PROCESS DEVELOPMENT FOR ANTIOXIDANT EXTRACTION FROM WET POMEGRANATE PEEL

X. Wu, C. Venkitasamy, T. McHugh, Z. Pan

HIGHLIGHTS

- A novel process for antioxidant extraction from wet pomegranate peel (WPP) was developed using water as solvent.
- Effects of extraction parameters on extract yield, quality, composition, and color were quantified.
- Extraction from WPP achieved 10% higher extract yield compared with dried peel.
- The WPP extraction process is more energy-efficient than extraction from dried peel.

ABSTRACT. *Pomegranate peel (PP) is underused as animal feed or discarded in landfills, causing environmental pollution, even though it has a unique polyphenol profile with health-promoting properties. Past research has focused on the potential utilization of dried pomegranate peel (DPP), involving extended processing time, energy usage, and potential quality loss. The objective of this study was to develop a process to extract water-soluble antioxidants from wet pomegranate peel (WPP) and compare its antioxidant attributes with that of DPP. The WPP was sliced and ground into two sizes, and phenolics were extracted in water at different temperatures (20°C, 30°C, 40°C, 50°C, and 60°C), solvent (water) ratios (1:1, 2:1, 4:1, 6:1, and 8:1), and extraction times (2, 3, 4, 5, and 6 min). WPP extraction was compared with DPP extraction using hot air (HA, 40°C) and infrared radiation (IR, 60°C). The total phenolic yield (TPY) from WPP ranged from 10.37% to 12.80%, and the extraction rate increased significantly with increasing extraction time, temperature, and solvent ratio. DPPH scavenging activity (DSA), an important indicator of antioxidant activity, was in the range of 5.366 to 6.591 g g^{-1} and decreased only with the increase in temperature. Extraction using WPP particles resulted in 10% more extract yield and 2.5% more TPY compared to DPP powders with similar DSA. This study demonstrated that WPP could be used for the extraction of phenolic compounds with high antioxidant activity and without energy-intensive drying.*

Keywords. Infrared drying, Peel composition, Polyphenol, Principal component analysis, Wet pomegranate peel.

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae. It has been grown since ancient times for its delicious fruit and as an ornamental plant for its red, orange, or occasionally creamy yellow flowers. The estimated global cultivation area for pomegranate is about 300,000 ha, with fruit production of 3.0 million metric tons (Kahramanoglu and Usanmaz, 2016). Spanish missionaries brought pomegranate to the Americas in the 1500s (LaRue, 1977; Pareek et al., 2015). Wonderful, a primary cultivar in the U.S., was discovered in Florida and brought to California in 1896. Since then, pomegranate has been grown abundantly in California and Arizona, where mild winters enable the fruits to reach the quality necessary for successful commercial production. In 2015, about 282,000 tons of pomegranate fruit were grown in California, with an economic value of $115.4 million (CDFA, 2014).

Research has shown that pomegranate fruit might be beneficial for its antioxidant, anti-mutagenic, and anti-hypertension activities and its ability to reduce liver injury (Al-Jarallah et al., 2013; Dhinesh and Ramasamy, 2016; Du et al., 1975; Gil et al., 1996; Lansky et al., 2000; Tsuda et al., 1994). It has also been studied for therapeutic purposes to alleviate ailments such as colic, colitis-diarrhea, dysentery, leucorrhea, paralysis, and headache (Sadeghi et al., 2009; Schubert et al., 1999). Pomegranates are considered beneficial for curing chronic stomach ailments and are also known for their anti-inflammatory and anti-atherosclerotic activities against osteoarthritis, prostate cancer, heart disease, and HIV-I (Malik et al., 2005; Sumner et al., 2005).

Pomegranate juice processing generates two solid by-products: pomegranate peel (PP) and pomegranate seed (PS). Teh (2016) studied the physicochemical characteristics of PP and PS from five pomegranate cultivars (Wonderful, Molla Nepes, Parfianka, Purple Heart, and Vkusnyi) grown in California and determined the components of dried PP and PS through proximate analysis. The peel portion consisted

The authors are **Xingzhu Wu,** Doctoral Student, Department of Biological and Agricultural Engineering, University of California, Davis, California; **Chandrasekar Venkitasamy,** Adjunct Assistant Professor, Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa; **Tara McHugh,** Director, USDA-ARS Western Regional Research Center, Albany, California; **Zhongli Pan,** Adjunct Professor, Department of Biological and Agricultural Engineering, University of California, Davis, California. **Corresponding author:** Zhongli Pan, 3018 Bainer Hall, University of California, Davis, CA 95616; phone: 530-752-4367; e-mail: zlpan@ucdavis.edu.

of 38.77% to 53.01% carbohydrate as the principal constituent, making up 91% of PP, followed by 3.5% protein, 1.7% fat, and 3.7% ash or minerals. The PS contained higher proportions of protein and fat and included 60% to 72% carbohydrate, 11% to 17% protein, 15% to 21% fat, and 2% to 3% minerals. PP is non-edible and comprised mainly of bioactive compounds, such as hydrolyzable tannins (pedunculagin, punicalin, punicalagin, and ellagic and gallic acids) at concentrations ranging from 27 to 172 g kg^{-1} (Fischer et al., 2011), flavonoids (catechins, anthocyanins, and other complex flavonoids), and complex polysaccharides (Ismail et al., 2014). Therefore, PP is an excellent source of phenolic compounds, tannins, flavonoids, sterols, fatty acids, dietary fiber, minerals, and vitamins. Pomegranate byproducts have been used for the production of single-cell proteins, industrial enzymes, and lovastatin with diversified market potential and several economic and waste management benefits. PP extracts have been used in the fortification of food commodities and functional novel products and contribute to increased health benefits and improved quality, as well as extended shelf-life (Charalampia and Koutelidakis, 2017). However, the peel generated from juice processing is often underused as animal feed or discarded, resulting in solid waste pollution (Shabtay et al., 2008). Creating value-added products from the byproducts of pomegranate processing would be an outstanding solution to reduce solid waste and increase the value of pomegranates.

High molecular weight polyphenols are the major high-value phytochemicals in pomegranate peel. They have demonstrated the likelihood of reducing risks of chronic diseases (Heber, 2011), including type-2 diabetes (Banihani et al., 2013) and cardiovascular diseases (Hamoud et al., 2014). Among all the polyphenols in pomegranate peel, gallic acid, ellagic acid, and punicalagin are most likely responsible for these health benefits (Aviram et al., 2008; Pai et al., 2011). Punicalagin has also demonstrated significant *in vitro* antioxidant activities (Fischer et al., 2011) with abundant hydroxyl groups, which can trap peroxyl radicals to reduce oxidation. Therefore, it is of great interest to extract punicalagin, ellagic acid, and gallic acid for nutrient fortification and other applications. Extraction is a mass transfer process influenced by the matrix properties of the plant part as well as the solvent, temperature, and time (Hernandez et al., 2009). The particle size and solvent type play an essential role in extraction. Particle size reduction results in higher mass transfer efficiency and product yield, as smaller particles have a higher surface-to-volume ratio and less internal path (Adapa et al., 2011). The solvent type impacts extraction through different polarities and affinity to the compounds of interest (Alothman et al., 2009). However, better understanding is needed of the effects of extraction conditions on antioxidant yield and antioxidant activity.

Most previous research on the extraction of bioactive compounds used organic solvents to improve the extraction rate. However, inappropriate use or recycling of organic solvents in food applications can cause pollution and raise safety concerns. Green extraction of natural products, focusing on modified extraction processes with reduced energy consumption, alternative solvents, and renewable natural products, is in demand (Armenta et al., 2015). Water is a universal solvent that is generally recognized as safe (GRAS). Therefore, deionized (DI) water was applied for extraction in this study.

Nearly all extraction studies of plant-based bioactive compounds have used dried material for extended research time (Azmir et al., 2013). However, drying increases the energy and time consumption, in addition to the excessive preparation requirements. Therefore, a novel extraction process using fresh wet pomegranate peel (WPP) was studied in this research.

In summary, this work aimed to investigate the effects of extraction conditions on the isolation of bioactive compounds and compare the extraction rates of wet and dry pomegranate peel particles. The optimal extraction parameters were determined based on the antioxidant yield and activity to provide more insight for industrial-scale production.

MATERIALS AND METHODS
RAW POMACE AND CHEMICALS
Pomegranate pomace of Wonderful variety was collected from SunnyGem LLC (Buttonwillow, Cal.). The wet pomace was stored at -18°C and thawed to room temperature before use. The chemicals used in the experiments, including Folin-Ciocalteu reagent, analytical standards of tannic, gallic, and ellagic acids, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma-Aldrich (St. Louis, Mo.). Methanol, HPLC-grade o-phosphoric acid (85), and analytical-grade sodium hydroxide and sodium carbonate were obtained from Fisher Scientific (Pittsburgh, Pa.). A mixture of punicalagin α and punicalagin β (51.54% α and 48.46% β) was purchased from ChromaDex Co. (Irvine, Cal.).

PREPARATION OF PEEL PARTICLES
Pomegranate juice had been extracted by carving the peels and separating the peels from the arils. Therefore, the collected pomace contained about 96% (wt/wt, d.b.) peels and 4% (wt/wt, d.b.) seeds. Because the percentage of seeds in the pomace was negligible, further separation of seeds was not performed. The WPP was sliced into small pieces of less than 5 mm using a continuous-feed food processor (Hobart, Troy, Ohio). The sliced peel pieces were then ground into two groups of fine particles in a Comitrol processor (model 3600, Urschel Laboratories, Chesterton, Ind.) using 0.024 and 0.012 in. (~0.60 and 0.38 mm) cutting heads. The moisture contents of the two groups were determined by drying about 10 g of particles at 105°C in a hot-air oven until a constant weight was obtained (APHA, 1998).

To compare the polyphenol extraction yield and antioxidant activity of polyphenols produced from WPP and DPP, the WPP samples were dried using infrared radiation (IR) and hot air (HA). IR drying was performed by heating a single layer of peels at a surface temperature of 60°C, and HA drying was performed using hot air at 40°C until the moisture content was less than 10% (wt/wt, d.b.). DPP samples produced by IR and HA drying were milled in a sample mill (Stein M-2, Hoffman Manufacturing, Corvallis, Ore.) to less than 0.38 mm size for polyphenol extraction.

COMPOSITION OF WET AND DRIED PEEL

Analytical measurements were used to determine the compositions of WPP and DPP. Ash content was measured by gravimetric method by heating to 550°C (AOAC Method 942.05, AOAC, 1990). Crude fat was determined based on Randall modification of the standard Soxhlet extraction method (AOAC Method 2003.05, AOAC, 1990). Total protein content was quantified by nitrogen conversion using AOAC Method 990.03 (AOAC, 1990). Carbohydrate content was calculated by difference using equation 1:

$$\text{Carbohydrate} = \text{dry matter} - (\text{protein} + \text{fat} + \text{ash}) \qquad (1)$$

Total dietary fiber and soluble and insoluble fiber were quantified using AOAC Method 991.43 (AOAC, 1990). Results were reported on a 100% dry basis of individual dry matter according to NFTA 2.2.2.5 (Shreve et al., 2006).

EFFECTS OF EXTRACTION PARAMETERS

Experiments were conducted using WPP particles of both sizes to determine the effects of single factors, including particle size, solvent ratio, extraction temperature, and extraction time, on the extraction of polyphenols. To study the effect of extraction time, 5 g of WPP was mixed with 40 mL of DI water and extracted at 20°C for 2, 3, 4, 5, and 6 min with a stirring speed of 1200 rpm. The effect of solvent ratio was investigated by extracting 5 g of WPP powder with 5, 10, 20, 30, and 40 mL of DI water for 6 min at 20°C. The effect of temperature was tested by adding 5 g of WPP powder to 40 mL of DI water and extracting for 6 min at temperatures of 20°C, 30°C, 40°C, 50°C, and 60°C. All extractions were conducted in triplicate using a Corning hot plate stirrer (Corning, N.Y.) properly shielded from light to avoid light-induced quality loss. After extraction, the extract mixture was separated by centrifugation at 4400×g at 4°C for 15 min (Centrifuge 5804R, Eppendorf, Hamburg, Germany). The liquid extract (supernatant) was collected for determination of physicochemical qualities.

ANALYSIS ASSAY
Determination of Phenolic Yield and Content

Total extract yield (TEY) was determined by drying 5 mL of liquid extract at 60°C under 20 kPa in a vacuum oven (Lindberg/Blue M VO1218C, Thermo Scientific, Columbia, Md.) and was expressed as g dry extract g^{-1} DPP. According to the Folin-Ciocalteu method (Li et al., 2006), total phenolic yield (TPY) in the extract was expressed as g tannic acid equivalent g^{-1} dry peel, while total phenolic content (TPC) was expressed as g tannic acid equivalent g^{-1} dry extract. To quantify the tannic acid equivalent, a 0.6 mL extract sample was mixed thoroughly with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 2 mL of 7.5% (wt/wt) Na_2CO_3 using a vortex mixer (Vortex-Genie 2, Scientific Industries, Bohemia, N.Y.). After 30 min of 25°C incubation of the mixed solution, the absorbance was measured at 760 nm using a UV spectrophotometer (UV-3600 Plus UV-Vis-NIR spectrophotometer, Shimadzu Scientific, Columbia, Md.). For each liquid extract, the tests were conducted in triplicate, and the absorbance was read three times for each sample. A reference blank was prepared using the aforementioned proce-

dure with DI water rather than liquid extract. TEY, TPY, and TPC were calculated using equations 2, 3, and 4, respectively:

$$\text{TEY (\%)} = \frac{W_2}{100 W_1} \times 100 \qquad (2)$$

$$\text{TPY (\%)} = \frac{CV}{100 W_1} \times 100 \qquad (3)$$

$$\text{TPC (\%)} = \frac{CV}{100 W_2} \times 100 \qquad (4)$$

where
W_1 = dry weight of sample (g)
W_2 = dry weight of extract (g)
C = phenolic concentration (g mL^{-1})
V = total volume of liquid extract (mL).

Determination of Antioxidant Activity

Antioxidant activity of the extract was determined as DPPH scavenging activity (DSA, g DPPH equivalent g^{-1}) using equation 5 (Qu et al., 2014a):

$$\text{DSA} = \frac{n V_t \left[C_c - (C_s - C_b) \right]}{C_t V_t} \qquad (5)$$

where
C_c = DPPH conc. equivalent in control solution (g L^{-1})
C_s = DPPH conc. equivalent in sample solution (g L^{-1})
C_b = DPPH conc. equivalent in blank solution (g L^{-1})
n = dilution factor of liquid extract.

Liquid extract (60 µL) or DI water (control group) was mixed thoroughly with 3 mL of DPPH solution in methanol (0.05 g L^{-1}) using a vortex mixer and kept in a 25°C water bath for 20 min. Liquid extract (60 µL) was also mixed with 3 mL of methanol and used as a blank solution. Absorbance at 517 nm was noted. Three measurements were conducted for each liquid sample, and each test was replicated three times.

PHENOLIC COMPOSITION OF WET PEEL EXTRACT

The phenolic composition of wet peel extract was determined using a Prominence HPLC system with a photodiode array detector (PAD) (Shimadzu Scientific, Columbia, Md.). Chromatography was achieved using a 4.6 × 100 mm Kinetex 2.6 lm C-18 column (Phenomenex, Torrance, Cal.) equipped with a KrudKatcher Ultra in-line column filter. Instrument control and data acquisition were accomplished using Shimadzu LCsolution software. The HPLC method developed by Qu et al. (2012) was used with slight modification. Two mobile phases, including 0.1% (v/v) H_3PO_4 in HPLC water (A) and 0.1% (v/v) H_3PO_4 in acetonitrile (B), were filtered through Whatman 0.45 µm nylon membrane. The analysis was conducted at a constant temperature of 30°C using a flow rate of 1.8 mL min^{-1} and a sample injection volume of 10 µL. Detector wavelengths of 270 nm for gallic acid, 254 nm for ellagic acid, and 378 nm for punicalagin α and β were used. The PAD detector was set to scan from 210 to 600 nm. The elution conditions were as follows:

isocratic elution 1% B (0 to 4.5 min), linear gradient from 1% B to 4.5% B (4.5 to 9.0 min), isocratic elution 4.5% B (9.0 to 15.0 min), linear gradient from 4.5% B to 7.0% B (15.0 to 19.5 min), isocratic elution 7.0% B (19.5 to 25.5 min), linear gradient from 7.0% B to 25% B (25.5 to 41.19 min), to 90% B (41.19 to 43.17 min), and to 1% B (43.17 to 49.17 min).

STATISTICAL ANALYSIS

The data were analyzed using ANOVA followed by Duncan's multiple range test in SAS (ver. 9.2., SAS Institute, Cary, N.C.), and significant differences among treatments were determined with a significance of $p < 0.05$. All experiments were replicated three times, and the mean values are reported. Principal component analysis (PCA) was performed based on the correlation matrix of the values of the characteristics, which means that the contribution of each variable was independent of the range of its values (Höft et al., 1999). In PCA, observations consisted of the average subsampling results from the 30 aforementioned extraction conditions at different extraction times, temperatures, and solvent ratios. Four vectors (F1 to F4) were estimated based on the eigenvectors of the correlation matrix of four indicators: total extract yield (TEY), total phenolic yield (TPY), total phenolic content (TPC), and DPPH scavenging activity (DSA).

RESULTS AND DISCUSSION

EFFECT OF CUTTING HEADS ON WPP PARTICLE SIZE

The size distribution of the two groups of ground WPP obtained using two different cutting heads is shown in figure 1. The group with large peel particles (LPP) had an average particle size of 0.60 mm, and the small peel particles (SPP) had an average size of 0.38 mm. The LPP had a moisture content of 70.37% ±0.27%, and the SPP had a moisture content of 70.53% ±0.60%. The two particle sizes (LPP and SPP) were used in the experiments to evaluate the effects of particle size on extraction yield and quality.

EFFECTS OF EXTRACTION TIME

The effects of extraction time on the TEY, TPY, TPC, DSA, and color of the extract are shown in figure 2. When
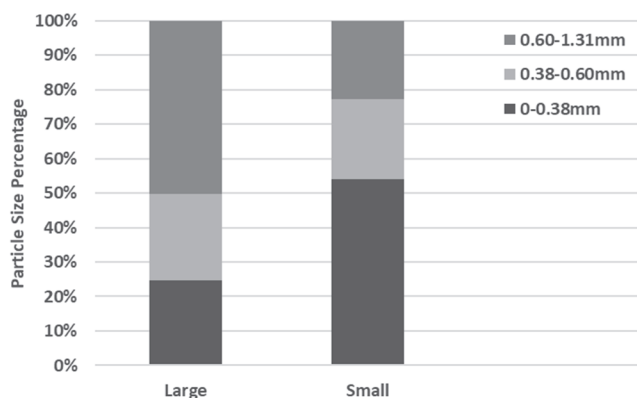

Figure 1. Size distribution of peel particles ground by large (0.024 in., ~0.60 mm) and small (0.012 in., 0.38 mm) cutting heads.

the extraction time was extended from 2 to 6 min, the TEY of SPP (fig. 2a) increased slightly from 53.74% to 54.47%. The TPY and TPC (fig. 2b) of SPP ranged from 10.28% to 10.71% and from 19.14% to 19.69%, respectively. The DSA (fig. 2c) of the SPP extract ranged from 6.63 to 6.78 g g^{-1}. The TPY, TPC, and DSA were not significantly different among the extraction times. These results indicate that the extraction of antioxidants reached equilibrium after 2 min at 20°C. These findings are in agreement with the results of Wang et al. (2011), who reported that the TPY was 6.55%, 9.14%, and 11.92% after 2 min of extraction at 25°C, 60°C, and 95°C, respectively. The extraction of LPP resulted in similar TEY, TPY, TPC, and DSA values, and no significant difference in these values was found for extraction with LPP and SPP.

Increasing the extraction time significantly reduced the color characteristics of the liquid extracts of both SPP and LPP (figs. 2d to 2f). The lightness (L^*) of the extract, which is related to light transmission, decreased from 34.98 to 33.20. This result is consistent with the findings of Nour et al. (2015), who reported a negative correlation between TPC and lightness. The chroma (C^*) value, which is used to quantitatively describe the intensity or saturation of color, decreased from 31.88 to 29.09. Hue ($H°$) is a qualitative attribute representing colors, including red-purple (0°), yellow (90°), bluish-green (180°), and blue (270°). The $H°$ for both peel particle sizes decreased with increasing extraction time, indicating a color shift toward less intense red-purple due to the accumulation of phenolic compounds with increased time. The color characteristics of the SPP extract reached equilibrium after 4 min, while the LPP extract required 5 min. This result proves that the smaller particles had a more shattered cell structure and contained more liquid, which could be more easily extracted. To evaluate the effects of other parameters, an extraction time of 6 min was used in the subsequent experiments.

EFFECTS OF EXTRACTION TEMPERATURE

The effects of extraction temperature on the TEY, TPY, TPC, DSA, and color of the extract are shown in figure 3. As shown in figure 3a, the TEY significantly ($p < 0.05$) increased from 54.47% to 57.83% when the temperature increased from 20°C to 60°C. The TPY and TPC (fig. 3b) followed a similar trend as TEY and significantly increased ($p < 0.05$) from 10.71% to 12.80% and from 19.69% to 22.06%, respectively. This result demonstrates that extraction at higher temperatures promoted the solubility of phenolic compounds and the diffusion coefficient. Wang et al. (2011) reported similar findings and concluded that extraction was sensitive to a temperature increase in the range of 25°C to 95°C. On the contrary, the DSA (fig. 3c) significantly decreased ($p < 0.05$) from 6.75 to 5.54 g g^{-1} with the increase in temperature, which was consistent with the findings of Qu et al. (2010), who compared extractions at 25°C, 40°C, 60°C, 80°C, and 95°C and observed a significant drop ($p < 0.05$) in DSA from 6.2 to 3.1 g g^{-1}.

The L^* value decreased from 33.20 to 28.56 with an increase in the extraction temperature. This result agrees with the results of Qu et al. (2014b). The a^*, b^*, and C^* values also decreased as the extraction temperature increased from
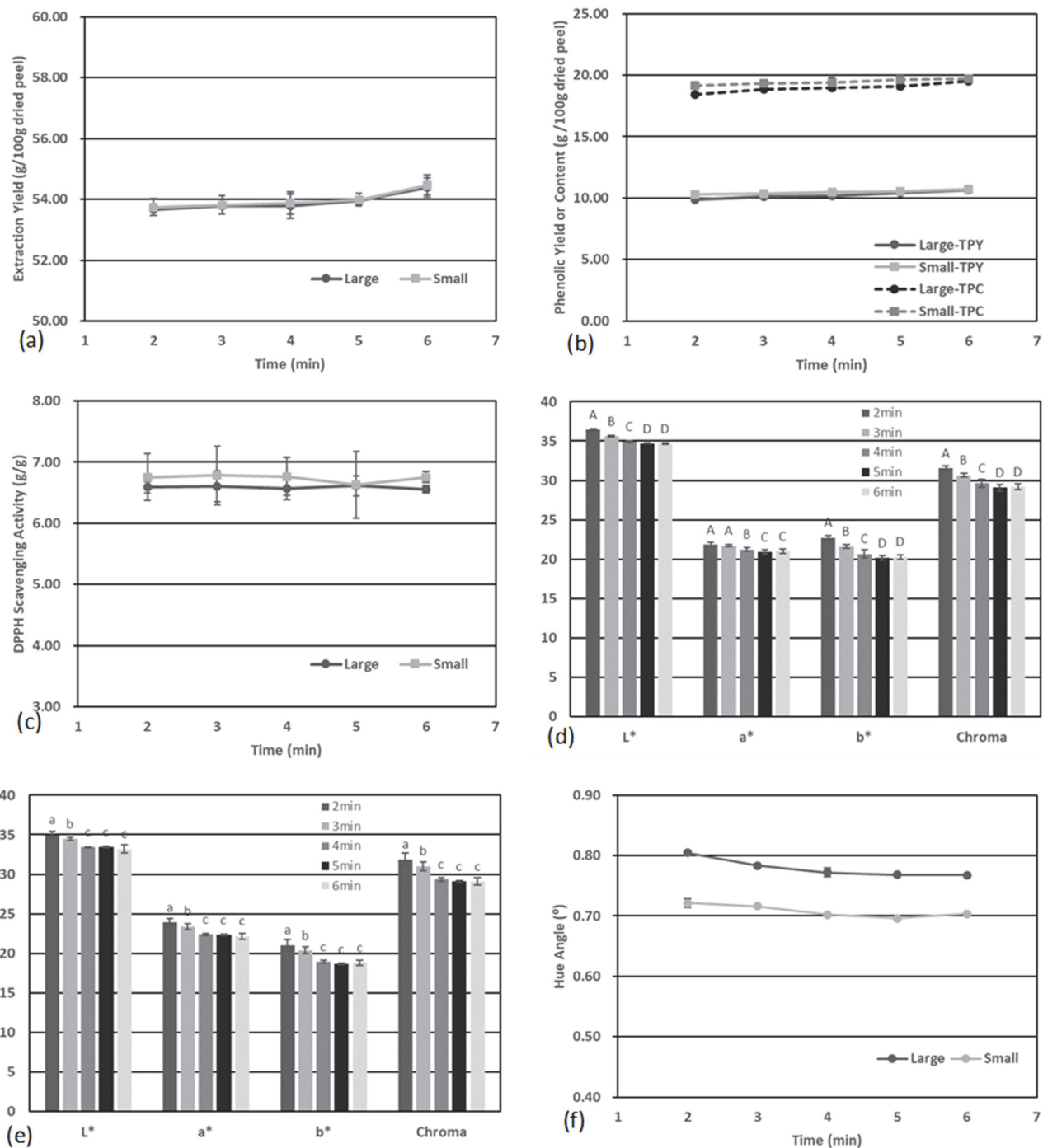
Figure 2. (a) Total extract yield, (b) total phenolic yield or content, (c) DPPH scavenging activity, (d) *L**, *a**, *b**, and chroma for large particles, (e) *L**, *a**, *b**, and chroma for small particles, and (f) hue angle of wet peel particle (WPP) extract for 2 to 6 min at 20°C with a solvent (water) ratio of 8:1. Triplicates were tested and analyzed.

20°C to 40°C, but no definite trend was observed when the temperature was above 40°C. The lower values of the color characteristics occurred because most of the phenolic compounds are heat-sensitive. No significant differences in the yield and color parameters were observed for extraction using LPP and SPP with the increase in extraction temperature. Therefore, to obtain phenolic extract of desirable quality, including DSA and color characteristics, an extraction temperature of 20°C, which required less energy, was applied in the subsequent experiments.

EFFECTS OF SOLVENT RATIO

The effects of the ratio of solvent (water) to peel particles on the TEY, TPY, TPC, and DSA of the extract are shown in figure 4. When the solvent ratio increased from 1:1 to 4:1, the TEY, TPY, and TPC significantly increased ($p < 0.05$) from 27.53% to 53.20%, from 4.42% to 10.53%, and from 16.06% to 19.53%, respectively. SPP achieved significantly higher TEY, TPY, and phenolic content than LPP, showing that the finer cell structure facilitated mass transfer consistent with Fick's law (Waterman and Sutton, 2003). The
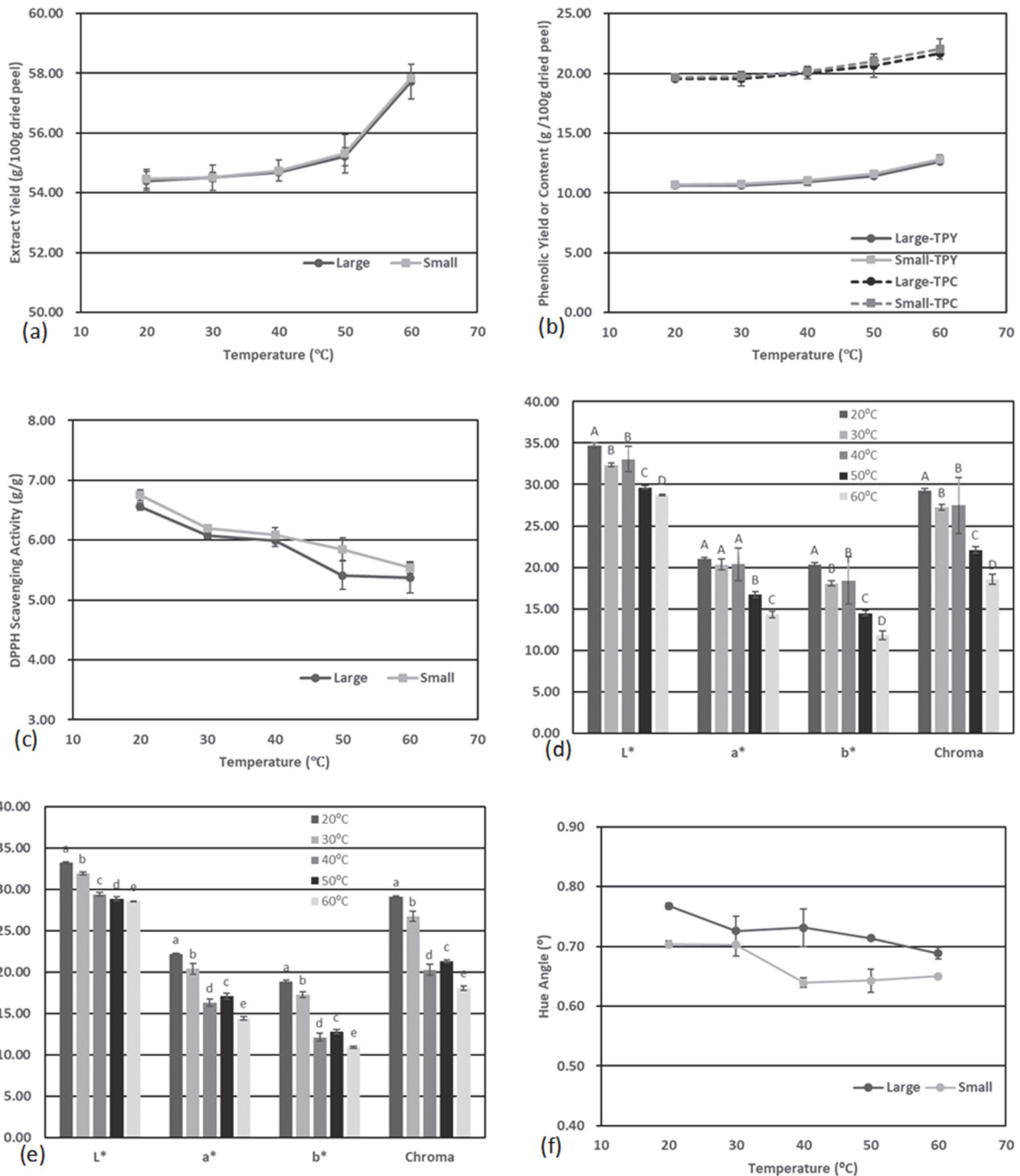
Figure 3. (a) Total extract yield, (b) total phenolic yield or content, (c) DPPH scavenging activity, (d) *L**, *a**, *b**, and chroma for large particles, (e) *L**, *a**, *b**, and chroma for small particles, and (f) hue angle of wet peel particle (WPP) extract at 20°C to 60°C for 6 min with a solvent (water) ratio of 8:1. Triplicates were tested and analyzed.

DSA (fig. 4c) was not significantly different between the two particle groups. Laroze et al. (2010) studied the extraction kinetics of polyphenols from raspberry pomace. They observed that small particles (0.15 to 1 mm) resulted in much higher extraction efficiency compared to large particles (1 mm) with methanol extraction at a 20:1 solvent ratio. The reason could be that smaller particles allowed the solvent to access solutes with less resistance to mass transfer. Smaller particles were also related to more cell breakage, which promoted the release of phytochemicals.

Increasing the solvent ratio to greater than 20:1 resulted in a slightly increased TEY, but it was not significantly different, indicating that equilibrium was reached. A possible reason could be that a higher concentration gradient was

built up with the increasing solvent ratio, which increased diffusion from the internal structure, thus increasing the extraction rate. Similar findings were observed for the extraction of phenolics from date seeds (Al-Farsi and Lee, 2008) and dried sage (Durling et al., 2007). The results demonstrated that a higher solvent ratio increased the antioxidant yield and content, with a limited effect on the DSA. The DSA was not significantly different regardless of the solvent

ratio and peel particle size at this phase. A higher solvent ratio requires more water consumption in the extraction and higher energy use for concentration of the extract. In summary, a solvent ratio of 4:1, which resulted in relatively high TEY and TPY, was optimal in terms of solvent usage. Therefore, a solvent ratio at 4:1 is recommended for industrial applications. The LPP had an extraction rate comparable to the SPP, showing that the particle size had no significant effect on the extraction rate.

In summary, when using WPP for antioxidant extraction, higher TEY, TPY, and TPC can be achieved by increased the extraction temperature, time, and solvent ratio. The DSA is independent of the extraction time and solvent ratio but decreased with the increase in extraction temperature. The two groups of peel particles, with average particle sizes of 0.60 and 0.38 mm, respectively, had no significantly different effects on the extraction of phenolics with varied extraction temperature, time, and solvent ratio. The highest TEY, TPY, and TPC obtained were 57.83%, 12.80%, and 22.06%, respectively. The DSA ranged from 5.37 to 6.35 g g^{-1}. The results indicated that extraction of phenolics from peel particles, produced by grinding to less than 0.6 mm with a large cutting head, at a temperature 20°C for 6 min using a solvent (water) ratio of 4:1 could be the most economical and sustainable approach for industrial-scale production.

PHENOLIC COMPOSITION IN EXTRACT

The effects of extraction conditions on the phenolic composition were investigated for the five groups of extraction conditions shown in table 1. The parameters and standard curves of the HPLC analysis of the extract are shown in table 2.

The chromatogram obtained from the HPLC, showing the peaks of major compounds in the peel extract, including gallic acid, ellagic acid, punicalagin α, and punicalagin β, is shown in figure 5a. The content of the four major phenolic compounds produced by the five groups of extraction conditions (G1 to G5) is shown in figure 5b. As shown in figure 5b, the gallic acid content significantly improved, from 0.17 to 0.29 mg g^{-1} ($p < 0.05$), with the increase in solvent ratio from 1:1 to 4:1. Except for the group with 1:1 solvent ratio (G1), the gallic acid contents obtained with the other groups with different solvent ratios varied from 0.27 to 0.35 mg g^{-1} and were not significantly different, regardless of the time and temperature. The ellagic acid content varied from 0.77
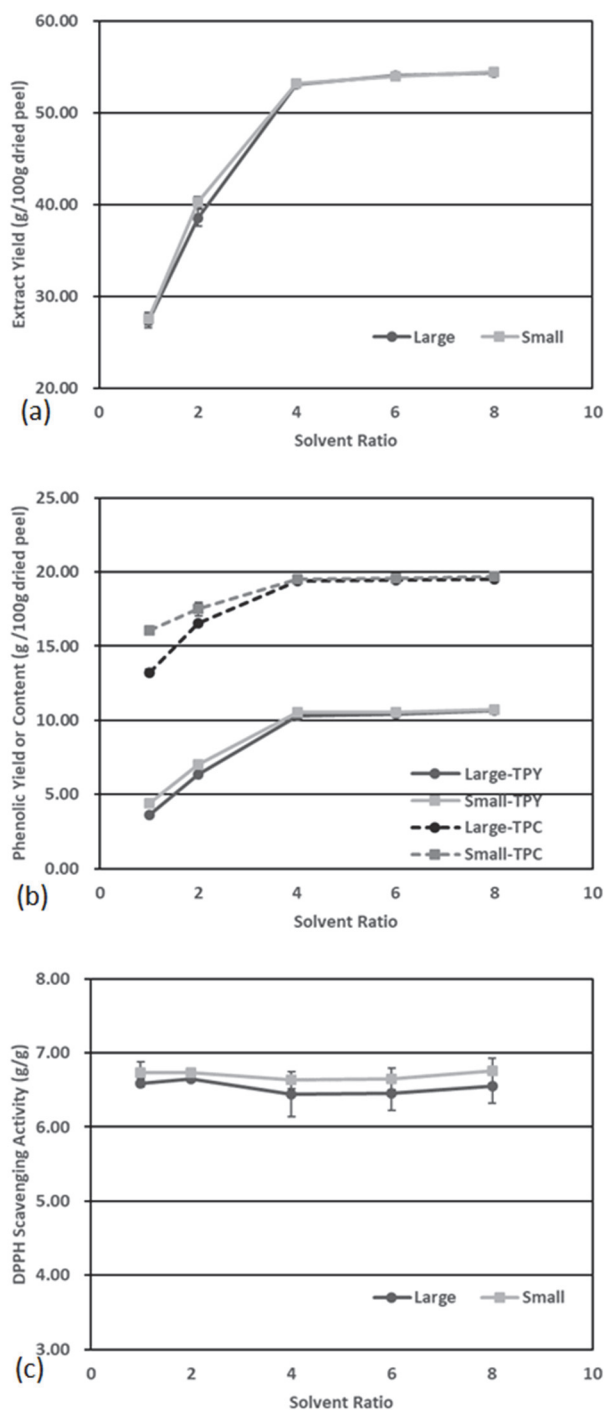
Figure 4. (a) Total extract yield, (b) total phenolic yield or content, and (c) DPPH scavenging activity of wet peel particles extracted for 2 to 6 min at 20°C with a solvent (water) ratio of 8:1. Triplicates were tested and analyzed.

Table 1. Groups of extraction conditions used in HPLC analysis.

	G1	G2	G3	G4	G5
Time	6 min	6 min	6 min	6 min	2 min
Temp.	20°C	20°C	20°C	60°C	20°C
Ratio	1:1	4:1	8:1	8:1	8:1

Table 2. Parameters and standard curves of HPLC analysis.

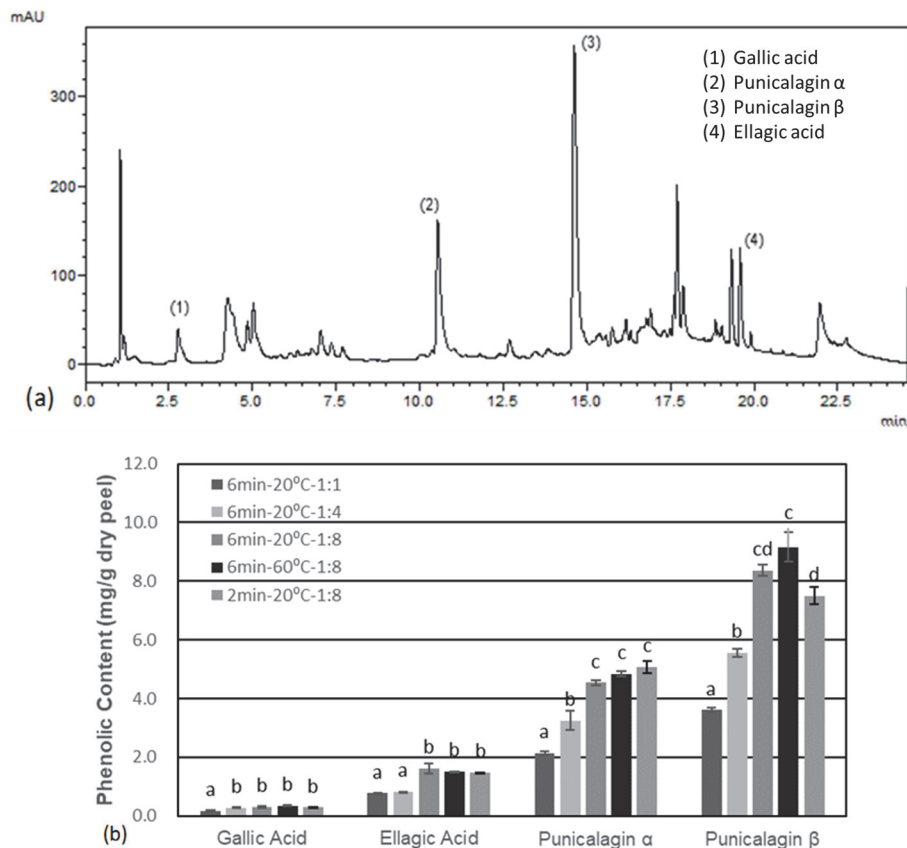| Standard	Retention Time (min)	λ (nm)	Equation ($y = ax + b$)	Adj. R^2
Gallic acid	2.772	270	$y = 12914.2x + 474352.9$	0.997
Ellagic acid	19.563	254	$y = 32581.9x - 1456409.3$	0.996
Punicalagin α	10.535	378	$y = 1791.3x - 41611.3$	0.995
Punicalagin β	14.613	378	$y = 4335.7x - 66014.9$	0.997

Figure 5. (a) HPLC analysis of pomegranate peel extract and (b) composition of four major phenolic compounds in pomegranate peel extract. Triplicates were tested and analyzed.

to 1.51 mg g^{-1}, with no significant differences among groups G3, G4, and G5. The solvent ratio mostly influenced punicalagin α. At the same extraction conditions, the punicalagin α content increased from 2.13 to 4.48 mg g^{-1} when the solvent ratio increased from 1:1 to 8:1. In addition to the solvent ratio (G1: 3.61 mg g^{-1}; G3: 8.28 mg g^{-1}), the extraction time and temperature affected the extraction of punicalagin β, which can be seen from the increased punicalagin β content from 7.51 mg g^{-1} (G5) to 9.17 mg g^{-1} (G4). The punicalagin purity ranged in order of G2 > G4 > G5 > G3 > G1, and the corresponding values were 88.99%, 88.27%, 87.73%, 87.64%, and 85.93%, respectively.

Qu et al. (2012) compared the gallic acid, punicalagin α, punicalagin β, and ellagic acid concentrations of different pomegranate products. The results presented in this study showed significantly higher retention of punicalagin than the pomegranate peel extract using dried peel particles (50:1 solvent ratio, 25°C, 90 min) used by Qu et al. (2012). The WPP extraction in this study also achieved slightly higher phenolic concentrations compared to Langers 100% pomegranate juice.

COMPOSITION, PHENOLIC CONTENT, AND QUALITY OF WET AND DRIED POMEGRANATE PEEL

To study the effects of drying, DPP was produced using HA drying and IR drying, and the peel compositions and phenolic extraction conditions were compared between DPP and WPP. The compositions of WPP and DPP on a dry basis are shown in table 3. The WPP clearly had higher contents of protein, ash, and crude fat than the DPP. The loss of these contents was reduced by avoiding the drying process. On the other hand, the HA DPP maintained higher total dietary fiber (TDF) at 25.44%, and the ratio of insoluble dietary fiber to soluble dietary fiber (IDF/SDF) was 8.84. This was higher than the values for WPP, which maintained 17.19% TDF and an IDF/SDF ratio 4.93. Morais et al. (2016) compared the compositions of raw, freeze-dried, and oven-dried papaya peels. They reported similar findings for the differences in fiber content, but the differences were not statistically significant.

Figure 6 shows the TEY, TPY, DSA, and color characteristics of IR DPP, HA DPP, and WPP. The DPP resulted in significantly lower TEY, TPY, and TPC than the WPP. The DSA values were similar for both DPP and WPP at about 6.41 g g^{-1}. In other words, extraction from WPP resulted in 10% more extract yield and 2.5% more TPY with

Table 3. Composition of fresh and hot-air dried pomegranate peel (values are in g per 100 g, d.b.).

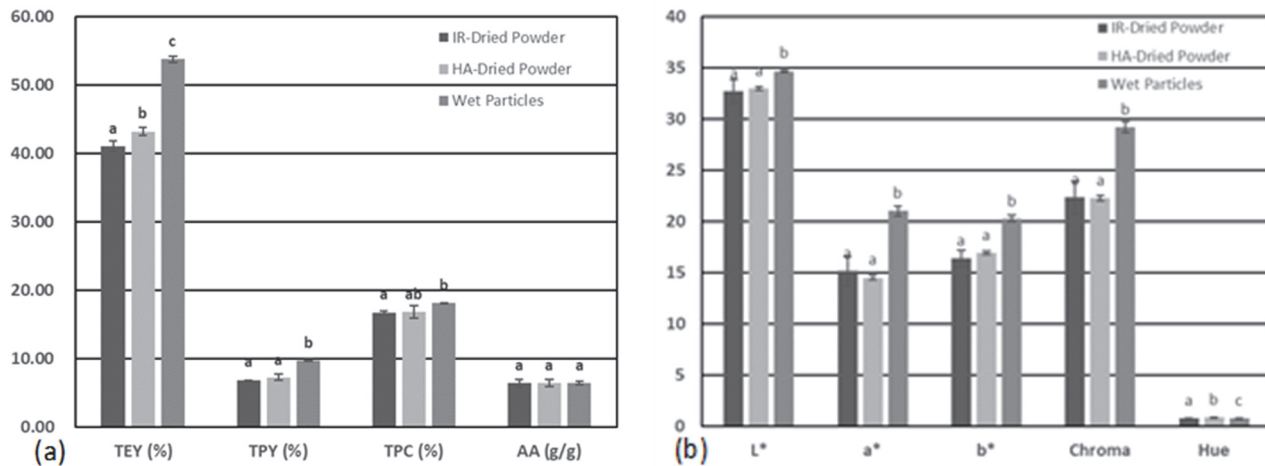|	DM	Protein	Ash	Crude Fat-Rinse	Carbohydrate	Total Dietary Fiber	Insoluble Dietary Fiber	Soluble Dietary Fiber
Fresh WPP	26.30 ±0.40	4.60 ±0.20	4.75 ±0.51	0.71 ±0.27	89.94 ±0.67	17.19	15.47	1.75
Hot-air DPP	82.23 ±0.06	4.43 ±0.06	4.18 ±0.12	0.56 ±0.03	90.83 ±0.19	25.44	21.16	4.28

Figure 6. (a) Total extract yield (TEY), total phenolic yield (TPY), total phenolic content (TPC), and DPPH scavenging activity (AA) and (b) color characteristics of extracts from wet pomegranate peel and from infrared (IR) and hot-air (HA) dried pomegranate peel. Extractions were performed at 20°C for 6 min with a solvent (water) ratio of 4:1. Triplicates were tested and analyzed.

similar DSA compared to DPP. Loizzo et al. (2016) investigated the phytochemical contents of extracts from fresh and processed peel and pulp. Comparing to steamed, baked, and microwaved pulp, extraction with fresh pulp achieved up to 0.9% higher TEY and twice the TPC. Their results demonstrated that extraction with fresh peel or pulp could reduce the phytochemical loss that occurs during the drying process. Similarly, Mphahlele et al. (2016) compared the bioactive compounds in fresh peel and hot-air dried peel at 40°C, 50°C, and 60°C.

As for color, extraction of WPP achieved significantly higher *L** (6.08%), *a** (38.71%), *b** (23.81%), and *C** (30.86%) values. The results demonstrated statistically higher retention of *L** (29.83%) and *a** (18.58%) values using WPP. According to Cadena et al. (2013), reduced color characteristics indicate the formation of caramel-colored pigments resulting from nonenzymatic processes, which are related to lower sensory acceptance. Therefore, extraction from WPP could be a more suitable method for the extraction of polyphenols for use in food product development and supplementation.

PRINCIPAL COMPONENT ANALYSIS

PCA was applied to explore the interdependence among variables. Observations consisted of the average subsampling results from the 30 aforementioned extraction conditions at different extraction times, temperatures, and solvent ratios. Four vectors (F1 to F4) were estimated based on the eigenvectors of the correlation matrix of four variables: total extract yield (TEY), total phenolic yield (TPY), total phenolic content (TPC), and DPPH scavenging activity (DSA). The eigenvalues of F1 to F4 were 2.548, 0.899, 0.544, and 0.008, and the first three PCs accounted for 63.70%, 22.48%, and 13.61% of the sample variance, respectively, and represented 99.79% of the total variance in cumulation (table 4). Biplots of the observations and variables (fig. 7) show the data distributions of F1-F2 and F1-F3. Confidence ellipses with 99% confidence intervals were used for each set of experiment groups, and no outliers were detected. PCA weighting scores of the four measured variables (TEY, TPY,

Table 4. Factor loadings for interpretation of figure 7.

Variable	F1	F2	F3	F4
Total extract yield (TEY)	0.649	0.747	0.138	-0.039
Total phenolic yield (TPY)	-0.760	0.574	-0.302	0.045
Total phenolic content (TPC)	0.982	0.030	0.171	0.069
DPPH scavenging activity (DSA)	-0.764	0.102	-0.636	0.011
Eigenvalue	2.548	0.899	0.544	0.008
Explained variance	63.70	22.48	13.61	0.21

TPC, and DSA) are listed in table 4. Higher scores (absolute values) indicate a closer relationship between the variable and the factor. For instance, TEY and TPC were positively correlated with the F1 axis, whereas TPY and DSA were negatively correlated. In addition, TEY and TPY were positively correlated with the F2 axis, while DSA was negatively correlated with the F3 axis. Figure 7 also shows the data distribution for F1-F2 and F1-F3. For instance, the data point with a higher F1-axis value had higher TPC, which was extracted at 60°C for 6 min using a solvent ratio of 8:1. This was in accordance with the experimental results, indicating that PCA can be applied for future condition prediction.

CONCLUSIONS

Extraction of bioactive compounds from waste fruit peel is an efficient approach to improve food system sustainability and improve industry profitability. This study developed a novel green process for antioxidant extraction from wet pomegranate peel (WPP) and investigated the effects of extraction conditions on polyphenol yield and quality, including phenolic composition, DPPH scavenging activity, and color characteristics. PCA condensed the multivariable analysis into three factors, which explained 99.79% of the variance and could be suitable for future process development. Three parameters, including drying preparation, extraction temperature, and solvent (water) ratio, significantly influenced the extraction rate. Considering water usage and energy consumption, WPP extraction at 20°C for 6 min with a solvent ratio of 4:1 is recommended as an economic and sustainable process, resulting in 10.53% total phenolic yield with 88.93% punicalagin purity.
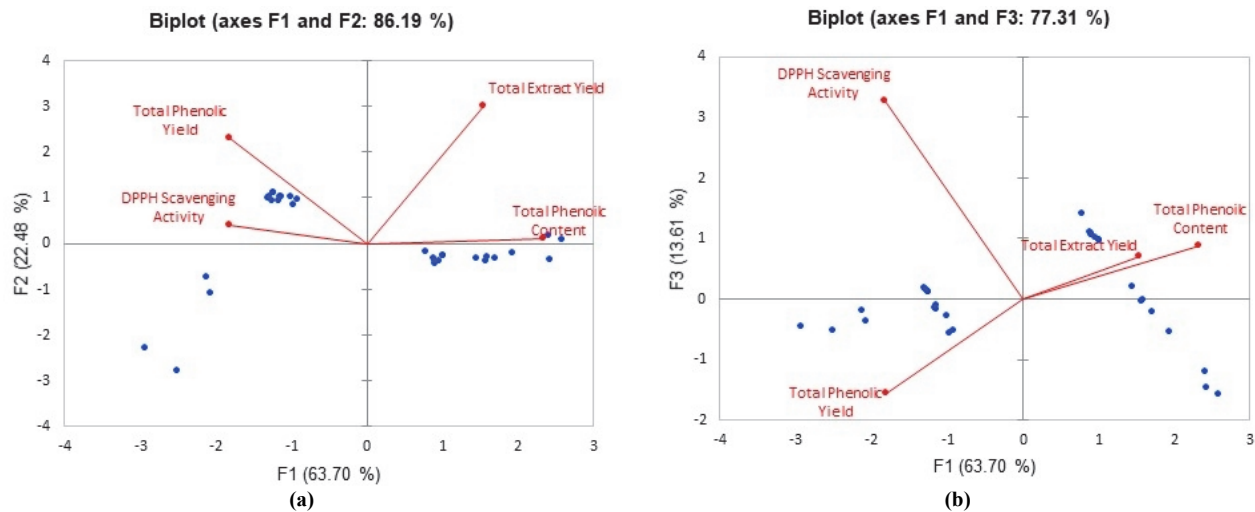
Figure 7. Biplots of objects and vectors for (a) factors F1 and F2 and (b) factors F1 and F3 generated from correlation matrix PCA.

REFERENCES

Adapa, P., Tabil, L., & Schoenau, G. (2011). Grinding performance and physical properties of non-treated and steam exploded barley, canola, oat, and wheat straw. *Biomass Bioenergy, 35*(1), 549-561. https://doi.org/10.1016/j.biombioe.2010.10.004

Al-Farsi, M. A., & Lee, C. Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chem., 108*(3), 977-985. https://doi.org/10.1016/j.foodchem.2007.12.009

Al-Jarallah, A., Igdoura, F., Zhang, Y., Tenedero, C. B., White, E. J., MacDonald, M. E., ... Trigatti, B. L. (2013). The effect of pomegranate extract on coronary artery atherosclerosis in SR-BI/APOE double knockout mice. *Atherosclerosis, 228*(1), 80-89. https://doi.org/10.1016/j.atherosclerosis.2013.02.025

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem., 115*(3), 785-788. https://doi.org/10.1016/j.foodchem.2008.12.005

AOAC. (1990). *Official methods of analysis*. Washington, DC: Association of Official Analytical Chemist.

APHA. (1998). *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association.

Armenta, S., Garrigues, S., & de la Guardia, M. (2015). The role of green extraction techniques in green analytical chemistry. *Trends Anal. Chem., 71*, 2-8. https://doi.org/10.1016/j.trac.2014.12.011

Aviram, M., Volkova, N., Coleman, R., Dreher, M., Reddy, M. K., Ferreira, D., & Rosenblat, M. (2008). Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: Studies *in vivo* in atherosclerotic apolipoprotein e-deficient (E0) mice and *in vitro* in cultured macrophages and lipoproteins. *J. Agric.*

Food. Chem., 56(3), 1148-1157. https://doi.org/10.1021/jf071811q

Azmir, J., Zaidul, I. S., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... Omar, A. K. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng., 117*(4), 426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Banihani, S., Swedan, S., & Alguraan, Z. (2013). Pomegranate and type 2 diabetes. *Nutr. Res., 33*(5), 341-348. https://doi.org/10.1016/j.nutres.2013.03.003

Cadena, R. S., Cruz, A. G., Netto, R. R., Castro, W. F., Faria, J. d. A. F., & Bolini, H. M. (2013). Sensory profile and physicochemical characteristics of mango nectar sweetened with high-intensity sweeteners throughout storage time. *Food Res. Intl., 54*(2), 1670-1679. https://doi.org/10.1016/j.foodres.2013.10.012

CDFA. (2014). California agricultural statistics: 2013 crop year. Sacramento, CA: California Department of Food and Agriculture. Retrieved from https://www.nass.usda.gov/Research_and_Science/Cropland/metadata/metadata_ca13.htm

Charalampia, D., & Koutelidakis, A. E. (2017). From pomegranate processing by-products to innovative value-added functional ingredients and bio-based products with several applications in food sector. *BAOJ Biotech, 3*(1), article 025. Retrieved from https://bioaccent.org/biotechnology/biotechnology25.pdf

Dhinesh, K. V., & Ramasamy, D. (2016). Pomegranate processing and value addition. *J. Food Proc. Tech., 7*(3), article 1000565. https://doi.org/10.4172/2157-7110.1000565

Du, C. T., Wang, P. L., Francis, F. J. (1975). Anthocyanins of pomegranate, *Punica granatum*. *J. Food Sci., 40*(2), 417–418. https://doi.org/10.1111/j.1365-2621.1975.tb02217.x

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y., & Perry, N. B. (2007). Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. *Food Chem., 101*(4), 1417-1424. https://doi.org/10.1016/j.foodchem.2006.03.050

Fischer, U. A., Carle, R., & Kammerer, D. R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril, and differently produced juices by HPLC-DAD-ESI/MSn. *Food Chem., 127*(2), 807-821. https://doi.org/10.1016/j.foodchem.2010.12.156

Gil, M. I., Artes, F., & Tomas-Barberan, F. A. (1996). Minimal processing and modified-atmosphere packaging effects on

pigmentation of pomegranate seeds. *J. Food Sci., 61*(1), 161-164. https://doi.org/10.1111/j.1365-2621.1996.tb14749.x

Hamoud, S., Hayek, T., Volkova, N., Attias, J., Moscoviz, D., Rosenblat, M., & Aviram, M. (2014). Pomegranate extract (POMx) decreases the atherogenicity of serum and of human monocyte-derived macrophages (HMDM) in simvastatin-treated hypercholesterolemic patients: A double-blinded, placebo-controlled, randomized, prospective pilot study. *Atherosclerosis, 232*(1), 204-210. https://doi.org/10.1016/j.atherosclerosis.2013.11.037

Heber, D. (2011). Chapter 10: Pomegranate ellagitannins. In I. F. Benzie & S. Wachtel-Galo (Eds.), *Herbal medicine: Biomolecular and clinical aspects* (2nd Ed.). Boca Raton, FL: CRC Press. http://www.ncbi.nlm.nih.gov/books/NBK92772/

Hernandez, Y., Lobo, M. G., & Gonzalez, M. (2009). Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. *Food Chem., 114*(2), 734-741. https://doi.org/10.1016/j.foodchem.2008.10.021

Höft, M., Barik, S. K., & Lykke, A. M. (1999). Quantitative ethnobotany: Applications of multivariate and statistical analyses in ethnobotany. Paris, France: UNESCO. Retrieved from http://pure.au.dk/portal/files/17477574/hoeftbariklykke1999.pdf

Ismail, T., Akhtar, S., Riaz, M., & Ismail, A. (2014). Effect of pomegranate peel supplementation on nutritional, organoleptic, and stability properties of cookies. *Intl. J. Food Sci. Nutr., 65*(6), 661-666. https://doi.org/10.3109/09637486.2014.908170

Kahramanoglu, I., & Usanmaz, S. (2016). *Pomegranate production and marketing.* Boca Raton, FL: CRC Press. https://doi.org/10.1201/b20151

Lansky, E., Shubert, S., & Neeman, I. (2000). Pharmacological and therapeutic properties of pomegranate. *Proc. Symp. Production, Processing, and Marketing of Pomegranate in the Mediterranean Region: Advances in Research and Technology Seminaires Mediterraneens* (pp. 231-235). Paris, France: Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM)

Laroze, L. E., Díaz-Reinoso, B., Moure, A., Zúñiga, M. E., & Domínguez, H. (2010). Extraction of antioxidants from several berries pressing wastes using conventional and supercritical solvents. *European Food Res. Tech., 231*(5), 669-677. https://doi.org/10.1007/s00217-010-1320-9

LaRue, J. (1977). Growing pomegranates in California. Davis, CA: University of California, Fruit and Nut Research and Information Center. Retrieved from http://ucce.ucdavis.edu/files/programs/5419/Growing_Pomegranates_in_California.htm

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem., 96*(2), 254-260. https://doi.org/10.1016/j.foodchem.2005.02.033

Loizzo, M. R., Bonesi, M., Menichini, F., Tenuta, M. C., Leporini, M., & Tundis, R. (2016). Antioxidant and carbohydrate-hydrolysing enzymes potential of *Sechium edule* (Jacq.) Swartz (cucurbitaceae) peel, leaves and pulp fresh and processed. *Plant Foods Human Nutr., 71*(4), 381-387. https://doi.org/10.1007/s11130-016-0571-4

Malik, A., Afaq, F., Sarfaraz, S., Adhami, V. M., Syed, D. N., & Mukhtar, H. (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc. Natl. Acad. Sci., 102*(41), 14813-14818. https://doi.org/10.1073/pnas.0505870102

Morais, D. R., Rotta, E. M., Sargi, S. C., Bonafe, E. G., Suzuki, R. M., Souza, N. E., ... Visentainer, J. V. (2016). Proximate composition, mineral contents, and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. *J. Brazilian Chem. Soc., 28* (2), 308-317. https://doi.org/10.5935/0103-5053.20160178

Mphahlele, R. R., Fawole, O. A., Makunga, N. P., & Opara, U. L. (2016). Effect of drying on the bioactive compounds, antioxidant, antibacterial, and antityrosinase activities of pomegranate peel. *BMC Complem. Altern. Med., 16*(1), 143. https://doi.org/10.1186/s12906-016-1132-y

Nour, V., Trandafir, I., & Cosmulescu, S. (2015). Central composite design applied to optimize the hydroalcoholic extraction of bilberry (*Vaccinium myrtillus* L.) fruits. *J. Food Biochem., 39*(2), 179-188. https://doi.org/10.1111/jfbc.12119

Pai, V., Chanu, T. R., Chakraborty, R., Raju, B., Lobo, R., & Ballal, M. (2011). Evaluation of the antimicrobial activity of *Punica granatum* peel against the enteric pathogens: An in vitro study. *Asian J. Plant Sci. Res., 1*(2), 57-62.

Pareek, S., Valero, D., & Serrano, M. (2015). Postharvest biology and technology of pomegranate. *J. Sci. Food Agric., 95*(12), 2360-2379. https://doi.org/10.1002/jsfa.7069

Qu, W., Breksa III, A. P., Pan, Z., & Ma, H. (2012). Quantitative determination of major polyphenol constituents in pomegranate products. *Food Chem., 132*(3), 1585-1591. https://doi.org/10.1016/j.foodchem.2011.11.106

Qu, W., Li, P., Hong, J., Liu, Z., Chen, Y., Breksa III, A. P., & Pan, Z. (2014a). Thermal stability of liquid antioxidative extracts from pomegranate peel: Sterilization and storage stability of pomegranate peel extract product *J. Sci. Food Agric., 94*(5), 1005-1012. https://doi.org/10.1002/jsfa.6361

Qu, W., Pan, Z., & Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *J. Food Eng., 99*(1), 16-23. https://doi.org/10.1016/j.jfoodeng.2010.01.020

Qu, W., Shi, S., Li, P., Pan, Z., & Venkitasamy, C. (2014b). Extraction kinetics and properties of proanthocyanidins from pomegranate peel. *Intl. J. Food Eng., 10*(4). https://doi.org/10.1515/ijfe-2014-0034

Sadeghi, N., Janat, B., Oveysi, M., Haji, M. M., & Fotovat, M. (2009). Antioxidant activity of Iranian pomegranate (*Punica granatum* L.) seed extracts. *J. Agric. Sci. Tech., 11*, 633-638.

Schubert, S. Y., Lansky, E. P., & Neeman, I. (1999). Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J. Ethnopharmacol., 66*(1), 11-17. https://doi.org/10.1016/S0378-8741(98)00222-0

Shabtay, A., Eitam, H., Tadmor, Y., Orlov, A., Meir, A., Weinberg, P., ... Kerem, Z. (2008). Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. *J. Agric. Food. Chem., 56*(21), 10063-10070. https://doi.org/10.1021/jf8016095

Shreve, B., Thiex, N., & Wolf, M. (2006). NFTA reference method: Dry matter by oven drying for 3 hours at 105°C. Omaha, NE: National Forage Testing Association.

Sumner, M. D., Elliott-Eller, M., Weidner, G., Daubenmier, J. J., Chew, M. H., Marlin, R., ... Ornish, D. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *American J. Cardiol., 96*(6), 810-814. https://doi.org/10.1016/j.amjcard.2005.05.026

Teh, H. E. (2016). Extraction and characterization of functional components from fruit and vegetable processing waste. PhD diss. Davis, CA: University of California, Department of Food Science.

Tsuda, T., Watanabe, M., Ohshima, K., Norinobu, S., Choi, S.-W., Kawakishi, S., & Osawa, T. (1994). Antioxidative activity of the anthocyanin pigments cyanidin 3-O-beta-D-glucoside and cyanidin. *J. Agric. Food. Chem., 42*(11), 2407-2410. https://doi.org/10.1021/jf00047a009

Wang, Z., Pan, Z., Ma, H., & Atungulu, G. G. (2011). Extract of phenolics from pomegranate peels. *Open Food Sci. J., 5*(1), 17-25. https://doi.org/10.2174/1874256401105010017

Waterman, K. C., & Sutton, S. C. (2003). A computational model for particle size influence on drug absorption during controlled-release colonic delivery. *J. Controlled Release, 86*(2-3), 293-304. https://doi.org/10.1016/S0168-3659(02)00418-2

NOMENCLATURE

DSA = DPPH scavenging activity
DPP = dried pomegranate peel
IDF = insoluble dietary fiber
LPP = large peel particles
PP = pomegranate peel
PS = pomegranate seed
SDF = soluble dietary fiber
SPP = small peel particles
TDF = total dietary fiber
TEY = total extract yield
TPC = total phenolic content
TPY = total phenolic yield