5.5 g/g) at extraction time of 60 min, temperature of 25 ± 2 °C, and water/peel ratio of 50/1, w/w. CUAE and PUAE increased the antioxidant yield by 24% and 22% and reduced the extraction time by 90% and 87%, respectively, compared to CE. PUAE saved 50% of electrical energy compared to CUAE. Because of low electrical energy consumption, high extraction time reduction, antioxidant yield increase and antioxidant activity, PUAE was clearly superior to CUAE. A second-order kinetic model was successfully applied to describe the mechanism of ultrasound-assisted extraction.

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