

1 **Chemical composition of dietary fiber and polyphenols of five different varieties of wine**
2 **grape pomace skins**

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1 **Abstract**

2 The skins of two white wine grape pomace (WWGP) and three red wine grape pomace
3 (RWGP) from US Pacific Northwest were analyzed for their dietary fiber (DF) and phenolics
4 composition. DF was measured by gravimetric-enzymatic method with sugar profiling by HPLC-
5 ELSD. Insoluble DF composed of Klason lignin (7.9-36.1% DM), neutral sugars (4.9-14.6%
6 DM), and uronic acid (3.6-8.5% DM) weighed more than 95.5% of total DF in all five WGP
7 varieties. WWGP was significantly lower in DF (17.3-28.0% DM) than those of RWGP (51.1-
8 56.3%), but extremely higher in soluble sugar (55.8-77.5% DM vs. 1.3-1.7% DM) ($p < 0.05$).
9 Soluble polyphenols were extracted by acidified 70% acetone and measured
10 spectrophotometrically. Compared with WWGP, RWGP had higher values in total phenolics
11 content (21.4-26.7 mg GAE/g DM vs. 11.6-15.8 mg GAE/g DM) and DPPH radical scavenging
12 activity (32.2-40.2 mg AAE/g DM vs. 20.5-25.6 mg AAE/g DM) ($p < 0.05$). The total flavanol
13 and proanthocyanidin contents were ranged from 31.0 to 61.2 mg CE/g DM and 8.0 to 24.1 mg/g
14 DM, respectively for the five WGP varieties. This study demonstrated that the skins of WGP can
15 be ideal sources of DF rich in bioactive compounds.

16

17 **Key words:** wine grape pomace (WGP), dietary fiber (DF), polyphenols, radical scavenging
18 activity (RSA)

1 **1. Introduction**

2 The United States is the 4th largest wine producing region in the world. It is estimated that
3 over 4 million metric tons of grapes are harvested and processed into wine products in the US
4 annually (USDA, 2006) and that amount is expected to increase. Wine grape production in
5 Washington and Oregon was 156,000 and 40,200 tons in 2009, respectively; their production
6 being 2nd and 4th among all states (USDA-NASS, 2010a, b). Therefore, tremendous amounts of
7 wine grape pomace (WGP) are available annually in the Pacific Northwest region of the United
8 States. WGP is primarily composed of seeds, skins and stems, and is commonly used for the
9 extraction of grape seed oils (Mattick & Rice, 1976), and the production of citric acid, methanol,
10 ethanol, and xanthan via fermentation (Hang & Woodams, 1985; Hang, Lee & Woodams, 1986;
11 Couto & Sanromán, 2005). The large amounts of WGP available at relatively low cost provide
12 an opportunity for value-added product development through innovative technologies.

13 Dietary fiber (DF), defined as “edible parts of plants or analogous carbohydrates that are
14 resistant to digestion and absorption in the human small intestine with complete or partial
15 fermentation in the large intestine” (AACC, 2001), is abundant in plant products such as fruits,
16 vegetables, and grains. DF is widely recognized as being a beneficial component of a healthy
17 diet, its consumption being correlated with reductions in risks associated with cardiovascular
18 disease, cancer, and diabetes (Cho & Dreher, 2001). Furthermore, grape skins, which comprise,
19 on average, 82% of the wet weight of WGP (Jiang, Simonsen & Zhao, 2011), contain multiple
20 types of polyphenols, including 39 types of anthocyanins, hydroxycinnamic acids, catechins, and
21 flavonols (Kammerer, Claus, Carle & Schieber, 2004). These polyphenols have been claimed to
22 have antioxidant activity (González-Paramás et al., 2004) and inhibit low-density lipoprotein
23 (LDL) oxidation (Yildirim, Akçay, Güvenç, Altindişli & Sözmen, 2005).

1 The DF content of WGP from grape varieties in Spain, such as Manto Negro, Cencibel, and
2 Airén, has been published (Valiente, Arrigoni, Esteban & Amado, 1995; Bravo & Saulix-Calixto,
3 1998; Llobera & Cañellas, 2007). However, little is known of the composition of WGP resulting
4 from the processing of grapes common to the US Pacific Northwest. These varieties include
5 Pinot Noir, which accounts for >50% of the total wine grape production in Oregon (USDA-
6 NASS, 2009), and Cabernet Sauvignon and Merlot, the latter two being the top red wine varieties
7 in Washington (USDA-NASS, 2010). The chemical composition of WGP is known to vary
8 depending on the grape cultivar, growth climates, and processing conditions. Therefore, the aim
9 of this study was to characterize the DF and polyphenol fractions of WGP skins common to the
10 US Pacific Northwest. The information obtained from this study will be helpful in the
11 development of value added products derived from such WGP.

12

13 **2. Material and methods**

14 **2.1. Sample preparation**

15 In this study, skins of WGP were abbreviated as WGP. Two white wine grape pomace
16 (WWGP) samples, *Vitis vinifera* L. cv. Morio Muscat and cv. Muller Thurgau, were donated by
17 a private winery in Corvallis, Oregon, USA. One red wine grape pomace (RWGP), *Vitis vinifera*
18 L. cv. Cabernet Sauvignon, was from a commercial winery in Kennewick, Washington, USA.
19 The other two RWGP samples, *Vitis vinifera* L. cv. Pinot Noir and cv. Merlot, were received
20 from the Oregon State University Research Winery (Corvallis, OR, USA). All WGP samples
21 were processed in fall 2008, packed in the polyethylene terephthalate pails (Ropak Corp.,
22 Cincinnati, OH, USA) and stored at -24 °C until used.

1 Dietary fiber samples were prepared from the skins contained within the WGP using the
2 procedures of Park, Jiang, Simonsen & Zhao (2009). Briefly, WGP was thawed at room
3 temperature, stems and seeds were removed manually and the remaining WGP skins were
4 ground in a disintegrator (M8A-D, Corenco, Inc., Sebastopol, CA, USA) to pass a 0.11 mm mesh
5 screen. The resulting preparation was then dried in an environmental chamber (T10RS, Tenney
6 Environmental, Williamsport, PA, USA) at 10% RH and 70 °C for 48 h. The dried WGP was
7 then milled (Thomas Scientific, Swedesboro, NJ, USA) to pass a 20-mesh screen. The resulting
8 powders were stored in Ziploc® storage bags (S.C. Johnson & Son, Inc., Racine, WI, USA) at -
9 24 °C until used.

10

11 **2.2. Chemicals and reagents**

12 D-(+)-glucose, D-(+)-xylose, D-(+)-galactose, L-(+)-arabinose, and D-(+)-mannose were of
13 HPLC grade and purchased from Sigma-Aldrich (MO, USA). Dimethylphenol reagent, protease
14 (P-5459), Folin-Ciocalteu reagent, gallic acid, and (+)-catechin hydrate were all purchased from
15 Sigma-Aldrich (MO, USA). Anthrone and vanillin were supplied by Alfa Aesar (MA, USA).
16 Disodium ethylenedinitrilo tetraacetic acid (Na₂-EDTA) and ascorbic acid were obtained from
17 Mallinckrodt Baker, Inc. (NJ, USA) while galacturonic acid was from Spectrum Chemical Co.
18 (CA, USA). Ammonium sulfate was provided by EMD Chemicals (USA). In addition, 1,1-
19 diphenyl-2-picrylhydrazyl (DPPH) was purchased from Kasel Kogyo Co. Ltd (Tokyo, Japan).
20 All other solvents and reagents were of analytical grade.

21

22 **2.3. Analysis of ash, fat and crude protein contents**

1 Ash, fat and crude protein contents of the WGP powders were determined at a certified
2 commercial laboratory (Bodycote Testing Group, Portland, OR, USA). One measurement was
3 carried out for each WGP variety and the results were expressed as percentage of dry matter
4 (DM).

6 **2.4. Analysis of soluble sugars**

7 A representative 0.5 g sample of WGP powder in a 35 mL centrifuge tube was extracted
8 three times in succession with 25 mL of 80% ethanol for 15 min at room temperature in an
9 ultrasonic unit (Branson B – 220H, SmithKline Co., Shelton, CT, USA) (AOAC 994.13, 2007).
10 Each extraction was terminated by centrifugation ($10,000 \times g$ for 10 min) and collection of the
11 resulting supernatant. The combined supernatants, from the three extractions, were evaporated at
12 $50\text{ }^{\circ}\text{C}$ under vacuum by a rotary evaporator (Brinkmann Instruments, Westbury, NY, USA) to
13 remove ethanol and then diluted to 50 mL with water. The sugar content was then determined by
14 thoroughly mixing 1 ml of the soluble sugar-containing solution with 2 mL 75% H_2SO_4 and 4
15 mL anthrone reagent consisted of 0.5 mg anthrone in 250 mL 75% H_2SO_4 , incubating the
16 reaction mixture at $100\text{ }^{\circ}\text{C}$ for 15 min, cooling to room temperature, and measuring absorbance
17 at 578 nm (UV160U, Shimadzu, Kyoto, Japan) (Gerhardt, Wood, Krieg & Murray, 1994).
18 Absorbance values were converted to “glucose equivalents” using a calibration curve prepared
19 with a D-(+)-glucose as specified above. Results were expressed as a percent of WGP DM.

21 **2.5. Fractionation of extractable pectins**

22 WGP extractable pectins were fractionated into water soluble pectin (WSP), chelator soluble
23 pectin (CSP), and hydroxide soluble pectin (HSP) as described by Silacci & Morrison (1990).
24 WGP powder, 1.0 g, was first homogenized (PT 10-35, Kinematica, Littau, Switzerland) in 20 g

1 deionized water for 10 min. The homogenate was then filtrated (Whatman No.1 filter paper) and
2 the retentate and filtrate collected. WSP was obtained as the precipitate that resulted from the
3 addition of 95% ethanol to the filtrate (filtrate: 95% ethanol = 1:5) and then allowing it to stand
4 overnight in the refrigerator. CSP was obtained from the water extracted residue by first boiling
5 the residue with 95% ethanol for 10 min and then doing three successive extractions of the
6 resulting residue with 50 mL, 20 mM Na₂-EDTA, pH 8.0. Following each extraction the
7 suspension was filtered and the filtrates combined. The residue obtained from the Na₂-EDTA
8 extractions was then extracted with 50 mL of 50 mM NaOH for 15 min at room temperature; the
9 suspension was filtered and the filtrate collected for measurement of HSP.

10 WSP, CSP, and HSP were quantified as galacturonic acid equivalents (GUAE) based on a
11 colorimetric assay (AOAC 994.13, 2007) using galacturonic acid for preparation of the
12 calibration curve. Briefly, 250 µL boric acid-sodium chloride solution (content) and 250 µL of
13 sample (or standard) were mixed with 4 mL of 96% H₂SO₄ and incubated at 70 °C for 40 min.
14 Next, 200 µL of dimethyphenol reagent (100 mg of 3, 5-dimethyphenol in 100 mL of glacial
15 acetic acid) was added with mixing and the absorbance read at 400 nm and 450 nm, respectively.

16

17 **2.6. Determination of dietary fiber**

18 **Separation of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF).** A
19 representative 0.5 g sample of WGP powder was defatted by two successive extractions with 25
20 mL petroleum ether for 10 min in an ultrasonic water bath at room temperature; the liquid and
21 solid phases being separated by centrifugation for 10 min at 10,000 × g. The defatted residue was
22 then extracted three times in succession with 80% ethanol, as described above, to remove soluble
23 lower molecular weight saccharides. The resulting residue was dried at 40 °C for 16 h. The dried

1 residue was then treated with 0.0275 mL protease in 0.05 M phosphate buffer, pH 7.5, for 30 min
2 at 60 °C (AOAC, 1985). Liquid and solid phases were separated by centrifugation at 10,000 × g
3 for 10 min. The supernatant was saved for determination of SDF. The residue was washed with
4 two portions of 10 mL deionized water; the supernatants from these washings were combined
5 with the supernatant obtained following the protease treatment for determination of SDF. The
6 resulting residue was washed first with 95% ethanol and then twice with acetone followed by
7 drying at 40 °C for 16 h (Prosky, Asp, Furda & DeVries, 1985). This residue was used for IDF
8 analyses.

9 **Analysis of SDF.** The soluble dietary fiber (SDF) was purified by dialysis in order to prevent
10 error caused by precipitating dietary fiber with ethanol (Mañas, 1994), the supernatant containing
11 soluble dietary fiber was sequentially dialyzed in a dialysis tube of which the molecular weight
12 cutoff was 12,000-14,000 (Spectrum Laboratories, Inc., Rancho Dominguez, CA., USA) in 1 L
13 of DI water in the refrigerator. DI water was changed at 4, 16, 28, 30, and 36 h, respectively and
14 the dialysis was finished at 48 h of which the conductivity of dialysate was around 2-6 µS/cm,
15 very close to the value of DI water (~1 µS/cm).

16 The dialyzed SDF preparation was freeze-dried and then acid hydrolyzed in 6% sulfuric acid
17 at 121 °C for 1 h (Bravo & Saura-Calixto, 1998). Uronic acids (UA) in the resulting hydrolysate
18 were quantified by colorimetry as described above. Neutral sugars (NS) were determined by
19 HPLC following neutralization by addition of calcium carbonate. D-(+)-glucose, D-(+)-xylose,
20 D-(+)-galatose, L-(+)-arabinose, and D-(+)-mannose were employed as standards. HPLC was
21 done using a Shimadzu 20A series instrument equipped with Shimadzu-LT II evaporative light
22 scattering detector (ELSD) (Shimadzu, Columbia, MD, USA) and a Biorad Aminex HPX-87P
23 column (Bio-Rad Laboratories, Hercules, CA, USA). The nitrogen pressure of the ELSD was

1 maintained at 50.6 to 52.0 psi and column temperature at 85 °C. Distilled/deionized water was
2 used as the mobile phase with a flow rate of 0.6 mL/min and injection volume of 20 µL (Sluiter,
3 et al., 2008). The sum of NS and UA was taken as the amount of SDF in WGP. All the results
4 were expressed as percentage per DM.

5 **Analysis of IDF.** The dry residue was hydrolyzed in a two-stage manner. The first stage
6 involves the addition of 3 mL 72% sulfuric acid to the residue, with stirring, and subsequent
7 incubation for 1 h at 30 °C. The second stage consists of diluting the first-stage hydrolysate to
8 2.5% sulfuric acid by the addition of 84 g deionized water, followed by incubation of the
9 resulting suspension at 121 °C for 1 h. The hydrolyzed mixture is then filtrated (fritted crucible;
10 Pyrex[®] 30 mL M, Corning, Inc., USA). The filtrate is used for quantifying UA and NS as
11 described above. The crucible containing the residue is used for the gravimetric determination of
12 Klason lignin (KL) as described by Sluiter et al. (2008). KL was the weight of residue after
13 drying at 105 °C for 16 h, ashing for 5 h at 525 °C, and subtracting resistant protein (RP).
14 Resistant protein (RP) in the WGP powder, defined as the protein after protease treatment and
15 acid hydrolysis in this study, was determined by the micro-Kjedahl method using a nitrogen-to-
16 protein conversion factor of 6.25 (AOAC 960.52, 1995). IDF was the total of NS, UA and KL,
17 and expressed as percentage per DM.

18

19 **2.7. Determination of bound condensed tannins (CT)**

20 Residue analogous to that used for the IDF analyses, prepared from 0.5g WGP as discussed
21 above, was used for determination of bound CT using the method described by Reed (1982). The
22 residue was incubated with 50 mL 5% HCl-butanol (v/v) in a capped Erlenmeyer flask at 100 °C
23 for 3 h. After cooling to room temperature, the absorbance of the liquid phase at 553 nm was

1 determined. Standards for CT are not commercially available; therefore, a CT preparation from
2 red wine grape skin (~80.4% by weight CT) was used as the standard in companion analyses
3 (Kennedy & Jones, 2001). The extinction coefficient used for quantification was based on
4 incubating a 5.0 mg sample of the CT standard with 10 mL 5% acid-butanol at 100 °C for 30 min
5 and subsequently determining the liquid phase absorbance at 553 nm. The bound CT of WGP
6 was expressed as percentage per DM.

7

8 **2.8. Extraction and quantification of soluble phenolic compounds**

9 **Phenolics extraction from fresh WGP.** Two methods were compared for extraction of
10 soluble phenolics in WGP. Frozen WGP were ground to a fine powder in liquid nitrogen and
11 subsequently used for extraction experiments as WGP. Preliminary data (not shown), as well as a
12 survey of the literature (Spigno & Faveri 2007; Spigno, Tramelli & Faveri, 2007), indicated that
13 1:4 (w/v) ratio of WGP to solvent was sufficient for extraction of phenolic compounds, thus both
14 methods were carried out at this ratio. In the first method (method “A”), WGP was extracted
15 with 0.1% HCl/70% acetone/29.9% water (v/v/v) by placing the suspension in an ultrasonic unit
16 for 1 h at room temperature; the temperature of the ultrasonic bath was kept below 45 °C by
17 exchanging water when necessary. The liquid and solid phases were then separated by
18 centrifugation at $10,000 \times g$ for 15 min. This procedure was done three times per sample (total of
19 three extractions per sample). The combined supernatant from the three extractions was
20 concentrated on a rotary evaporator at 40 °C under vacuum and then brought to 50 mL with DI
21 water and stored at -70 °C until analyzed. In the second method (method “B”), the solvent was
22 70% acetone/30% water and the extraction was done in an environmental shaker at room
23 temperature with constant agitation (125 rpm). This procedure was done two times per sample

1 (total of two extractions per sample). The combined supernatant from the two extractions was
2 concentrated, brought to volume and stored as in method “A”. The thawed supernatents resulting
3 from extraction methods A and B (referred to below as “extracts”) were used as the starting
4 material for the analysis of total phenolics, antiradical scavenging activity, total flavanol,
5 extractable proanthocyanidins, and anthocyanins.

6 **Analysis of total phenolic content (TPC).** The total phenolic content of extracts was
7 measured using the Folin–Ciocalteu (FC) reagent-based colorimetric assay as described by
8 Singleton & Rossi (1965). Phenolic content was calculated as gallic acid equivalents (GAE) and
9 reported as mg/g DM. Briefly, 0.5 mL appropriately diluted extract (or gallic acid standard at 0,
10 50, 100, 150 or 200 ppm) was mixed with 0.5 mL of 2N FC reagent (Sigma Chemical Co., MO,
11 USA) and 7.5 mL deionized water and allowed to stand for 10 min at room temperature; then 3
12 mL of 20% (w/v) Na₂CO₃ was added to the reaction mixture and it was placed in a 40 °C water
13 bath for 20 min. After the 20 min reaction period, the samples were cooled to room temperature
14 and the absorbance measured at 765 nm.

15 **Analysis of antiradical scavenging activity (RSA).** The RSA of extracts was determined by
16 the 1,1–diphenyl–2–picrylhydrazyl (DPPH)-based assay using ascorbic acid as the calibration
17 standard and reported as mg ascorbic acid equivalents (AAE) per g DM (Brand-Williams, 1995).
18 Briefly, 0.5 mL appropriately diluted extract (or ascorbic acid standard at 0, 0.01, 0.02, 0.03,
19 0.04 mg/mL) was mixed thoroughly with 1.5 mL of DPPH–methanol reagent (9 mg DPPH in
20 100 mL methanol) and allowed to stand at room temperature for 5 min prior to measuring the
21 solutions absorbance at 517 nm.

22 **Determination of total flavanol content (TFC).** The total flavanol content of extracts was
23 determined by the vanillin colorimetric assay (Price, 1978) using (+)-catechin hydrate as the

1 calibration standard. A 1.0 mL aliquot of appropriately diluted extract (or the catechin standard
2 at 0, 0.06, 0.12, 0.18, 0.24, 0.30 mg/ml) was mixed with 5.0 mL vanillin reagent (0.5% vanillin
3 in 4% HCl-methanol, w/v). This mixture was allowed to react for 20 min at 30 °C and then the
4 absorbance measured at 500 nm. The blank, included in each assay, was treated as above except
5 the blank-vanillin reagent contained no vanillin. Concentrations were determined based on taking
6 the difference between the absorbance of the corresponding assay and blank samples. TFC was
7 expressed as mg catechin equivalents (CE) per g DM.

8 **Determination of extractable proanthocyanidins (PAC).** Extractable PAC was measured
9 by the colorimetric assay of Porter (1986) using a red wine grape skin CT powder as the
10 calibration standard. Briefly, 0.5 mL extract (or standard solution) was mixed (vortexed) with 3
11 mL acid butanol (50 mL 12 N HCl with 950 mL n-butanol) and 0.1 mL iron reagent (2% ferric
12 ammonium sulfate in 2 N HCl). The mixture was then placed in boiling water for 50 min
13 followed by the measurement of its absorbance at 553 nm. Extractable PAC was expressed as mg
14 CT equivalents per g DM.

15 **Analysis of anthocyanin content (ACY).** The ACY content of extracts was determined by
16 the pH differential method (Guisti & Wrolstad, 2001). Briefly, extract was diluted to the same
17 extent in 0.025 M potassium chloride (final pH = 1.0) and in 0.4 M sodium acetate (final pH =
18 4.5) and the absorbance of the two solutions was measured at 520 nm and 700 nm. A solution of
19 malvidin-3-glucoside (Mvd-3-glu), molar absorptivity of 28,000 L/cm/mol and molar mass of
20 529 g/mol (Thimothe, Bonsi, Padilla-Zakour & Koo, 2007), was used as the calibration standard.
21 ACY was calculated as:

22
$$\text{ACY mg/liter} = ((A_{520} - A_{700})_{\text{pH } 1} - (A_{520} - A_{700})_{\text{pH } 4.5}) \times 529 \times \text{dilution factor} \times 1000 / 28000,$$

23 and expressed as mg Mvd-3-glu equivalents/g DM.

1 **2.9. Data Analysis**

2 All analyses, with the exception of those specified as being done by a commercial laboratory,
3 were done in triplicate. Results were expressed as mean \pm SD. Pair t-test was used to compare
4 the differences between the two phenolic extraction methods (A and B). One way ANOVA was
5 used to analyze the differences among WGP samples based on LSD test at 95% confidence level
6 (SAS 9.1, SAS Institute Inc., Cary, NC, USA).

8 **3. Results and discussion**

9 **3.1. Ash, protein and fat**

10 Overall, RWGP had higher crude protein, fat and ash contents than those of WWGP (Table
11 1), which was consistent with the results found by Baumgärtel, Kluth, Epperlein & Rodehutschord
12 (2007). Among WWGP varieties, Muller Thurgau had higher values in crude protein and fat but
13 lower value in ash than those in Morio Muscat. Regarding to RWGP varieties, Cabernet
14 Sauvignon had relatively high crude protein, fat and ash content, an indication of high amino
15 acids and minerals in the pomace sample.

17 **3.2. Soluble sugar**

18 In this study, soluble sugar was defined as mono- or di-saccharides dissolving in 80% ethanol.
19 The two WWGP varieties had significantly ($p < 0.05$) higher soluble sugar contents than those of
20 RWGP, about 56% for Muller Thurgau and 78% for Morio Muscat (Table 1), while it was
21 between 1.3-1.7% for RWGP. The high soluble sugar content of WWGP was not reported in
22 previous publications and probably due to different winemaking procedures applied and different
23 varieties of the wine grape. WWGP was obtained right after pressing juice while RWGP was

1 after fermented with juice for several days in order to extract color and polyphenols. Hence, the
2 unfermented juice residue attaching to WWGP rendered the considerably higher amount of
3 soluble sugar compared with those in RWGP. Soluble sugar content of RWGP from this study
4 was relatively lower than those commercial RWGP in Europe, such as Cencibel, Manto Negro,
5 and Blauer Portugieser (Bravo & Saura-Calixto, 1998; Llobera & Cañellas, 2007; Baumgärtel,
6 Kluth, Epperlein & Rodehutschord, 2007), but closer to the data reported in US wine grape
7 varieties, such as Concord, Ives, Baco Noir, and Cascade (Rice, 1976).

8

9 **3.3. Extractable pectins**

10 Overall, RWGP had higher total extractable pectins (TEP) (50.6-56.4 mg GUAE/g DM) than
11 that of WWGP (32.3-41.2 mg GUAE/g DM) (Fig. 1) ($p < 0.05$). However, the similar trend was
12 not observed in respect to individual fraction of the pectin (Fig. 1), in which the distributions of
13 three pectin fractions varied depending on the variety of WGP. Among the three fractions of TEP,
14 the highest proportion was chelator soluble pectin (CSP) (79.47-94.50% of TEP). CSP of RWGP
15 was higher than that of WWGP. Cabernet Sauvignon was of the highest value of CSP (about 50
16 mg GUAE/g DM), followed by Merlot and Pinor Noir. For WWGP, CSP of Muller Thurgau was
17 10 mg GUAE/g DM, higher than that of Morio Muscat ($p < 0.05$). Relatively, only small amounts
18 of water soluble pectin (WSP) and hydroxide soluble pectin (HSP) were observed in both
19 WWGP and RWGP. WSP of Merlot was significantly ($p < 0.05$) higher than the values in any
20 other varieties, about 4.5 mg GUAE/g DM followed by Pinot Noir with a value of about 2.5 mg
21 GUAE/g DM. The smallest proportion was HSP with the lowest amount in Morio Muscat (0.42
22 mg GUAE/g DM) and the highest amount in Merlot (0.89 g GUAE/g DM), which reflected a
23 small amount of high esterified pectin extracted from WGP (Košťálová, Hromádková &

1 Ebringerová, 2010). On the other hand, pectin matrix, cellulose and xyloglucan form the
2 supporting structure in the plant cell wall materials (Carpita & Gibeaut, 1993). Within the pectin
3 matrix, two chains of low-esterified or non-esterified galacturonic acids are mainly connected
4 with calcium ions on their carboxyl groups (Morris, Powell, Gidley & Rees, 1982; Carpita &
5 Gibeaut, 1993). Because Na₂-EDTA solution is able to chelate with calcium ions so that
6 destroying the connections between the chains, the highest proportion of CSP in TEP indicated
7 that pectic polysaccharides in WGP mainly constitute cell wall materials. Insufficient studies on
8 fractionating pectic polysaccharides of WGP into WSP, CSP and HSP have been reported.
9 According to Silacci & Morrison (1990), winemaking procedures did not have largely impact on
10 the distributions of three fractions except that the WSP might be reduced due to the compression
11 step.

12

13 **3.4. Dietary fiber (DF)**

14 Total dietary fiber (TDF) (Table 2) was the predominant composition in the RGWP but not
15 in the WWGP in which soluble sugar took the largest proportion. TDF of 51.1-56.3% on dry
16 matter in the RWGP were similar to those reported by Veliente, Arrigoni, Esteban & Amado
17 (1995) and Bravo & Saura-Calixto (1998) (about 50-60% of TDF).

18

19 **3.4.1. Neutral sugar and uronic acid**

20 Major fraction of TDF was insoluble dietary fiber (IDF) which dominated up to 98.5% of the
21 TDF (Table 3). RWGP had significantly ($p<0.05$) higher NS content than those of WWGP.
22 Regarding to NS in IDF, Merlot had at least 9% more than other two RWGP varieties, and
23 Muller Thurgau had 46% higher amount of NS than that of Morio Muscat. Among the NS,

1 glucose was of the highest amount for all five WGP varieties, followed with xylose, while
2 arabinose was traced to be the lowest amount. Since cellulose structured by large amount of
3 glucose subunits, substantial amount of glucose existing in IDF indicated that cellulose was the
4 major constituent of IDF in the WGP. This finding was consistent with the results by Bravo &
5 Saura-Calixto (1998) and Valiente, Arrigoni, Esteban & Amado (1995). The ratio of xylose to
6 galactose varied from 0.8 in Morio Muscat to 2.1 in Merlot, which, to some extent, indicated the
7 amount of hemicellulose in IDF. This ratio is within the range of other findings (Valiente,
8 Arrigoni, Esteban & Amado, 1995; Bravo & Saura-Calixto, 1998). Based on the fact that the
9 major constituent of hemicellulose in grape skins is xyloglucans, structured by the glucan
10 backbone linked with β (1 \rightarrow 4) linkage and 75% of glucose siding with xylose and 35% galactose
11 (Thompson & Fry, 2000), it was predictable that a promising amount of hemicellulose in the
12 WGP skins.

13 The majority of uronic acid (UA) was detected in IDF fraction in WGP (89.4% to 96.9% of
14 the total UA) (Table 3), illustrating that a large proportion of pectic polysaccharides composed of
15 cell wall materials. Similarly, UA of RWGP was 7-134% higher than that of WWGP. Cabernet
16 Sauvignon had the highest UA value, while Muller Thurgau had the lowest among the five
17 varieties. Since UA is the backbone of pectic polysaccharides in plant cell walls, it was
18 concluded that RWGP generally had higher contents of pectic polysaccharides. By considering
19 the neutral sugar profiling data, it could be confirmed that WGP skins contain a large amount of
20 homogalacturonan (HG) consisting of backbone galacturonic acids with some xylose and
21 glucose and a small amount of hairy pectic polysaccharides (rhamnogalacturonan I and
22 rhamnogalacturonan II) (Arnous & Meyer, 2009).

1 Unlikely in IDF, SDF took only small portion of TDF in all WGP varieties. In SDF, the NS
2 was mainly associated with UA to form the pectic polysaccharides. The NS and UA values
3 varied from 0.4-1.0% DM and 0.3-0.7% DM, respectively. NS in SDF was the lowest in Morio
4 Muscat (~0.4% DM) and the highest in Pinot Noir (1.0% DM), and UA in SDF were the highest
5 in Merlot and Pinot (0.73% DM and 0.72% DM, respectively) while the lowest in Morio Muscat
6 and Cabernet Sauvignon (0.26% DM and 0.27% DM, respectively). Glucose was the
7 predominant sugar in all five WGP varieties with the highest in Pinot Noir (0.43% DM) and the
8 lowest in Morio Muscat (0.15% DM), but only 0.02-0.04% xylose was detected in the SDF of
9 WGP. The NS data in SDF did not agree with the findings by Bravo & Saura-Calixto (1998) in
10 which only arabinose was detected in SDF of WGP, but agreed with the data from Valiente,
11 Arrigoni, Esteban & Amado (1995). That the UA value close to NS might indicate that the hairy
12 pectic polysaccharides were the major portion of the SDF. The low UA content detected in the
13 WGP differed from the work by Bravo & Saura-Calixto (1998) and Valiente, Arrigoni, Esteban
14 & Amado (1995), possibly owing to the discrepancies in grape berry maturity at the time of
15 harvest, the WGP varieties, and the winemaking procedures.

16 Assuming that all pectic polysaccharides were completely released from WGP by the harsh
17 acid hydrolysis, TEP (Fig.1) took 81.9%, 79.3%, 63.3%, 76.9% and 88.4% of the total pectic
18 polysaccharides in Muller Thurgau, Morio Muscat, Cabernet Sauvignon, Merlot and Pinot Noir,
19 respectively. It revealed that the major amount of pectins were soluble in water, chelator, and
20 hydroxide in WGP. In addition, the values of WSP (Fig.1) were lower than UA values in SDF
21 (Table 3), and the differences between them were 0.35%, 0.12%, 0.18%, 0.25%, and 0.49% DM
22 in Muller Thurgau, Morio Muscat, Cabernet Sauvignon, Merlot and Pinot Noir, respectively. It

1 indicated that after protease treatment and sulfuric acid hydrolysis, more WSP was released from
2 WGP matrix.

3

4 **3.4.2. Klason lignin, condensed tannins and resistant protein**

5 Considerable amount of Klason lignin (KL) was observed in IDF fraction of the WWGP, but
6 significantly lower than those in the RWGP (Table 3). Pinot Noir possessed the highest amount
7 of KL among RWGP, 4.4% and 7.7% higher than that of Cabernet Sauvignon and Merlot,
8 respectively. In terms of WWGP, KL of Muller Thurgau was nearly double that of Morio Muscat.
9 The ratios of KL to TDF ranged from 54.9% in Morio Muscat to 64.0% in Pinot Noir.
10 Differences in lignifications of cell wall materials, wine grape varieties and the attachment of
11 berry pulps could all contribute to the various KL values in the WGP. KL values in Muller
12 Thurgau and Morio Muscat obtained from this study were smaller than that in another reported
13 WWGP skins (Airén) (38% DM), while the KL percentages of RWGP were similar to the
14 reported values of RWGP skins (Cencibel and Mento Negro) (Bravo & Saura-Calixto, 1998;
15 Llobera & Cañellas, 2007). The high KL content in IDF of WGP skins make it suitable for
16 various applications, such as phenolic resins and adhesives (Stewart, 2008).

17 In this study, resistant proteins (RP) and bound condensed tannins (CT) were also quantified
18 (Table 4). Expectedly, the CT and RP values generally had the same trend as KL. Both bound
19 CT and RP values of the RWGP were higher than those of WWGP. Pinot Noir had the highest
20 amount of bound CT (19.89% DM) and highest RP (4.92% DM), while Morio Muscat was the
21 lowest in both values, 6.04% DM for bound CT and 1.50% DM for RP.

22 Bound CT values in RWGP in this study were generally lower than those in cv. Cencibel and
23 cv. Mento Negro which were 26.8% and 22.3% DM, respectively, meanwhile WWGP had

1 smaller amount of bound CT than that in WWGP skins (Airén) (20.5% DM) (Bravo & Saura-
2 Calixto, 1998; Llobera & Cañellas, 2007). The bound CT in the WGP was significantly higher
3 than those in fruit, fruit peels and dark chocolates of which the bound CT value ranged from 0-8%
4 DM (Goñi, Díaz-Rubio, Pérez-Jiménez, Saura-Calixto, 2009).

5 RP values of WGP ranged from 1.5% to 4.9% DM (Table 4) and were lower than that of
6 RWGP skins (Cencibel) and WWGP skins (Airén) (Bravo & Saura-Calixto, 1998) but close to
7 Mento Negro (Llobera & Cañellas, 2007). In addition, the percentage RP of crude protein (Table
8 4) ranged from 32.4% to 40.6% in RWGP and 27.9% to 35.2% in WWGP, lower than those
9 described in the previous studies, such as 80.6% in RWGP skins (Cencibel) and 78.6% in
10 WWGP skins (Airén) (Bravo & Saura-Calixto, 1998). Bound CT is the polymeric polyphenols
11 and usually precipitating proteins to form CT-protein matrix in plant cell wall during the grape
12 berry maturation. Therefore, lower values of bound CT might contribute the lower values of
13 resistant protein in this study. Furthermore, RP was measured after protease treatment and the
14 harsh acid hydrolysis which could partially hydrolyze proteins into amino acids hence caused the
15 smaller values of detected RP.

16

17 **3.5. Effect of different extraction conditions on the soluble phenolic compounds**

18 Results in the soluble phenolic compounds extracted by using two different extracting
19 methods were reported in Table 5. Method A was superior to method B in almost all phenolic
20 compounds for all varieties of WGP ($p < 0.05$) except TPC of Morio Muscat and Merlot, ACY of
21 Cabernet Sauvignon and Pinot Noir, and PAC of Morio Muscat. By using method A, TPC values
22 of RWGP and Muller Thurgau increased from 39% to 110%, RSA and TFC values increased
23 from 53% to 125% and 24% to 65%, respectively for all the WGP varieties, and PAC values also

1 remarkably increased from 49% to 130% for all varieties except for Morio Muscut. This study
2 demonstrated the influence of extraction conditions on the yield of phenolic compounds from
3 WGP. First, the ultrasound assisted extraction yielded higher content of polyphenols than the
4 extraction carried out at room temperature with low speed shaking. This observation agreed with
5 results from previous publications (Kalia, Sharma, Singh & Singh, 2008; Khan, Abert-Vian,
6 Fabiano-Tixier, Dangles & Chemat, 2009). Secondly, HCl acidified aqueous solvent extraction
7 enhanced the extraction yield of TPC and RSA but little on ACY (p value was from 0.010 to
8 0.180), which was consistent with the findings from Maisuthisakul & Gordon (2009) and Vatai,
9 Škerget & Knez (2009). Increased TPC and RSA values as a result of acid hydrolysis solvent
10 extraction might be explained by the fact that the hydrolysis step helped the release of
11 polyphenols from plant cell wall materials (Krygier & Sosulski, 1982; Maisuthisakul & Gordon,
12 2009). Finally, the three times extraction might also promote the efficiency of phenolics
13 extraction from the WGP.

14

15 **3.6. Soluble polyphenols**

16 Since method A worked better than method B in respect to extraction of phenolic compounds,
17 only results from method A are discussed here. TPC values of RWGP were significantly higher
18 than those of WWGP ($p < 0.05$) due to the lack of ACY in WWGP. No markedly difference in
19 TPC values between Muller Thurgau and Morio Muscat was observed. Among RWGP, TPC of
20 Pinot Noir was lower than that in Merlot and Cabernent Sauvignon ($p < 0.05$) (Table 5), which
21 may be attributed to the thinner fruit skin of Pinot Noir, retaining less TPC.

22 Table 6 shows the total polyphenols reported in previous publications on wine grape pomace,
23 grape berry, and other fruit byproducts. TPCs detected in this study were within the range of the

1 published data (9.7-54.0 mg GAE/g DM). The higher TPC values in other publications were
2 owing to inclusion of grape seeds into WGP which possessed higher content of phenolics. The
3 TPC values of fresh wine grape berry were also reported in Table 6. TPC of Pinot Noir was 38.4
4 mg/g based on fresh weigh, which was significantly higher than value from this study (21.4 mg
5 GAE/g DM). The reduction of TPC in WGP was due to the extraction of polyphenols from red
6 wine grape skins into wine via winemaking processing.

7 Similar to the TPC values, the RSA values of RWGP were higher than those of WWGP
8 ($p < 0.05$). RSA of Pinot Noir was of the lowest among the RWGP, while no difference between
9 two WWGP varieties. The TPC and RSA values were linearly correlated ($R = 0.989$) (Fig. 2),
10 which was in agreement with the results by Dunonne et al. (2009).

11 Anthocyanins (ACY) was not detected in the WWGP but in the RWGP with the highest
12 content in Merlot (1.42 mg Mal-3-glu/g DM) followed by Cabernet Sauvignon (0.89 mg Mal-3-
13 glu/g DM) and Pinot Noir (0.29 mg Mal-3-glu/g DM). As was stated previously, due to the
14 thicker skins of Merlot and Cabernet Sauvignon, ACY values of those two varieties were
15 significantly higher than that of Pinot Noir. Reported ACY values in grape pomace varied
16 depending on the extraction and analytical methods and grape varieties. Thimothe, Bonsi,
17 Padilla-Zakour & Koo (2007) reported that ACY of Pinot Noir and Cabernet Franc was 5.9 and
18 67.2 mg mal-3-glu/g of lyophilized extract, respectively. Kammerer, Claus, Carle & Schieber
19 (2004) quantified the total amount of ACY in 14 wine grape varieties ranged from 11.47 to 29.82
20 mg/g DM by using HPLC.

21 The total flavanol content (TFC) and proanthocyanidins (PAC) differed significantly among
22 WGP varieties (Table 5). The TFC values in this study represented the amount of monomeric
23 flavanols (free or terminal of PAC) in the aqueous solution whilst the PAC values indicated the

1 amount of oligomeric and polymeric flavan-3-ols which were linked by C – C bonds (Muller-
2 Harvey & McAllan, 1992), were determined by the acid butanol assay. Merlot, Muller Thurgau
3 and Cabernet Sauvignon were of the highest content of TFC, followed by Pinot Noir and Morio
4 Muscat ($p < 0.05$) (Table 5). Likewise, Merlot possessed the highest PAC, followed by Muller
5 Thurgau, Cabernet Sauvignon, Pinot Noir and Morio Muscat ($p < 0.05$). Although TPC for
6 WWGP were lower than those for RWGP, TFC and PAC values did not show significant
7 difference between WWGP and RWGP varieties ($p > 0.05$) (Table 5). Furthermore, neither linear
8 correlation between RSA and TFC ($R = 0.653$) nor between RSA and PAC ($R = 0.667$) was
9 observed (Fig. 2), which was consistent with the previous study by Bozan, Tosun & Özcan
10 (2008).

11 Overall, WGP had high TPC values comparing with other fruit byproducts. Among the five
12 WGP varieties evaluated in this study, Merlot ranked the first in all phenolic compounds. Muller
13 Thurgau also displayed relatively high values of TFC and PAC.

14

15 **4. Conclusion**

16 This study was the first one that successfully characterized dietary fiber and quantified
17 polyphenols of WGP skins from the Pacific Northwest. Results provided the baseline data for
18 developing innovative utilizations of the WGP skins. Overall, the RWGP skins had high contents
19 of TPC, RSA, ACY, TFC, PAC, and dietary fiber, making them excellent candidates for
20 nutraceutical, medical, and food applications. They are also good sources of Klason lignin,
21 cellulose, and hemicellulose, hence are of great potentials of being environmental-friendly
22 supporting materials. The distinctively high amount of soluble sugars in the WWGP skins may
23 enable them to form innovative biodegradable packaging materials with excellent flexibility.

1 Nevertheless, more studies on WGP should be carried out, for instance, to further investigate
2 the differences in chemical compositions due to the different wine making processing, to study
3 more varieties of WGP in order to profile the chemical composition of WGP from US Pacific
4 Northwest region. On the other hand, more efforts should be made to improve WGP preparation
5 procedures for obtaining bright color, appreciative aroma, more soluble fractions and nutrient
6 compounds.

7

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- 29

1 **Table 1**

2 Crude protein, fat and ash content of five varieties of wine grape pomace skins (WGP)

| Composition (% DM) | Muller Thurgau | Morio Muscat | Cabernet Sauvignon | Merlot | Pinot Noir |
|-----------------------|-------------------|--------------|-----------------------|--------|------------|
| Crude protein | 6.54 | 5.38 | 12.34 | 11.26 | 12.13 |
| Fat | 2.64 | 1.14 | 6.33 | 3.35 | 4.74 |
| Ash | 2.53 | 3.31 | 7.59 | 7.19 | 6.17 |

3

1 **Table 2**

2 Major carbohydrate fractions in five varieties of wine grape pomace skins (WGP)

| Composition (% DM) | Muller Thurgau | Morio Muscat | Cabernet Sauvignon | Merlot | Pinot Noir |
|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Soluble sugar | 55.77±2.12 ^b | 77.53±1.01 ^a | 1.71±0.49 ^c | 1.34±0.92 ^c | 1.38±0.93 ^c |
| Total dietary fiber | 28.01±1.36 ^e | 17.28±0.21 ^d | 53.21±0.38 ^b | 51.09±0.58 ^c | 56.31±1.47 ^a |

3 Data were expressed as mean ± SD (n=3). The different superscripts in the same row were
4 significant different (p <0.05).

1 **Table 3**

2 Dietary fiber content in five varieties of wine grape pomace skins (WGP)

| Dietary fiber | Muller Thurgau | Morio Muscat | Cabernet Sauvignon | Merlot | Pinot Noir |
|---|---------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Glucose | 4.59±0.57 ^c | 3.16±0.07 ^d | 7.33±0.51 ^b | 9.16±0.46 ^a | 8.43±0.39 ^a |
| Xylose | 0.91±0.09 ^c | 0.45±0.04 ^d | 1.94±0.11 ^b | 2.13±0.11 ^a | 2.09±0.08 ^a |
| Galactose | 0.67±0.05 ^c | 0.54±0.03 ^d | 0.97±0.02 ^b | 1.02±0.03 ^b | 1.17±0.08 ^a |
| Arabinose | 0.43±0.04 ^c | 0.34±0.02 ^d | 0.76±0.05 ^a | 0.75±0.05 ^a | 0.58±0.06 ^b |
| Mannose | 0.49±0.05 ^c | 0.36±0.01 ^d | 1.48±0.04 ^a | 1.54±0.05 ^a | 1.12±0.07 ^b |
| IDF % DM | NS _{IDF} | 7.08±0.80 ^d | 4.85±0.04 ^e | 12.25±0.39 ^c | 14.59±0.39 ^a |
| | UA _{IDF} | 4.81±0.66 ^{cd} | 3.64±0.21 ^d | 8.53±0.82 ^a | 6.62±0.25 ^b |
| | NS _{IDF} + UA _{IDF} | 11.89±1.20 ^c | 8.49±0.17 ^d | 20.78±0.79 ^a | 21.20±0.61 ^a |
| | KL | 15.40±0.27 ^d | 7.94±0.06 ^e | 31.62±0.48 ^b | 28.38±0.67 ^c |
| | IDF | 29.59±1.46 ^d | 17.93±0.24 ^c | 56.92±0.37 ^b | 53.24±0.33 ^c |
| Glucose | 0.20±0.07 ^c | 0.15±0.04 ^c | 0.33±0.03 ^b | 0.38±0.1 ^{ab} | 0.43±0.39 ^a |
| Xylose | 0.02±0.00 ^c | 0.02±0.01 ^c | 0.03±0.01 ^{ab} | 0.04±0.01 ^a | 0.02±0.00 ^c |
| Galactose | 0.12±0.04 ^{ab} | 0.13±0.04 ^a | 0.07±0.03 ^b | 0.15±0.06 ^a | 0.18±0.02 ^a |
| Arabinose | 0.07±0.02 ^c | 0.11±0.01 ^b | 0.07±0.02 ^b | 0.13±0.02 ^{ab} | 0.16±0.01 ^a |
| SDF % DM | Mannose | 0.05±0.01 ^{bc} | 0.01±0.00 ^c | 0.05±0.06 ^{bc} | 0.09±0.03 ^b |
| | NS _{SDF} | 0.46±0.13 ^c | 0.42±0.10 ^c | 0.54±0.08 ^c | 0.78±0.22 ^b |
| | UA _{SDF} | 0.43±0.06 ^b | 0.26±0.03 ^c | 0.27±0.04 ^c | 0.73±0.07 ^a |
| | SDF | 0.72±0.14 ^c | 0.84±0.07 ^c | 0.81±0.06 ^c | 1.51±0.14 ^b |
| (NS _{IDF} + NS _{SDF}) % DM | 7.54±0.74 ^d | 5.27±0.11 ^e | 12.79±0.34 ^c | 15.37±0.48 ^a | 14.39±0.29 ^b |
| (UA _{IDF} + UA _{SDF}) % DM | 5.07±0.64 ^{cd} | 4.07±0.25 ^d | 8.80±0.79 ^a | 7.34±0.2 ^b | 5.72±0.72 ^c |

3 KL, Klason lignin; NS, neutral sugar; UA, uronic acid, expressed as mg galacturonic acid
 4 equivalents (GUAE)/g DM; IDF, insoluble dietary fiber; SDF, soluble dietary fiber. IDF = NS_{IDF}
 5 + UA_{IDF}+KL; SDF = NS_{SDF} + UA_{SDF}. Data were expressed as mean ± SD (n=3). The different
 6 superscripts in the same row were significant different (*p* < 0.05).

1 **Table 4**
 2 Bound condensed tannis (CT) and resistant protein (RP) of insoluble dietary fiber (IDF) residue (%
 3 DM) in five varieties of wine grape pomace skins (WGP)

| | Muller Thurgau | Morio Muscat | Cabernet Sauvignon | Merlot | Pinot Noir |
|----------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| Bound CT % DM | 8.53±0.33 ^c | 6.04±0.09 ^d | 16.14±0.24 ^b | 16.26±0.58 ^b | 19.89±0.37 ^a |
| RP % DM | 2.30±0.01 ^b | 1.50±0.04 ^b | 4.52±0.10 ^a | 3.65±0.03 ^a | 4.92±0.57 ^a |
| RP% of crude protein | 35.17 | 27.88 | 36.63 | 32.42 | 40.56 |

12 CT, condensed tannins; RP, resistant protein. Values were presented as mean ± SD (n=3). The
 13 different superscripts in the same row were significant different ($p < 0.05$).

Table 5

Soluble phenolic compounds of five different varieties of wine grape pomace skins (WGP) extracted by method A and B

| WGP | TPC mg GAE/g DM | | | RSA mg AAE /g DM | | | ACY mg Mal – 3 – glu /g DM | | | TFC mg CE/g DM | | | PAC mg/g DM | | |
|--------------------|------------------------|-----------------------|----------|-----------------------|-----------------------|----------|-------------------------------|------------------------|----------|-----------------------|------------------------|----------|-----------------------|-----------------------|----------|
| | <u>A</u> | <u>B</u> | <i>p</i> | <u>A</u> | <u>B</u> | <i>p</i> | <u>A</u> | <u>B</u> | <i>p</i> | <u>A</u> | <u>B</u> | <i>p</i> | <u>A</u> | <u>B</u> | <i>p</i> |
| Muller Thurgau | 15.8±0.3 ^c | 11.4±0.9 ^b | 0.001 | 25.6±1.9 ^c | 11.4±1.2 ^b | <0.001 | - | - | - | 58.9±1.6 ^a | 40.3±5.0 ^a | 0.004 | 19.4±2.3 ^b | 10.5±2.6 ^b | 0.010 |
| Morio Muscat | 11.6±1.0 ^c | 11.4±1.7 ^b | 0.870 | 20.5±3.3 ^c | 10.9±1.6 ^b | 0.01 | - | - | - | 31.0±1.7 ^c | 25.0±3.3 ^c | 0.050 | 8.0±1.3 ^c | 7.7±1.5 ^b | 0.750 |
| Cabernet Sauvignon | 26.7±1.8 ^a | 12.7±0.5 ^b | <0.001 | 39.7±2.6 ^a | 17.7±2.3 ^a | <0.001 | 0.89±0.02 ^b | 0.85±0.03 ^b | 0.140 | 54.3±4.6 ^a | 42.0±3.7 ^a | 0.022 | 17.2±2.3 ^b | 7.7±1.8 ^b | 0.005 |
| Merlot | 25.0±3.6 ^a | 18.3±3.0 ^a | 0.060 | 40.2±1.9 ^a | 19.7±1.7 ^a | <0.001 | 1.42±0.14 ^a | 1.09±0.03 ^a | 0.010 | 61.2±5.0 ^a | 37.0±2.6 ^{ab} | 0.001 | 24.1±3.8 ^a | 15.8±1.2 ^a | 0.020 |
| Pinot Noir | 21.4±4.3 ^{ab} | 11.2±0.2 ^b | 0.010 | 32.2±4.5 ^b | 21.1±2.3 ^a | 0.019 | 0.29±0.01 ^c | 0.26±0.03 ^c | 0.180 | 42.6±5.4 ^b | 32.5±3.1 ^{bc} | 0.050 | 11.9±1.4 ^c | 8.0±1.0 ^b | 0.016 |

TPC, total phenolics content; RSA, DPPH radical scavenging activity; ACY, monomeric anthocyanins; TFC, total flavanol content; PAC, extractable proanthocyanidins content. Values were presented as mean ± SD (n=3). Different superscripts shown within the method A column indicated the differences among WGP varieties based on LSD test ($p<0.05$); the comparison between method A and B employed pair t – test ($p<0.05$) to compare the differences in the values of the same parameter obtained by the two methods.

Table 6

The documentary values of total phenolic compounds from WGP and/or WGP skins, fresh wine grape berry, and other fruit byproducts

| Byproducts | Total phenolic content | Bibliography |
|---|--|---|
| WGP and/or WGP skins (varieties) | | |
| RWGP, skins (Cabernet Sauvignon, Merlot and Pinot Noir) | 21.4 to 26.7 mg GAE/g DM | This study |
| WWGP, skins (Muller Thurgau and Morio Muscat) | 11.6 to 15.8 mg GAE/d DM | This study |
| Wine grape marc (Cabernet Sauvignon and Merlot) | 20.2 mg GAE/g DM | Vatai, Škerget & Kenz, 2009 |
| RWGP (Manto Negro) | 26.3 mg GAE/g DM | Llobera & Cañellas, 2007 |
| WWGP, skins (Cencibel) | 37.6 mg GAE/g DM | Bravo & Saura – Calixto, 1998 |
| RWGP, skins (Airén) | 44.8 mg GAE/g DM | Bravo & Saura – Calixto, 1998 |
| WWGP and WWGP skins (Roditis) | 48.3 and 9.7 mg GAE/g DM | Makris, Boskou & Andrikopoulos, 2007 |
| RWGP and RWGP skins (Agiorgitiko) | 54.0 and 36.25 mg GAE/g DM | Makris, Boskou & Andrikopoulos, 2007 |
| WWGP (Prensal Blanc) | 34.9 mg GAE/g DM | Llobera & Cañellas, 2008 |
| Fresh wine grape berry | | |
| Red wine grape, skins (Pinot Noir) | 38.4 mg/g FW * | Mané, et al., 2007 |
| White wine grape, skins (Muscat of Alexandria) | 1.2 mg GAE/g FW | Poudel, Tamura, Kataoka & Mochioka, 2008 |
| Other fruit byproduct | | |
| Cider apple pomace | 6.46 × 10 ⁻³ mg GAE/g DM 5.5 × 10 ⁻³ to 10.9 × 10 ⁻³ mg GAE/g DM | Suárez, et al., 2010 García, Valles & Lobo, 2009 |
| Orange peel | 2.76 mg GAE/g FW | Khan, et al., 2010 |
| Acerola residues (juice production) | 6.81 mg GAE/g DM | Oliveira, et al., 2009 |
| Pineapple residues (juice production) | 2.75 mg GAE/g DM | Oliveira, et al., 2009 |
| Passion fruit residues (juice production) | 1.03 mg GAE/g DM | Oliveira, et al., 2009 |
| Strawberry residues (juice production) | 59.77 mg GAE/g extract corresponding to the yield of extract 17.1% DM | Peschel, et al., 2006 |
| Pear residues (juice production) | 12.90 mg GAE/g extract corresponding to the yield of extract 11.4% DM | Peschel, et al., 2006 |
| Red beet residues (juice production) | 91.74 mg GAE/g extract corresponding to the yield of extract 20.1% DM | Peschel, et al., 2006 |
| Cranberry juice pomace | About 11.0 GAE/g DM | Vattem, Lin & Shetty, 2005 |

Blueberry pomace

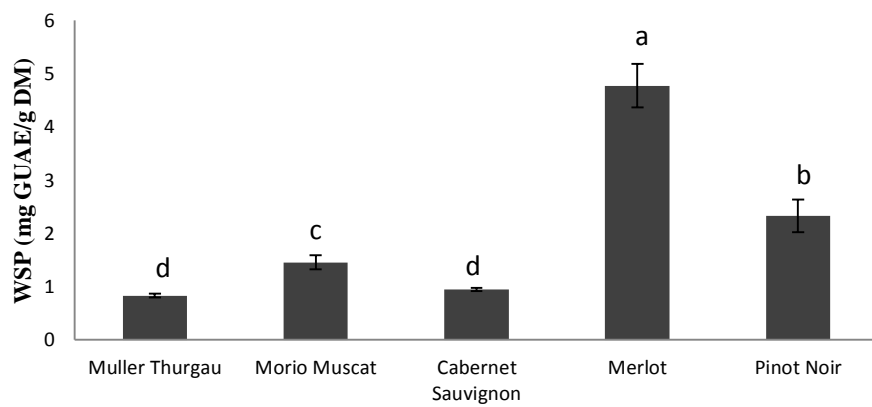
Juice pomace 11.9 mg GAE/g DM Su & Silva, 2006

Wine pomace 10.9 mg GAE/g DM Su & Silva, 2006

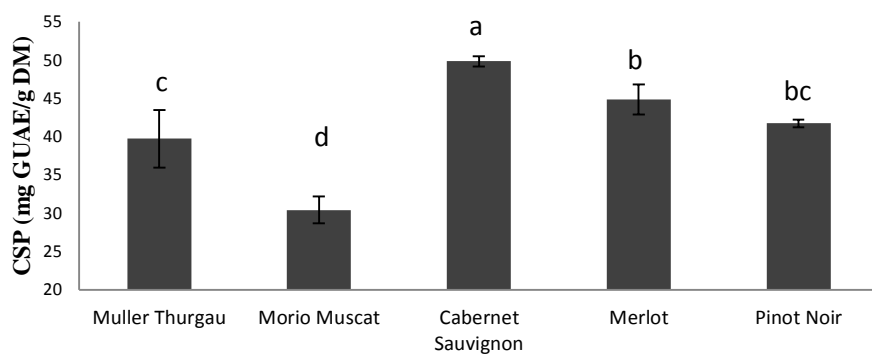
Vinegar pomace 2.3 mg GAE/g DM Su & Silva, 2006

* TPC was quantified by HPLC instead of Folin-Ciocalteu assay.

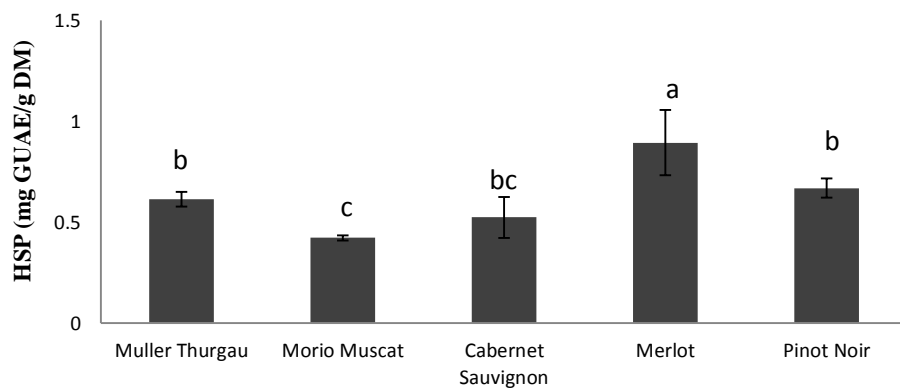
WGP, wine grape pomace; WWGP, white wine grape pomace; RWGP, red wine grape pomace; GAE, gallic acid equivalents.



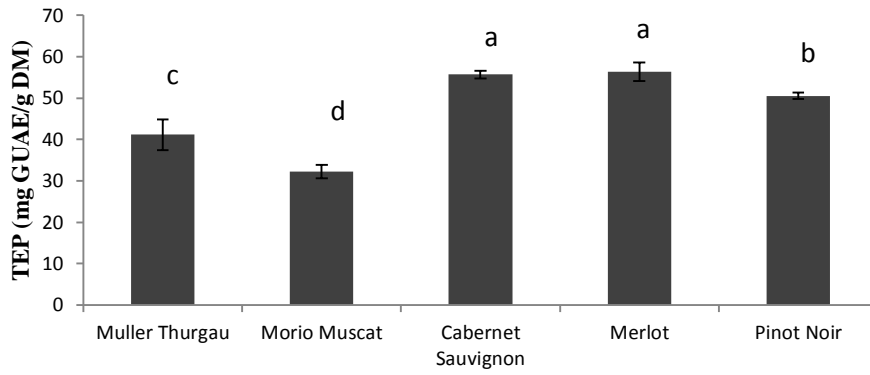
(a) Water soluble pectin (WSP)



(b) Chelator soluble pectin (CSP)



(c) Hydroxide soluble pectin (HSP)



(d) Total extractable pectins (TEP)

Fig.1 Fractionation of extractable pectins (water soluble, chelator soluble, hydroxide soluble pectin and total extractable pectins) from wine grape pomace (WGP) expressed as mg GUAE/g DM. Different letters on the top of each column showed the differences among the varieties ($p < 0.05$, $n=3$).

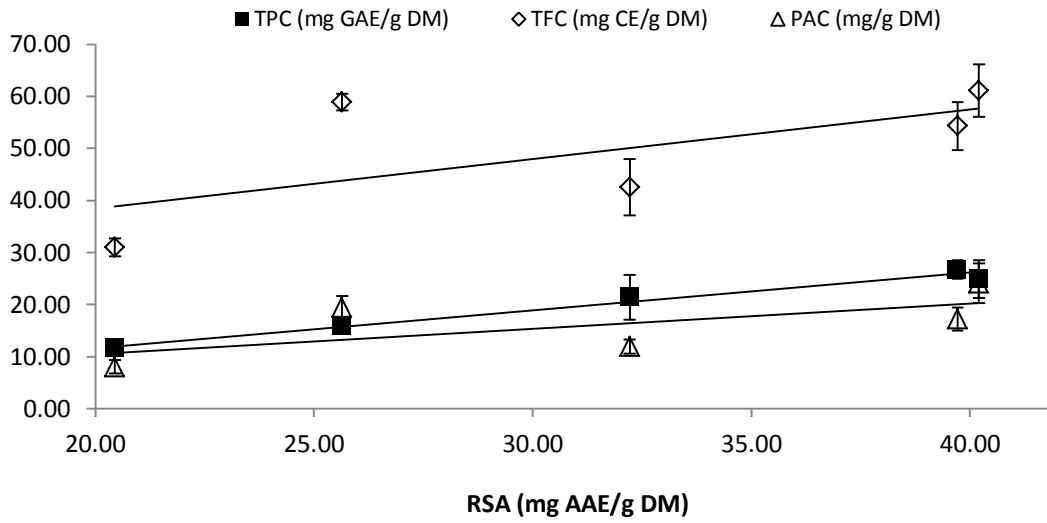


Fig. 2 Correlation between RSA and TPC ($R=0.989$), RSA and TFC ($R=0.653$), and RSA and PAC ($R=0.667$) for five WGP varieties ($n=3$).