XVII[th] World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

CONTINUOUS AND PULSED ULTRASOUND-ASSISTED EXTRACTIONS OF ANTIOXIDANTS FROM POMEGRANATE PEEL

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CSBE101304 – Presented at Section VI: Postharvest Technology and Process Engineering Conference

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 ºC 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%. PUAE resulted in the antioxidant yield of 14.5%, DPPH scavenging activity of 5.8 g/g, and energy saving of 50% compared to CUAE. 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 dry peel of pomegranate marc.

Keywords: Pomegranate peel, Total phenolics, Ultrasonic extraction, Antioxidant activity, Kinetics.

INTRODUCTION Reported researches have shown that pomegranate juice has nutritional and medical benefits such as antioxidant, anticancer, and antimutagenic efficacy (Adams et al., 2006; Adhami et al., 2006; Faria et al., 2007; Heber et al., 2007; Yasoubi et al., 2007). Because of the 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 feed or directly disposed as waste. Our measurement showed that pomegranate marc contained 78% peel and 22% seeds on wet basis (w.b.) (Qu et al., 2009). The results from our previous studies had shown that the peel had higher content of antioxidants than the seeds and could be a good source for producing high-value antioxidants (Qu et al., 2009). Thus, the peel was used in this study for further improving the extraction performance.

Our previous research demonstrated that water was an environmentally friendly and efficient extraction solvent for producing antioxidants from pomegranate marc (Qu et al., 2009). 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 (Kang et al., 2006). For instance, the applications of ultrasonic technique in the extraction of bioactive compounds for producing functional additives or nutraceuticals have been reported (Li et al., 2002; Palma et al., 2002; Rodrigues et al., 2007; Rodrigues et al., 2008). 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 (Miller, 1981; Paniwnyk et al., 2001; Riera et al., 2004; Torres et al., 2008; Vinatoru et al., 1999). However, no research has been found in the literature on the ultrasound-assisted extraction of antioxidants from pomegranate marc.

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 (Rakotondramasy-Rabesiaka et al., 2007; Rakotondramasy-Rabesiaka et al., 2009) 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.

MATERIALS AND METHODS

Materials 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 ºC until use. Prior to the experiment, pomegranate marc was thawed at 4 ºC and then dried at 40 ºC 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 ºC (Apha et al., 1998) was 11.7% (w.b.).

Reagents 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.

Equipment 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 0.635 cm^2, and intensity levels (power per unit area of the sonicator probe) ranged from 2.4 W/cm^2 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 ºC 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 separated from the residue by centrifugation (Marathon 21000R, Fisher scientific Inc., Pittsburgh, PA, USA) at 3500 rpm at 4 ºC for 20 min. The amount of antioxidants (in terms of tannic acid equivalents) in the liquid extract was analyzed to quantify the yield and DPPH scavenging activity in the Analysis assay.

Extraction performance of continuous ultrasound-assisted extraction The continuous ultrasound-assisted extraction (CUAE) of antioxidants was performed in the sonicator under continuous mode and the experimental design is shown in Table 1.

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 the pulsed mode following the experimental design shown in Table 1.

Comparison of extraction performance The performances of ultrasound-assisted extractions in continuous and pulsed modes were evaluated and compared with conventional extraction (CE) (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.

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

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 second-order rate law applied in conventional extraction study (Rakotondramasy-Rabesiaka et al., 2007; Rakotondramasy-Rabesiaka et al., 2009) was used. The general second-order model can be written as:

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

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 equation (2) or a linearized equation (3):

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

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

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

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

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).

Analysis assay 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 (Li et al., 2006). 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 ºC 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 equation (5):

$$Total\ \ phenolic\ \ yield = \frac{PC_t V_t}{100 W_0} \times 100\% \qquad (5)$$

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).

Determination of antioxidant activity The antioxidant activity was determined using the DPPH equivalent, according to an adapted colorimetric procedure (Singh et al., 2002). 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 ºC. 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 equation (6):

$$DPPH\ scavenging\ activity = \frac{n\, V_t\, [C_d - (C_e - C_f)]}{PC_t\ V_t} \qquad (6)$$

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.

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) (α=0.05).

RESULTS AND DISCUSSION

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 to 59.2 W/cm^2) and treatment time (2 to 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 to 82% with increased treatment times ranging from 10 to 60 min (Ma et al., 2008). Similar results were also found for extraction of anthocyanin and ascorbic acid (Tiwari et al., 2008). 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 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.

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 reported on the minimal degradation of anthocyanin content, color and ascorbic acid in orange juice caused by increased ultrasonic power (Tiwari et al., 2008; Tiwari et al., 2009). 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.

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/cm^2)										
	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	6.5dB	8.5cA	4.9f
10	5.0cF	5.3eF	6.3dE	7.6cD	8.0cD	9.2dC	10.6dB	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.

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/cm^2)										
	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.

Extraction performance of pulsed ultrasound-assisted extraction Figures 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 (figure 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. (2009) 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.

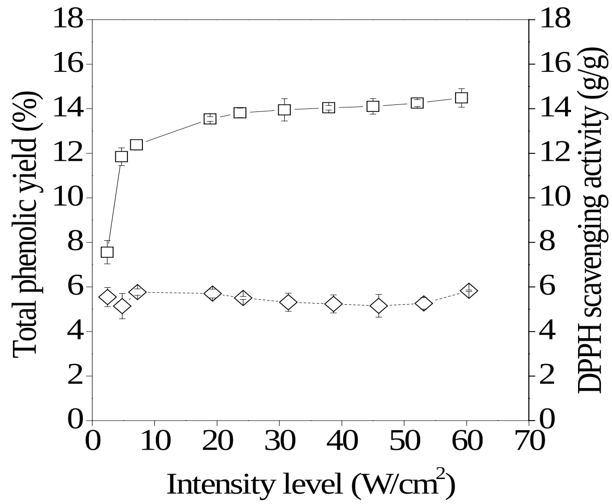

Figure 1. Total phenolic yields (solid line) and DPPH scavenging activities (dash line) of antioxidants at different intensity levels of pulsed ultrasound-assisted extraction.

The data of total phenolic yields clearly showed that the equilibrium was not reached with the combination of short pulse duration and interval (figure 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 recommended combinations of pulse duration and interval were 9 and 1, 5 and 5, 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% to 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.

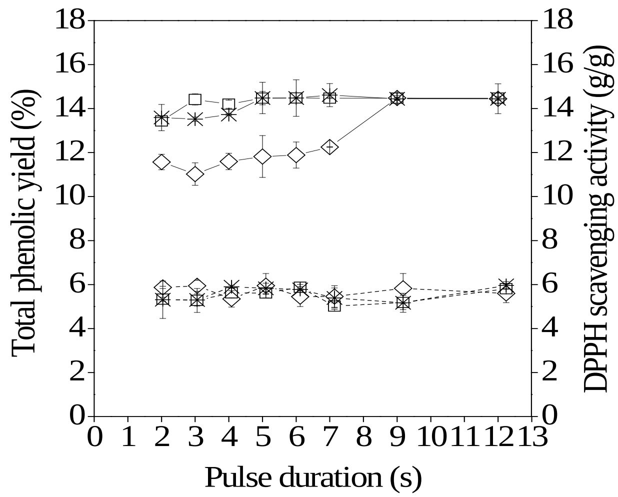

Figure 2. Total phenolic yields (solid lines) and DPPH scavenging activities (dash lines) of antioxidants at different pulse durations and intervals (◊ 1 s; ж 5 s; □ 15 s) of pulsed ultrasound-assisted extraction.

Regarding the effects of the numbers of pulse repetition (figure 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, 5 and 5, 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% to 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.

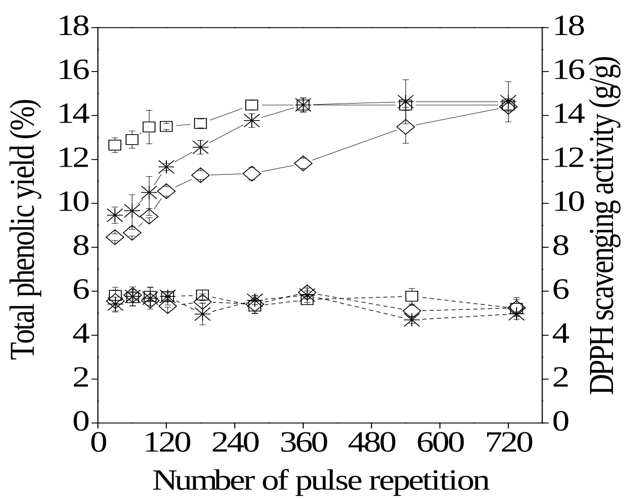

Figure 3. Total phenolic yields (solid lines) and DPPH scavenging activities (dash lines) of antioxidants at different numbers of pulse repetition of pulsed ultrasound-assisted extraction with three combinations of pulse durations and intervals, 5 and 1 (◊), 5 and 5 (ж), and 5 and 15 s (□).

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.

Performance comparison of different extraction methods Figure 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 (Coulson et al., 1991). 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 to 35% compared to conventional processing (Vilkhu et al., 2008). 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 above Section.

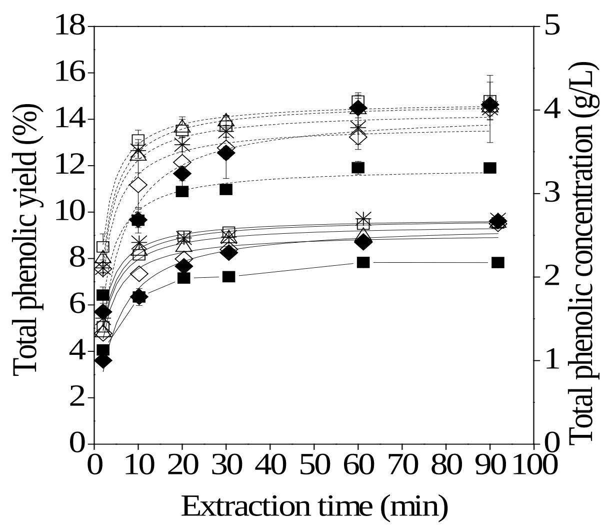

Figure 4. Comparison of total phenolic yields (dash lines) and concentrations (solid lines) of antioxidants from pomegranate peel at different extraction times for conventional extraction (CE, ■), continuous ultrasound-assisted extraction (CUAE, □), and pulsed ultrasound-assisted extraction (PUAE) under four conditions (◊ 30.8 W/cm^2, 5 s, 5 s; ж 59.2 W/cm^2, 2 s, 2 s; Δ 59.2 W/cm^2, 5 s, 5 s; ♦ 59.2 W/cm^2, 5 s, 15 s).

Figure 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.

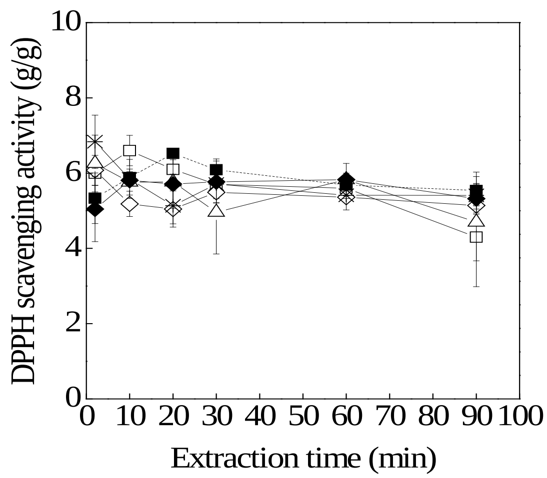

Figure 5. Comparison of DPPH scavenging activities of antioxidants from pomegranate peel at different extraction times for conventional extraction (CE, ■), continuous ultrasound-assisted extraction (CUAE, □), and pulsed ultrasound-assisted extraction (PUAE) under four conditions (◊ 30.8 W/cm^2, 5 s, 5 s; ж 59.2 W/cm^2, 2 s, 2 s; Δ 59.2 W/cm^2, 5 s, 5 s; ♦ 59.2 W/cm^2, 5 s, 15 s).

Table 4 shows the extraction times needed for all three methods to achieve total phenolic yield of 11.9% which was achieved by CE. 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.

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%)	14.8% (24%)
	45.0 W/cm^2	16 min (73%)	14.5% (22%)
	37.9 W/cm^2	18 min (70%)	14.3% (20%)

	30.8 W/cm^2	22 min (63%)	14.4% (21%)
	23.7 W/cm^2	29 min (52%)	14.1% (18%)
	18.9 W/cm^2	32 min (47%)	13.8% (16%)
	7.1 W/cm^2	50 min (17%)	13.1% (10%)
	4.7 W/cm^2	56 min (7%)	13.5% (13%)
	2.4 W/cm^2	>60 min	8.2%
PUAE	30.8 W/cm^2, 5 s, 5 s	12 min (80%)	14.4% (21%)
	59.2 W/cm^2, 2 s, 2 s	9 min (85%)	14.5% (22%)
	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%)	14.6% (23%)
CE		60 min	11.9%

[A] Extraction time denotes the extraction time at total phenolic yield of 11.9%.

[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.

Extraction kinetics of different extraction methods Figure 6 presents the linearized forms of the second-order model for the three different extraction methods.

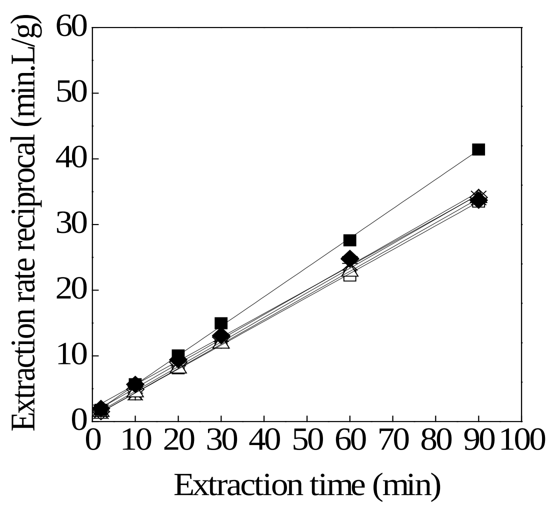

Figure 6. Comparison of extraction rate reciprocal (t/C_t) of antioxidants with different extraction times (t) for conventional extraction (CE, ■), continuous ultrasound-assisted extraction (CUAE, □), and pulsed ultrasound-assisted extraction (PUAE) under four conditions (◊ 30.8 W/cm^2, 5 s, 5 s; ж 59.2 W/cm^2, 2 s, 2 s; Δ 59.2 W/cm^2, 5 s, 5 s; ♦ 59.2 W/cm^2, 5 s, 15 s).

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.

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/cm2	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

CONCLUSION 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 ºC, 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.

Acknowledgements. This research was conducted at the Western Regional Research Center of USDA-ARS and Department of Biological and Agricultural Engineering, University of California, Davis, USA. The authors wish to thank Mr. Donald Olson for his technical support. The authors also wish to extend thanks to Stiebs pomegranate Inc. for providing the pomegranate marc materials.

REFERENCES

Adams, L. S., N. P. Seeram, B. B. Aggarwal, Y. Takada, D. Sand, and D. Heber. 2006. Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J. Agric. Food Chem. 54(3): 980-985.

Adhami, V. M., and H. Mukhtar. 2006. Polyphenols from green tea and pomegranate for prevention of prostate cancer. Free Radic. Res. 40(10): 1095-1104.

Apha, Awwa, and Wef.1998. Standard methods for the examination of water and wastewater. 20th ed. Washington, D.C. USA.

Coulson, J. M., J. Richardson, J. R. Backhurst, and J. H. Harker. 1991. Chemical Engineering, Particle Technology and Separation Processes. 2: 385.

Faria, A., R. Monteiro, N. Mateus, I. Azevedo, and C. Calhau. 2007. Effect of pomegranate (Punica granatum) juice intake on hepatic oxidative stress. Eur. J. Nutr. 46(5): 271-278.

Heber, D., N. P. Seeram, H. Wyatt, S. M. Henning, Y. J. Zhang, L. G. Ogden, M. Dreher, and J. O. Hill. 2007. Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. J. Agric. Food Chem. 55(24): 10050-10054.

Kang, Y., M. Li, J. Hou, Y. Ju, G. Liu, and S. Liu. 2006. Ultrasonic extraction of resveratrol from waste residue of grape spike-stalk. Chem. Ind. Eng. Prog. 25: 1362.

Li, Y. F., C. J. Guo, J. J. Yang, J. Y. Wei, J. Xu, and S. Cheng. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96(2): 254-260.

Li, H. Z., L. Pordesimo, and J. Weiss. 2004. High intensity ultrasound-assisted extraction of oil from soybeans. Food Res. Intern. 37(7): 731-738.

Ma, Y. Q., J. C. Chen, D. H. Liu, and X. Q.Ye. 2009. Simultaneous extraction of phenolic compounds of citrus peel extracts: Effect of ultrasound. Ultrason. Sonochem. 16(1): 57-62.

Ma, Y. Q., X. Q. Ye, Z. X. Fang, J. C. Chen, G. H. Xu, and D. H. Liu. 2008. Phenolic Compounds and Antioxidant Activity of Extracts from Ultrasonic Treatment of Satsuma Mandarin (Citrus unshiu Marc.) Peels. J. Agric. Food Chem. 56(14): 5682-5690.

Miller, D. L. 1981. Ultrasonic detection of resonant cavitation bubbles in a flow tube by their second-harmonic emissions. Ultrason. Sonochem. 19: 217-224.

Palma, M., and C. G. Barroso. 2002. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. Anal. Chim. Acta. 458(1): 119-130.

Paniwnyk, L., E. Beaufoy, J. P. Lorimer, and T. J. Mason. 2001. The extraction of rutin from flower buds of Sophora japonica. Ultrason. Sonochem. 8(3): 299-302.

Qu, W. J., Z. L. Pan, R. H. Zhang, H. L. Ma, X. G. Chen, B. N. Zhu, Z. B. Wang, and G. G. Atungulu. 2009. Integrated extraction and anaerobic digestion process for recovery of nutraceuticals and biogas from pomegranate marc. Transactions of the ASABE. 52(6): 1997-2006.

Rakotondramasy-Rabesiaka, L., J. L. Havet, C. Porte, and H. Fauduet. 2007. Solid–liquid extraction of protopine from Fumaria officinalis L.—Analysis determination, kinetic reaction and model building. Separation and Purification Technology. 54(2): 253-261.
Rakotondramasy-Rabesiaka, L., J. L. Havet, C. Porte, and H. Fauduet. 2009. Solid–liquid extraction of protopine from Fumaria officinalis L.—Kinetic modelling of influential parameters. Ind. Crops Prod. 29(2-3): 516-523.
Riera, E., Y. Golas, A. Blanco, J. A. Gallego, M. Blasco, and A. Mulet. 2004. Mass transfer enhancement in supercritical fluids extraction by means of power ultrasound. Ultrason. Sonochem. 11(3-4): 241-244.
Rodrigues, S., and G. A. S. Pinto. 2007. Ultrasound extraction of phenolic compounds from coconut (cocos nucifera) shell powder. J. Food Eng. 80(3): 869-872.
Rodrigues, S., G. A. S. Pinto, and F. A. N. Fernandes. 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (cocos nucifera) shell powder by response surface methodology. Ultrason. Sonochem. 15(1): 95-100.
Singh, R. P., K. N. C. Murthy, and G. K. Jayaprakasha. 2002. Studies on the antioxidant activity of pomegranate (punica granatum) peel and seed extracts using in vitro models. J. Agric. Food Chem. 50(1): 81-86.
Tiwari, B. K., C. P. O'Donnell, K. Muthukumarappan, and P. J. Cullen. 2009. Effect of sonication on orange juice quality parameters during storage. Intern. J. Food Sci. Technol. 44(3): 586-595.
Tiwari, B. K., C. P. O'Donnell, A. Patras, and P. J. Cullen. 2008. Anthocyanin and Ascorbic Acid Degradation in Sonicated Strawberry Juice. J. Agric. Food Chem. 56(21): 10071-10077.
Torres, R. A., C. Petrier, E. Combet, M. Carrier, and C. Pulgarin. 2008. Ultrasonic cavitation applied to the treatment of bisphenol A. Effect of sonochemical parameters and analysis of BPA by-products. Ultrason. Sonochem. 15(4): 605-611.
Vilkhu, K., R. Mawson, L. Simons, and D. Bates. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry — A review. Inn. Food Sci. Emerg. Technol. 9(2): 161-169.
Vinatoru, M., M. Toma, and T. J. Mason. 1999. Ultrasonically assisted extraction of bioactive principles from plants and their constituents, Adv. Sonochem. JAI Press, London. 5: 209-247.
Yasoubi, P., M. Barzegar, M. A. Sahari, and M. H. Azizi. 2007. Total phenolic contents and antioxidant activity of pomegranate (punica granatum L.) peel extracts. J. Agric. Sci. Technol. 9: 35-42.