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Monitoring selected monomeric polyphenol composition in pre- and post-fermentation products of *Vitis vinifera* L. cv. Airén and cv. Grenache noir

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ABSTRACT

A mass balance approach was used quantify select polyphenols in pre- and post-fermentation products resulting from the fermentation of *Vitis vinifera* cv. Grenache noir and Airén. For Grenache noir, the overall mass recovery was 102.7%. The main products were wine (78.3%), pomace (8.5%), lees (4.2%), and rachises (3.4%). Pomace was a rich source of all identified polyphenols. Lees sorbed significant amounts of gallic acid, catechin, epicatechin, malvidin-3-O-glucoside, malvidin-3-acetylglucoside, quercetin-3-O-glucoside, and quercetin. An approximately 200% increase in the total amount of gallic acid occurred during fermentation. For the Airén grapes, the overall mass recovery was >90%. The pomace, rachises, juice solids after settling, and lees constituted ~50% of the total mass of products obtained; pomace alone accounted for 40% of the total product mass. Over 90% of the total amount of gallic acid, catechin and epicatechin and ~50% of the quercetin-3-O-glucoside were found in the pomace.

1. Introduction

Grapes comprise one of the most valuable fruit crops worldwide (Boulton, Singleton, Bisson, & Kunkee, 1996). An underutilized processing co-product from wine and grape juice production is pomace, the macerated and pressed seeds, skins and rachises (bunch stems) remaining after free run wine or juice has been pressed off. Approximately 13–20% (w/w) or more of the total grape mass used in winemaking ends up as pomace (Torres et al., 2002) with the amount being dependent largely on winemaking processes. Currently, most pomace is composted and used as a fertilizer in vineyards (Ferrer et al., 2001). Grape pomace has also been used as an ingredient in livestock feeds (Brenes et al., 2008; Famuyiwa & Ough, 1982); as a natural fiber additive for foods (Llobera & Cañellas, 2008; Saura-Calixto, 1998); as a source of natural food colorants (Bocevska & Stevcevska, 1997; Braga, Lencart e Silva, & Alves, 2002); as a source of tartrates (Braga et al., 2002); and for extracting grape-seed oil (El-Shami, El-Mallah, & Mohammed, 1992). Grape pomace can also serve as a low cost nutrient source for solid state microbial fermentations used in the production of hydrolytic enzymes (Botella, Diaz, De Ory, Webb, & Blandino, 2009), bio-fuels (Hang & Woodams, 1985; Silva & Malcata, 1999), and citrates (Hang, Lee, Woodams, 1986).

There is considerable interest in utilizing grape skins and pomace as innovative food ingredients. Grape skins and pomace can be an excellent source of numerous polyphenolic compounds, with both functional and nutritional properties. Increased utilization of these co-products will improve the economic and environmental sustainability of the wine industry (Kammerer, Claus, Carle, & Schieber, 2004; Lu & Foo, 1998). Natural polyphenolics are of tremendous interest due to their antioxidant activity, free radical scavenging activity important in the inhibition of low-density lipoprotein oxidation and atherosclerotic plaque formation, as well as their antithrombic, antihypertensive and antiarrhythmic effects; and antiviral and carcinostatic properties (Birt, Hendrich, & Wang, 2001; Formica & Regelson, 1995; Hertog et al., 1995; Pérez-Jiménez et al., 2009; Rohn, Rawel, & Kroll, 2004; Siddiqui, Raisuddin, & Shukla, 2005). In grapes, polyphenols, including, phenolic acids, flavan-3-ols (or procyanidins), flavonols, and, in the red varieties anthocyanins, are

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principally located in the seeds, skins and rachises (Bocevska & Stevcevska, 1997; Braga et al., 2002). Recent studies have shown that some polyphenol compounds with antioxidant activity are associated with the grape pomace fiber matrix (i.e., cellulose, hemicellulose, and pectin), particularly that from the seeds and skins, leading to the development of the concept of "antioxidant dietary fiber" (Saura-Calixto, 1998, 2003). In addition, the antioxidant activity of the extractable polyphenols from grape pomace and seeds was found to be much greater than that extracted from wine (Saura-Calixto, 1998). This suggests that grape pomace could be a suitable source of both dietary fiber and bioactive polyphenols.

The qualitative and quantitative polyphenol profiles of whole grapes, grape fractions, wines and grape pomaces have been characterized for many grape varieties (Ginjom, D'Arcy, Caffin, & Gidley, 2010; Jensen, Demiray, Egebo, & Meyer, 2008; Kammerer et al., 2004; Lu & Foo, 1998). This composition is affected by many factors including: cultivar, vintage, vineyard location and climate, cultural practices, grape maturity level, extraction and processing technologies, as well as analysis methods (De Beer et al., 2004; Downey, Dokoozlian, & Krstic, 2006; Jackson & Lombard, 1993; Peña-Neira, Cáceres, & Pastenes, 2007; Romeyer, Macheix, Goiffon, Reminiac, & Sapis, 1983). In particular, fermentation conditions (e.g., skin contact time, extent of crushing/maceration prior to fermentation, temperature, pH, use of enzymes, etc.) can significantly impact the amount of polyphenols extracted from the grapes into the wine thereby influencing the polyphenol composition of the resulting co-products. In addition, the different classes of polyphenols may be differentially affected by processing conditions (Sacchi, Bisson, & Adams, 2005). As a result, winemaking conditions can potentially be manipulated to obtain wines and grape co-products of desired polyphenol composition.

White wine grapes do not have anthocyanins, which are present in red-colored varieties. However white grapes, particularly the skins and seeds, are rich in other monomeric polyphenol classes, including phenolic acids, flavan-3-ols, and flavonols (Adams, 2006; Singleton & Esau, 1969). During white wine production, juice is pressed from the grape skins and fermentation proceeds without skin and seed contact (Boulton et al., 1996). This leads to low polyphenol extraction into the wine from the skins and seeds and yields a pomace that remains rich in many polyphenols. Few studies have focused on the polyphenol composition of the resulting pomace and other co-products of white winemaking processes. During red wine processing, the grape skins and seeds are left in contact with the juice throughout fermentation resulting in extraction of polyphenols into the finished wines. However, this extraction is not exhaustive and depending on the variety and specific winemaking conditions, the pomace generally retains a relatively high total polyphenol content (Jensen et al., 2008). Manipulation of winemaking variables to obtain pomaces with polyphenol characteristics tailored to high value products and specific usages may be possible; however, this requires detailed knowledge of the effects of processing on polyphenol composition at each winemaking step. One approach toward obtaining such information is via component balance studies that systematically monitor the changes in polyphenol composition for each product and co-product formed during winemaking.

In this study we utilized a component balance approach to quantify the concentrations of select monomeric polyphenols in pre- and post-fermentation products obtained during processing of *Vitis vinifera* L. cv. Grenache noir grapes (red) and in cv Airén grapes (white); two of the most widely planted wine grape varieties worldwide (Boulton et al., 1996).

2. Materials and methods

2.1. Reagents and standards

All reagents and solvents were of analytical or HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA). Standards used for identification and quantification purposes were: gallic acid, (+)-catechin, (−)-epicatechin, cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, quercetin, quercetin rhamnoside, isorhamnetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-glucoside, quercetin 4'-*O*-glucoside, kaempferol 3-*O*-glucoside, and rutin (Extrasynthese, Lyon, France).

2.2. Grapes

Approximately 1600 kg of Grenache noir grapes and ~2500 kg of Airén grapes were hand harvested on 27 September, 2007 and 11 October, 2007, respectively. Grenache grapes were ~24 Brix at harvest while the Airén grapes were ~21 Brix at harvest. All grapes were sourced from the UC Davis Tyree Vineyard (Davis, CA) and after harvest they were immediately transported to the UC Davis Experimental Winery (Davis, CA) and held at 16 °C overnight prior to winemaking. Triplicate subsamples of whole grape clusters were taken for chemical analysis and prepared as described below.

2.3. Wine making

2.3.1. White wine

Standard white winemaking procedures were followed as outlined in Fig. 1. Briefly, rachises were removed from whole clusters using a Healdsburg 30 T/hour crusher/destemmer (Healdsburg Manufacturing, Healdsburg, CA) and the resulting grapes were crushed and pressed in pneumatic press (Bucher RPL 36, Chalonnes sur Loire, France) in two minute cycles of 0.2, 0.7, 0.9, 1.1, 1.4, and 1.6 bars, respectively, with cake breaking between each cycle. The juice was transferred to a 2000 L refrigerated, stainless steel tank (Paul Mueller Company, Springfield, MO), treated with 50 mg/L SO_2, cold settled at 10–15 °C for 24 h, and then racked to a new 2000 L refrigerated stainless steel tank. The juice was fermented to dryness (residual sugar ≤ 0.5% determined by Clinitest®) with Premier Cuvee yeast (Universal Food, Milwaukee, WI) and the temperature was maintained at 15–20 °C during fermentation. The alcoholic fermentation was completed in eight days. The wine was then pressed, cold settled for 30 days, transferred to 19 L glass carboys, purged with N_2 gas, and stored at 12 °C until analysis. SO_2 (50 mg/L) was added after pressing and prior to racking to the glass carboys. Weight loss during fermentation was monitored via pressure transducers (Rosemount, Model No. 300S1AFMS, Chanhassen, MN) mounted directly on the tank.

2.3.2. Red wine

The red winemaking process is outlined in Fig. 2. Rachises were separated from as described above and the crushed grapes were transferred to a 2000 L temperature controlled, stainless steel tank (Paul Mueller Company, Springfield, MO). The must was treated with 50 mg/L SO_2, inoculated with 3.8 kg Premier Cuvee yeast (Red Star Yeast and Products, Milwaukee, WI) and then fermented to dryness with two pump overs twice daily to ensure complete mixing and extraction of the cap during fermentation; fermentation temperature was maintained at 20–25 °C. After completion of alcoholic fermentation (≤0.5% residual sugar determined by Clinitest®), the wine was pressed in a pneumatic press (Bucher RPL 36, Chalonnes sur Loire, France) with six separate two minute press

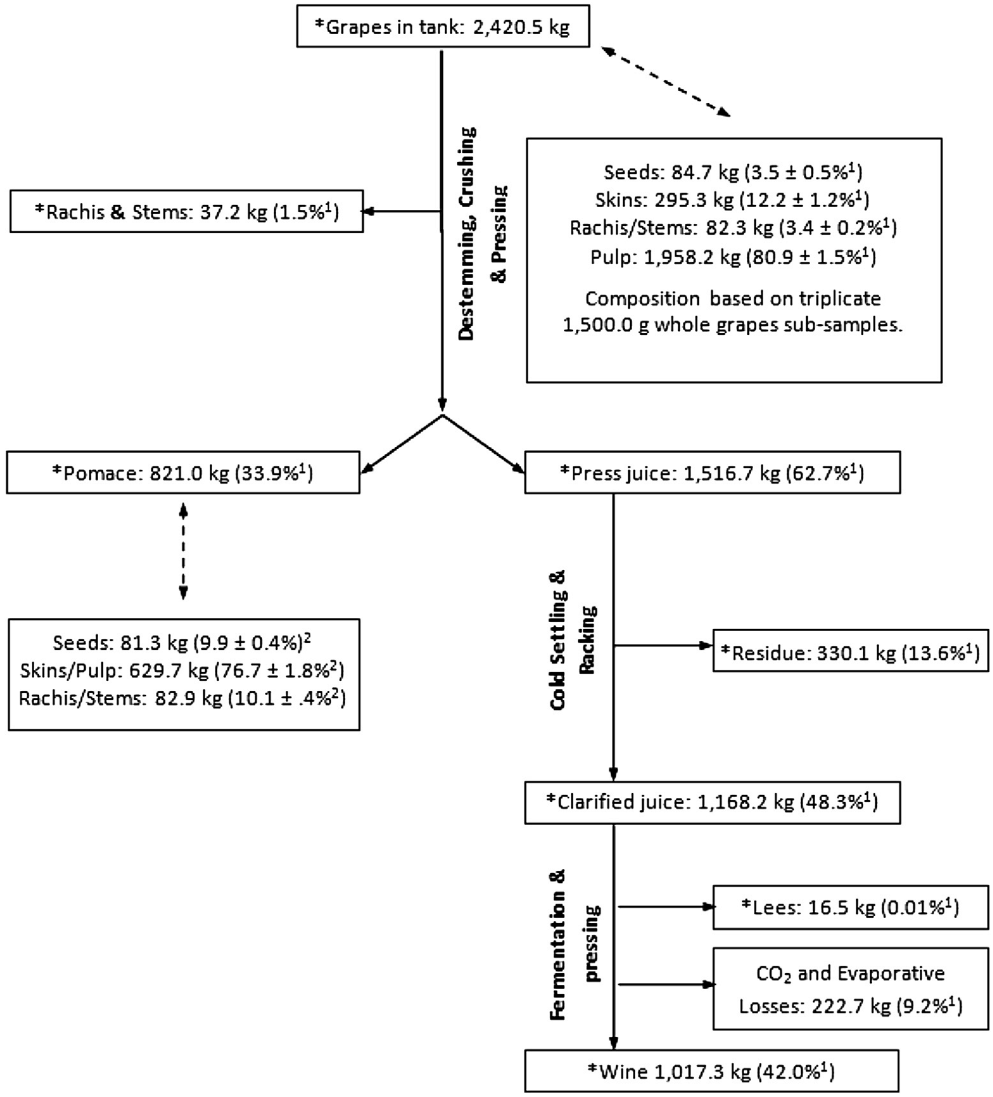

Fig. 1. Flow chart for the processing and sampling of *Airén* grapes. Seed, skin, and rachis composition of pomace is based on triplicate 1000.0 g sub-samples. Pulp was not removed from pomace skins prior to weighing and analysis. Samples collected for phenolic analysis are indicated with an *. [1]Percentage recovery based on weight of whole grapes; [2]percentage recovery based on weight of pomace.

cycles of 0.2, 0.7, 0.9, 1.1, 1.4 and 1.6- bars, respectively, with cake-breaking between cycles. The pomace was removed and the press wine was inoculated with *Oenococcus oenus* (1.5 g/250 L; Viniflora Oenos, Chr. Hansen, Horsholm, Denmark) to induce malolactic acid fermentation at 20 °C. After malolactic fermentation, the wine was cold settled (15 °C, 30 days) and racked to yield the finished wine and lees. Finished wine was transferred to 19 L glass carboys, purged with nitrogen gas, and stored at 15 °C until analysis. SO_2 (50 mg/L) was added after the malolactic fermentation (i.e., prior to cold settling) and immediately prior to racking. Weight loss during fermentation was monitored via pressure transducers (Rosemount, Model No. 300S1AFMS, Chanhassen, MN) mounted directly on the tank.

Triplicate samples were taken at all stages of processing for chemical analyses and weights were recorded for component balance calculations. Whole grapes and pomace were immediately separated into rachis, skin, seed, and pulp fractions prior to freezing and storage at −24 °C. Except for the wine samples which were stored at 15 °C as described above, all other samples were immediately frozen and stored at −24 °C and analyzed for phenol composition within six months.

Must and wine compositional analyses (Brix, pH, titratable acidity, residual sugar, ethanol) were performed using standard analytical methods (Ough & Amerine, 1988).

2.4. Grape sample preparation and extraction

For phenolic analysis of whole grapes, fresh grape bunches were sampled prior to the de-stemming and washed under running water, dried between cotton towels and three weighed replicates (2000 g) were separated into rachises and berries. The berries were further manually separated into skins, seeds and pulp, working rapidly and maintaining samples on ice to minimize oxidative and enzymatic reactions. Each of the grape fractions (whole grapes, rachises, skins, seeds and pulp) was weighed and their percentages (relative to the whole grapes) determined. Weighed triplicate portions (1000 g) of pomace were also manually separated into rachises, skins and seeds, (working on ice) and the resulting fractions were weighed. In the Airén grapes, the pulp of the grape could not be easily removed from the skins without causing further tissue damage. Therefore, this material was left attached to the skin fraction. Finally the separated rachises, skins, and seeds from both

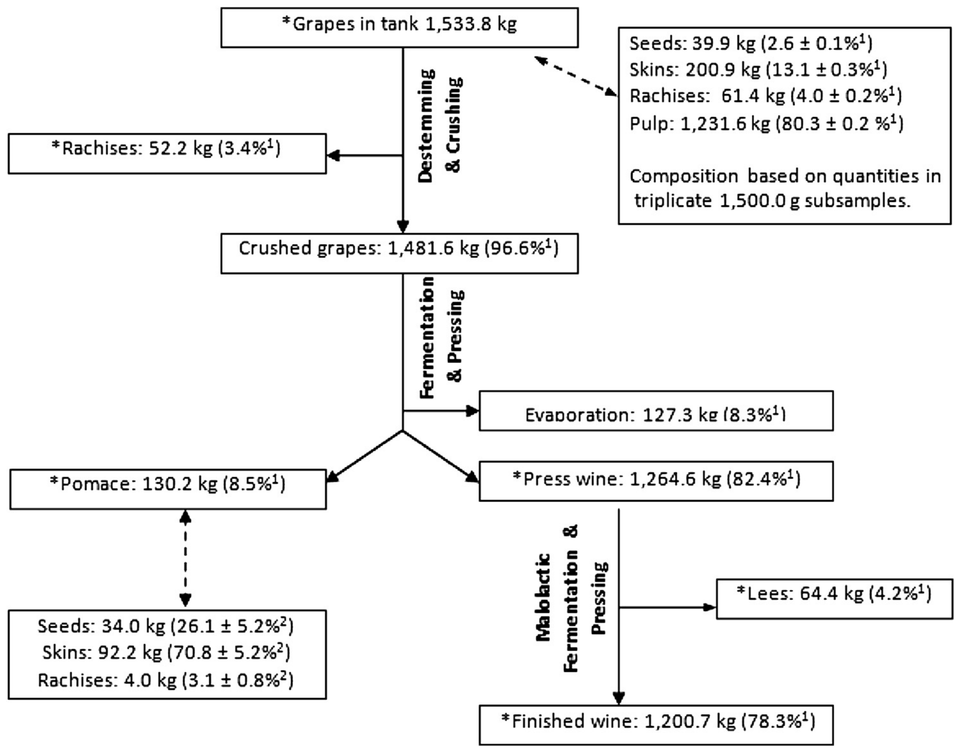

Fig. 2. Flow chart for the processing and sampling of Grenache noir grapes. Seed, skins, and rachis composition of pomace is based on triplicate 1000.0 g pomace subsamples. Samples collected for polyphenol analysis are indicated with an *.[1]Percentage recovery based on weight of whole grapes; [2]percentage recovery based on weight of pomace.

whole grapes and pomace were briefly placed under running water to remove sugars on the surface and were carefully dried between paper towels. All fractions were frozen and stored at −24 °C.

Prior to extraction and HPLC analysis, the triplicate portions of frozen rachises, skins, and seeds samples were lyophilized and the dry weight recorded. The lyophilized material was then ground in an IKA® M20 universal blade mill (K-IKA Werke, GMBH & Co., Staufen, Germany) and the powders screened through a 0.207 mm pore size sieve to obtain a powder of homogeneous particle size. The powders were either extracted immediately for HPLC analysis or stored at −24 °C prior to extraction and analysis.

The modified method of Kelm, Johnson, Robbins, Hammerstone, and Schmitz (2006) was used to remove the lipids, gums, chlorophylls, carotenoids, tocopherols, waxes, etc. that can interfere with the HPLC analysis of the monomeric polyphenols. To an accurately weighed mass (10 g) of the lyophilized, powdered sample, 45 mL of HPLC grade hexane was added and the mixture was vortexed and then sonicated for 30 min at a temperature < 45 °C. The sonicated mixture was centrifuged (4000 *g*, 30 min, 4 °C), the lipid carrying supernatant was decanted and discarded, the pellet was resuspended in a fresh hexane solvent, and the defatting process was repeated three times. After the final extraction, the pellet was dried under a very low nitrogen flow.

For polyphenol extraction from rachises and seeds, an accurately weighed defatted and dried sample (6.0 g) was vortexed and sonicated (30 min, temperature < 45 °C) with 45 mL extracting solvent consisting of acetone:water:acetic acid, 70:29.5:0.5 (v/v/v). The mixture was centrifuged (4000 *g*, 30 min, 4 °C) and the supernatant decanted and reserved. Then the pellet was resuspended in another fresh volume of the extracting solvent and the extraction repeated three times. The extracts were then combined and the volatile organic phase was evaporated in a rotary evaporator (temperature < 45 °C; Buchi EL 131, Buchi Labortechnik AG, Flawil, Switzerland), frozen (−24 °C) and lyophilized. An accurate mass of the lyophilized polyphenol extract (100 mg for rachis extracts, 50 mg for seed extracts) was dissolved in 1 mL acidified methanol (0.5% HCl), the solution filtered (0.45 μL nylon syringe filter, Fisher Scientific) and analyzed by HPLC.

For skins, the acetone:methanol:acetic acid (70:29.5:0.5 v/v/v; methanol was used instead of water to minimize extraction of sugars) extracts were combined and the volatile organic phase was evaporated to a volume of 5.0 mL in a rotary evaporator (temperature < 45 °C) filtered (0.45 μL nylon syringe filter), and analyzed by HPLC immediately after extraction.

2.5. Lees sample preparation and extraction

Triplicate lees samples were weighed, lyophilized, and powdered as described above. Sample weight after lyophilization was recorded. A weighed freeze-dried and powdered sample (10 g) was defatted with hexane as described above and 1.0 g of the defatted sample was extracted three times with fresh 45 mL volumes of the extracting solvent, acetone:methanol:acetic acid (70/29.5/0.5 v/v/v). The combined extracts were concentrated to 5.0 mL, filtered (0.45 μL nylon syringe filter), and immediately analyzed by HPLC.

2.6. Juice sample preparation and extraction

An accurately weighed mass (100 g) of thawed and centrifuged Airén juice (4000 *g*, 30 min, 4 °C) was de-sugared as follows. A volume of 10 mL was passed through a pre-conditioned (60 mL methanol, then 60 mL pure water) C_{18} prepsep cartridge (SPE C_{18} 10 g, Fisher Scientific, Fairlawn, NJ) and an equal volume of pure water passed through to elute sugars and other interfering soluble materials. The phenolic compounds that were sorbed on the solid phase were eluted with 20 mL of acidified (0.5% HCL) methanol. The procedure was repeated until all the juice was passed through the cartridge; the methanol extracts were combined, the organic solvent removed in a rotary evaporator (temperature < 45 °C). Finally the extract was lyophilized and redissolved in 1 mL acidified (0.5% HCl) methanol, filtered and analyzed by HPLC-DAD. Triplicate juice samples were extracted. To determine the recoveries of some of the phenolics analyzed, 100 mg L^{-1} solutions of gallic acid, (+)-catechin and (−)-epicatechin were treated similarly and 85–95% recoveries were obtained.

2.7. Wine sample preparation and extraction

2.7.1. White wine

Wine was concentrated as follows: a 100 mL volume of wine was de-alcoholized using a rotary evaporator (temperature < 45 °C). A 10 mL aliquot of the de-alcoholized wine was next passed through a pre-conditioned (60 mL methanol, then 60 mL pure water) C_{18} prepsep cartridge (SPE C_{18} 10 g, Fisher Scientific) to sorb the wine phenolics; these were eluted with 20 mL of acidified (0.5% HCl) methanol. The cartridge was reconditioned and the extraction process repeated until all the de-alcoholized wine was extracted. Finally, all the methanol extracts were combined and lyophilized. For HPLC-DAD analysis the lyophilized sample was dissolved in 1 mL acidified methanol and filtered (0.45 μm) prior to analysis. Triplicate wine samples were prepared for analysis.

2.7.2. Red wine

A 50 mL volume of wine sample was dealcoholized by rotary evaporation (<45 °C) and diluted with pure water to 60 mL. Then 5 mL of the dealcoholized wine was passed through a pre-conditioned (60 mL methanol, then 60 mL pure water) C_{18} prepsep cartridge (SPE C_{18} 10 g/60 mL, Fisher Scientific) to sorb the wine phenolics; the remaining water matrix was removed from the stationary phase before the polyphenols were eluted with 20 mL of acidified (0.5% HCl) HPLC grade methanol. The cartridge was reconditioned and the extraction process was repeated until all of the de-alcoholized wine polyphenols were extracted. Finally, all the extracts were combined, concentrated to a volume of 5 mL and immediately analyzed by HPLC. Triplicate wine samples were extracted.

2.8. Whole grape pulp and residue from juice cold settling

Pulp and juice residue of white wine were lyophilized and a lyophilized sample (10 g) was added to 50 mL of acetone:water:-acetic acid (70:29.5:0.5 v:v:v) extracting solvent and mixed at 17,000 rpm in a Waring blender (Model PB-5A; Waring Products Corporation/Conair, Stamford, CT) for 1 min. The mixture was then sonicated (30 min, temperature < 45 °C), centrifuged (30 min, 4 °C, 4000 *g*) and the supernatant was saved. The pellet was re-suspended in the extracting solvent, sonicated and the extraction process was repeated three times. All of the extracts were combined, the organic phase removed in the rotary evaporator and de-sugared as described for juice samples, lyophilized, and finally analyzed by HPLC-DAD.

2.9. HPLC-DAD analysis

All standard solutions and sample extracts were analyzed with a Waters 2690 Separations Module (Waters Corporation, Milford, MA) fitted with a DAD detector, internal degasser, quaternary gradient pump, thermo auto-sampler and column oven. The separations were performed on a RP C_{18} Zorbax Eclipse XDB-C_{18}, 4.6 mm i.d × 250 mm × 5 μm particle size column (Agilent Technologies, Santa Clara, CA) with a C_{18} ODS guard column (4.0 mm × 2.0 mm i.d.; Phenomenex Inc., Torrance, CA). The diode array detector was set to acquire in the range 200–600 nm at a rate of 1.25 scans/sec. The flow rate was 1 mL/min. The mobile phase consisted of 50 mM dihydrogen ammonium phosphate (pH 2.6) (A), 20% A and 80% C (B), and 0.02 M o-phosphoric acid, adjusted to pH 1.5 with concentrated NH_3 (C). The solvent gradient program was 100% A from 0 to 5 min; 92% A and 8% B at 8 min; 80% A and 20% B at 15 min; 14% B and 86% C at 20 min; 1.5% A, 16.5% B and 82.0% C at 25 min; 21.5% B and 78.5% C at 35 min; 50% B and 50% C at 60 min; and 100% A at 65–75 min. DAD spectra were extracted at 280 nm to measure select monomeric phenols and flavan-3-ols [herein gallic acid at 10.6 min, catechin at 22.5 min and epicatechin at 27.6 min]; 360 nm to measure flavonols [herein quercetin 3-glucoside at 39.7 min and quercetin at 51.8 min] and at 520 nm to measure anthocyanidins [herein malvidin 3-glucoside at 35.6 min].

Polyphenols were identified by comparing retention times and UV–Visible spectral data with those of pure standards and published spectra (Kammerer et al., 2004). For quantitation, external calibration curves were prepared by diluting accurate masses of polyphenol standards with acidified (0.5% HCl) HPLC grade methanol. When reference compounds were not available, the calibration of structurally related substances was used. To determine the recoveries of selected polyphenols, 100 mg/L solutions of gallic acid, (+)-catechin and (−)-epicatechin were treated as described for wine sample preparation and extraction and 85–95% recoveries were obtained.

2.10. Statistical analysis

Means, analytical standard deviations and relative standard deviations were calculated for all samples using Excel (Microsoft Corp., Redmond, WA).

3. Results and discussion

Although many studies have focused on polyphenol composition of wines throughout processing (Ginjom et al., 2010), few have focused on quantifying the concentrations and mass recoveries of polyphenolics in the various co-products. Nonetheless, co-products of wine processing are of increasing interest as sources of functional ingredients. Our objective therefore, was to quantitate changes in select monomeric polyphenols of Airén and Grenache noir grapes during winemaking to establish if a component balance approach could be used to measure the effects of wine processing on phenol composition in all post-fermentation products. Herein, only select monomeric polyphenols, for which we had authentic standards, were monitored so that absolute quantification could be used to validate this approach.

3.1. White wine

3.1.1. White wine making

Total mass recovered during Airén processing was 101% of the initial mass of grapes in the tank (calculated from sum of the mass of final wine, lees, residue from juice settling, pomace, and losses due to CO_2 production) (Fig. 1). The mass recovery indicates there were no significant, unaccounted sources of mass loss during processing.

The largest single product (by mass) of Airén fermentation was finished wine, however using standard white winemaking procedures this represented only 42% of the original mass of the starting grape material (Fig. 1). The co-products, including pomace, residues from juice settling, and lees accounted for ~50% of the starting grape mass; pomace constituted the largest mass of the co-products produced during winemaking (33.9%).

In whole grapes, seeds constituted 3.5% of the total mass (Fig. 1). The number of seeds per berry can vary according to grape variety so this relative percentage will vary correspondingly. Here we observed that the skin/pulp fraction of pomace made up ~77% of the total pomace weight compared to whole grapes where the skin contributed only ~12% of the total grape mass. During white wine processing significant amounts of pulp typically remain on the pomace skin and the skin and pulp were not further separated for quantitative analysis here, accounting for the differential mass percentages in whole grapes and pomace. Pressing conditions will significantly affect the amount of pulp remaining on the skins and the overall pomace yield. More severe pressing conditions (i.e., greater pressures, longer pressing times, and repeated pressing cycles) than the ones used in the present work will increase juice yield; conversely, the opposite effect can be achieved by applying less severe processing conditions (Threlfall, Morris, Howard, Brownmiller, & Walker, 2006). The equipment used here is typical of small-scale industrial wine production facilities and therefore can represent the co-products that would be characteristic of many general industrial practices.

During processing, the juice, with an initial Brix of 20.6 (wt/wt) was fermented to < 0.5% (g L^{-1}) residual sugar yielding 13.3% (v/v) ethanol in the final wine. Titratable acidity (TA) and pH remained constant in juice and wine: pH 3.83 (juice), 3.81 (wine); TA 3.51 g L^{-1} (juice), 3.54 g L^{-1} (wine).

3.1.2. Polyphenol composition of Airén grapes, wine and processing co-products

We quantified four of the major monomeric polyphenols in Airén grapes with authentic standards: gallic acid (a phenolic acid), (+)-catechin and (−)-epicatechin (flavan-3-ols), and quercetin-3-*O*-glucoside and quercetin aglycone. When monitoring HPLC response at 280 nm, numerous peaks were observed between the elution of gallic acid and quercetin but were not identified in this study. These results are consistent with those previously reported for Airén (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2010; Fernández de Simón, Hernández, & Estrella, 1993). Hydroxycinnamic acid esters have been reported in Airén must, although the levels were significantly lower than the measured levels of gallic acid, catechin, and epicatechin; the levels of these esters were also dependent on maturation with higher levels generally occurring prior to veraison (Fernández de Simón et al., 1993). Cinnamic acid esters, particularly caftaric acid (caffeoyl tartaric acid) are predominantly found in grape pulp (Adams, 2006) however they are highly unstable and major losses can occur during crushing and must preparation (Singleton, Salgues, Zaya, & Trousdale, 1985). Quantifiable levels of hydroxycinnamic acid esters were not obtained in this study and further work will be needed to fully characterize amounts of these polyphenols in pre- and post-fermentation products of Airén grapes.

Castillo-Muñoz et al. (2010) also reported significant levels of quercetin-3-*O*-glucuronide and low levels of rutin, quercetin-*O*-galactoside and kaempferol and isorhamnetin derivatives in Airén

Table 1
Component balances of monomeric polyphenols in pre- and post fermentation products throughout the fermentation process of *Airén grapes*. Relative standard deviation (%) of replicate analyses ($n = 3$) indicated in parentheses.

	Whole grapes				Destemmer rachises	Press juice	Pomace			Clarified juice	Lees	Wine
	Seeds	Skins	Rachis	Pulp			Seeds	Skins	Rachis			
Gallic acid												
Conc. (mg/kg)[a]	151.0 (7.5)	nd[c]	nd	nd	nd	nd	142.9 (7.0)	nd	nd	nd	nd	nd
Total (mg)[b]	12,792.3	0.0	0.0	0.0	0.0	0.0	11,614.8	0.0	0.0	0.0	0.0	0.0
% mass	100.0	0.0	0.0	0.0			100	0.0	0.0			
% mass relative to mass in	12,792.3 kg total in whole grapes in tank						11,614.8 kg total in pomace					
whole grapes	100				0.0		90.8			0.0	0.0	0.0
(+)-Catechin												
Conc. (mg/kg)[a]	1692.8 (3.7)	48.1 (8.9)	140.3 (5.8)	nd	140.3 (5.8)	nd	1666.8 (4.6)	22.6 (5.8)	138.3 (4.9)	nd	nd	nd
Total (mg)[b]	143,409.8	14,204.0	11,546.3	0.0	5216.4	0.0	135,475.8	14,231.4	11,468.0	0.0	0.0	0.0
% mass	84.8	8.4	6.8	0.0			84.0	8.8	7.1			
% mass relative to mass in	169,160.0 kg total in whole grapes in tank						161,175.2 kg total in pomace					
whole grapes	100				3.1	0.0	95.3			0.0	0.0	0.0
(−)-Epicatechin												
Conc. (mg/kg)[a]	1591.7 (6.5)	12.2 (4.8)	26.9 (10.4)	nd	26.9 (10.4)	nd	1562.7 (10.2)	5.3 (7.4)	26.2 (7.3)	nd	nd	nd
Total (mg)[b]	134,844.8	3602.7	2213.8	0.0	1000.1	0.0	127,014.7	3337.4	2172.6	0.0	0.0	0.0
% mass	95.9	2.6	1.6	0.0			95.8	2.5	1.6			
% mass relative to mass in	140,661.3 kg total in whole grapes in tank						132,524.7 kg total in pomace					
whole grapes	100				0.7	0.0	94.2			0.0	0.0	0.0
Quercetin-3-*O*-glucoside												
Conc. (mg/kg)[a]	nd	216.0 (8.1)	nd	0.2 (1.8)	nd	19.5 (9.0)	nd	50.9 (6.5)	nd	19.5 (9.0)	nd	nd
Total (mg)[b]	0.0	63,785.0	0.0	391.6	0.0	29,575.6	0.0	32,052.1	0.0	22,779.9	0.0	0.0
% mass	0.0	99.4	0.0	0.6			0.0	100.0	0.0			
% mass relative to mass in	64,176.7 kg total in whole grapes in tank						32,052.1 kg total in pomace					
whole grapes	100				0.0	46.1	49.9			35.5	0.0	0.0
Quercetin												
Conc. (mg/kg)[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	46.1 (6.9)	nd
Total (mg)[b]	0.0	nd	0.0	nd	0.0	nd	0.0	0.0	0.0	0.0	763	0.0
% mass	0.0	0.0	0.0	nd			0.0	0.0	0.0			
% mass relative to mass in	0.0 kg total in whole grapes in tank						0.0 kg total in pomace					
whole grapes	0				0.0	0.0	0.0			0.0	>100	0.0

[a] Concentration reported on a fresh weight basis.
[b] Total mass of phenolic compound in mg is calculated from fresh weight concentration multiplied by the total mass of the fraction from Fig. 1.
[c] nd = not detected, below limit of detection. No phenolic compounds were measured in the residue following settling and racking of press juice.

grape extracted in 50% aqueous methanol acidified with formic acid (1.5%). Isorhamnetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside could not be quantified in our samples. This may have resulted from a lower extraction efficiency of the 70% aqueous acetone acidified with acetic acid (0.5%) as compared to the aqueous methanol extraction solvent used by Castillo-Muñoz et al. (Pinelo, Laurie, & Waterhouse, 2006).

3.1.3. Component balance of polyphenols during Airén winemaking

Concentrations of each of the polyphenols in pre- and post-fermentation products were determined by HPLC-DAD and reported on a mg kg^{-1} fresh weight basis in Table 1. Using masses shown in Fig. 1, the total mass of each polyphenol in each of the pre- and post-fermentation products was determined and used to further calculate the component balance of the individual polyphenols throughout the fermentation. No monomeric polyphenols were found in the residue following cold settling of the press juice and so this information is not included in Table 1.

3.1.4. Gallic acid

Gallic acid was present in fresh whole Airén grapes and pomace with seeds being the main source of this polyphenol, consistent with results of Fernández de Simón et al. (1993) (Table 1). No gallic acid was present in the juice, lees, or the final wine. Overall recovery of gallic acid in pomace was >90% of the original mass in the grapes. Gallic acid is often esterified to other polyphenols, particularly polyphenols found in the seeds, and acid catalyzed hydrolysis during fermentation can lead to increased free gallic acid levels in post-fermentation products (De Freitas, Glories, Bourgeois, & Vitry, 1998; Núñez, Gómez-Cordovés, Bartolomé, Hong, & Mitchell, 2006). However, no significant amounts of galloylated compounds were observed in these samples and since the juice was not fermented in the presence of skins, extraction and hydrolysis of galloylated precursors did not occur during winemaking.

3.1.5. Catechin and epicatechin

In whole grapes at the beginning of fermentation, the flavan-3-ols, (+)-catechin and (−)-epicatechin, were found primarily in the seeds with small quantities in the rachises and skins (Table 1). These results are consistent with the findings of Fernández de Simón et al. (1993) in Airén grapes as well as in other white grape varieties (Adams, 2006). Of the initial amount of (+)-catechin and (−)-epicatechin present in the whole berries, > 94% was recovered in the pomace with only a small amount present in the rachises. Pulp did not contain any measurable flavan-3-ols. As indicated previously, the grape skins in the pomace contained significant amount of pulp, which resulted in an overall dilution of the actual flavan-3-ol concentrations (reported as mg kg^{-1}) in the pomace relative to concentrations in the skins separated from whole berries. However, the relative amounts (reported as %) of these compounds in seeds and rachises from both whole grapes and pomace fractions were quite similar, indicating virtually no compound extraction into the juice during de-stemming/crushing and pressing. The resulting levels of both of these flavan-3-ols in the press juice and subsequent wine products was therefore either below the limit of detection or absent (Table 1). Overall component recoveries were >95% for both compounds indicating minimal losses occurred during processing and analysis.

3.1.6. Quercetin-3-O-glucoside and aglycone

Almost all of the whole grape quercetin-3-*O*-glucoside was found in skins (99.4%) with a small portion (0.6%) being in the pulp. Skin is reported to be the major source of flavonols in Airén and other grape varieties (Adams, 2006; Castillo-Muñoz et al., 2010; Fernández de Simón et al., 1993) and the small amounts that were measured here in the pulp may be due to some extraction from the skins occurring during initial crushing and sample preparation prior to HPLC analysis. After pressing, 96% of whole grape quercetin-3-*O*-glucoside was accounted for; 50% being in pomace and 46% in press juice, consistent with extraction from the skins into the pulp due to the high aqueous solubility of quercetin glycosides (Mazauric & Salmon, 2006). During cold settling and fermentation, extensive losses occurred, possibly due to hydrolysis of the sugar moiety from the aglycone, resulting in no quercetin-3-*O*-glucoside in either the finished wine or in the lees (Table 1). Further study is needed to determine the fate of the quercetin-3-*O*-glycoside and its potential degradation products. No quercetin (aglycone) was detected initially in the whole grapes, pomace, or press juice, but small amounts (175.1 mg/kg) were measured in the lees (Table 1). This indicates that lees may sorb at least some aglycone formed from the hydrolysis of the quercetin-3-*O*-glucoside precursor. Sorption of polyphenols by yeast lees has been observed (Mazauric & Salmon, 2006; Rizzo, Ventrice, Varone, Sidari, & Caridi, 2006), however sorption of flavonols has not been well characterized. These results indicate that lees may provide a source of the bioactive flavonol, quercetin.

3.2. Red wine

3.2.1. Red wine making

During red wine processing, grape skins and seeds (and small amounts of rachises) are left in contact with the juice during fermentation. This results in extraction of polyphenols into the finished wine from the skins and seeds, however, the extraction is not complete and the skins and seeds remaining after pressing off the wine (i.e., pomace) can still contain significant concentrations of these bioactive compounds. Red wines also often undergo a second microbial fermentation (malolactic fermentation) following the alcoholic fermentation; this results in an additional co-product, lees, that consists of insoluble matter including microbial cells and grape particles that are removed from the wine after a second pressing (see also Fig. 2).

Overall material recovery throughout the red winemaking process was 102.7%, calculated relative to the mass of the initial whole grapes (Fig. 2). This overall recovery includes the mass of the rachises, pomace, lees, finished wine and CO_2 and evaporative losses. This high recovery indicates that there were no significant or unaccounted for losses throughout the winemaking procedures. Yeast growth during fermentation will increase the total product mass relative to the initial grape/must mass (i.e., increase of ~40 g/L or ~4% of the initial weight), however, in this study yeast weight could not be separated from the weight of pomace and lees during pressing and so is included in the overall product recovery.

Finished wine was the largest product, comprising 78.3% of the initial grape mass at the beginning of the fermentation (Fig. 2). Pomace and lees constituted the largest mass of co-products, accounting for 8.5% and 4.2% of the starting grape mass, respectively. In whole grapes, pulp was the largest fraction by weight, however, in the pomace following red wine fermentation and pressing, very little pulp remained attached to the skin so that grape skins were the largest component of the pomace (Fig. 2). No changes in mass occurred during the secondary malolactic fermentation and subsequent pressing off of the lees (i.e., overall recovery from the press wine to the finished wine and lees was 100.1%).

Grenache must, with an initial Brix of 24.1, was fermented to $\leq$ 0.5% residual sugar, resulting in 14.3% ethanol in the finished wine. Malolactic fermentation resulted in an increase in pH from 3.49 in the must to 4.00 in the finished wine; a corresponding decrease in titratable acidity from 4.07 g tartaric acid/L in the must to 3.35 g/L in the wine was observed.

Table 2
Component balances of monomeric polyphenols in pre- and post-fermentation products of Grenache noir grapes throughout the fermentation process. Relative standard deviation (%) of replicate analyses ($n = 3$) indicated in parentheses.

	Whole grapes[a]			Destemmer rachises	Press wine	Pomace			Lees	Finished wine
	Seeds	Skins	Rachises			Seeds	Skins	Rachis		
Gallic acid										
Conc. (mg/kg)[b]	261.8 (3.4)	nd[d]	45.0 (3.7)	47.4 (7.4)	12.3 (2.8)	207.4 (4.7)	16.1 (9.4)	40.2 (6.1)	21.7 (5.7)	12.1 (1.2)
Total (mg)[c]	10,440.8	nd	2763.0	2474.3	15,554.61	7051.6	1484.4	160.8	1397.5	14,528.4
% mass	79.1	nd	20.9			81.1	17.1	1.8		
% mass relative to mass in	13,203.8 kg total in whole grapes					8696.8 kg total in pomace				
whole grapes or [in press wine]	100			18.7	117.8	65.9			[9.0]	[93.4]
(+)-Catechin										
Conc. (mg/kg)[b]	2866.6 (0.9)	319.7 (2.1)	651.8 (3.3)	671.4 (2.9)	58.5 (1.3)	2052.5 (1.4)	241.7 (4.5)	278.1 (2.9)	160.6 (3.3)	48.2 (3.3)
Total (mg)[c]	114,377.3	64,227.7	40,020.5	35,047.1	73,979.1	69,785.0	22,284.7	1112.4	10,342.6	57,873.7
% mass	52.3	29.4	18.3			74.8	23.9	1.2	4.7	26.5
% mass relative to mass in	218,625.5 kg total in whole grapes					93,182.1 kg total in pomace				
whole grapes or [in press wine]	100			16.0	33.8	42.6			[14.8]	[78.2]
(−)-Epicatechin										
Conc. (mg/kg)[b]	2903.0 (7.2)	305.5 (3.9)	354.3 (9.2)	350.2 (6.3)	29.2 (8.6)	2037.7 (5.7)	207.4 (4.7)	251.0 (7.7)	130.4 (3.9)	22.8 (1.8)
Total (mg)[c]	115,829.7	61,375.0.0	21,754.0	18,280.4	36,926.3	69,281.8	19,122.3	1004.0	8397.8	27,375.9
% mass	58.2	30.9	10.9			77.5	21.4	1.1	4.2	13.8
% mass relative to mass in	198,958.7 kg total in whole grapes					89,408.1 kg total in pomace				
whole grapes or [in press wine]	100			9.2	18.6	44.9			[22.7]	[74.1]
Malvidin-3-*O*-Glucoside										
Conc. (mg/kg)[b]	nd	899.4	nd	nd	47.6 (3.6)	nd	172.1 (3.3)	184.5 (1.5)	114.9 (4.1)	43.7 (0.3)
Total (mg)[c]	0.0	180,721.7	0.0	0.0	60,195.0	0.0	15,867.6	738.0	7399.5	52,470.6
% mass	0.0	100.0	0.0			0.0	95.6	4.4	4.1	29.0
% mass relative to mass in	180,689.5 kg total in whole grapes					16,605.6 kg total in pomace				
whole grapes or [in press wine]	100			0.0	33.3	9.2			[12.3]	[87.2]
Malvidin-3-Acetylglucoside										
Conc. (mg/kg)[b]	nd	301.0 (6.0)	nd	nd	10.9 (7.9)	nd	82.4 (12.7)	80.2 (9.7)	71.5 (2.0)	5.9 (9.6)
Total (mg)[c]	0.0	60,470.9	0.0	0.0	13,784.1	0.0	7597.2	320.8	4604.6	7084.1
% mass	0.0	100.0	0.0			0.0	96.0	4.0		
% mass relative to mass in	60,470 kg total in whole grapes					7918.0 kg total in pomace				
whole grapes or [in press wine]	100			0.0	22.8	13.1			[33.4]	[51.4]
Delphinidin-3-*O*-glucoside										
Conc. (mg/kg)[b]	nd	48.0 (8.4)	nd	<LOQ	<LOQ	nd	2.4 (8.0)	nd	1.0 (8.2)	<LOQ
Total (mg)[c]	0.0	9643.2	0.0	<LOQ	<LOQ	0.0	221.3	0.0	64.4	<LOQ
% mass	0.0	100.0	–			0.0	100	0.9		
% mass relative to mass in	9648.2 kg total in whole grapes					221.3 kg total in pomace				
whole grapes or [in press wine]	100			<LOQ	<LOQ	2.3			[>100]	[<LOQ]
Petunidin-3-*O*-glucoside										
Conc. (mg/kg)[b]	nd	40.1 (12.0)	nd	nd	0.8 (1.2)	nd	3.6 (9.2)	<LOQ[e]	<LOQ	0.6 (0.6)
Total (mg)[c]	0.0	8056.1	0.0	0.0	1011.7	0.0	331.9	<LOQ	<LOQ	720.4
% mass	0.0	100.0	0.0			0.0	100%	<LOQ		
% mass relative to mass in	8056.1 kg total in whole grapes					331.9 kg total in pomace				
whole grapes or [in press wine]	100			0.0	12.6	4.1			[<LOQ]	[71.2]
Peonidin-3-*O*-glucoside										
Conc. (mg/kg)[b]	nd	157.4 (5.5)	nd	nd	1.7 (1.3)	nd	11.3 (10.3)	<LOQ	1.5 (5.1)	1.7 (1.3)
Total (mg)[c]	0.0	31,621.7	0.0	0.0	2149.8	0.0	1041.9	<LOQ	96.6	2041.2
% mass	0.0	100.0	0.0			0.0	100	<LOQ		
% mass relative to mass in	31,621.7 kg total in whole grapes					1041.9 kg total in pomace				
whole grapes or [in press wine]	100			0.0	6.8	3.3			[4.5]	[95.0]
Quercetin-3-*O*-glucoside										
Conc. (mg/kg)[b]	nd	60.6 (3.6)	nd	nd	7.1 (0.9)	nd	31.4 (7.2)	28.0 (8.2)	20.8 (6.6)	5.6 (7.5)
Total (mg)[c]	0.0	12,176.2	0.0	0.0	8978.7	0.0	2895.1	112.0	1339.5	6723.9
% mass	0.0	100.0	0.0			0.0	23.8	0.9		
% mass relative to mass in	12,172.6 kg total in whole grapes					3007.1 kg total in pomace				
whole grapes or [in press wine]	100.0				73.8	24.7			[14.9]	[74.8]
Quercetin										
Conc. (mg/kg)[b]	nd	nd	nd	nd	1.0 (0.1)	nd	nd	nd	175.1 (7.8)	1.6 (0.1)
Total (mg)[c]	0.0	0.0	0.0	0.0	1264.6	0.0	0.0	0.0	11,276.4	1921.2
% mass	0.0	0.0	0.0			0.0	0.0	0.0		
% mass relative to mass in whole grapes or [in press wine]	0.0			0.0	>100	0.0			[891.7]	[151.9]

[a] No polyphenols were quantified in the grape pulp and so no data is shown.
[b] Concentration reported on a fresh weight basis.
[c] Total mass of phenolic compound in mg is calculated from fresh weight concentration multiplied by the total mass of the fraction from Fig. 2.
[d] nd = not detected, below limit of detection.
[e] <LOQ = below limit of quantification.

3.2.2. Polyphenol composition of Grenache noir grapes, wine and processing co-products

Grenache noir is a relatively thin-skinned red grape variety and in comparison to other varieties has moderate amounts of total phenols (Guendez, Kallithraka, Makris, & Kefalas, 2005; Jensen et al., 2008; Landrault et al., 2001). Several polyphenols were identified and quantified with authentic standards in the Grenache noir samples including: gallic acid (a phenolic acid); (+)-catechin and (−)-epicatechin (flavan-3-ols); delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, malvidin 3-*O*-acetylglucoside, and malvidin 3-*O*-glucoside (anthocyanins); and quercetin and quercetin 3-*O*-glucoside (flavonols) Table 2. These results are consistent with previous reports for monomeric polyphenol composition of Grenache noir grapes and wines from a variety of locations and vintages (Guendez et al., 2005; Jensen et al., 2008; Landrault et al., 2001; Rentzsch, Schwarz, Winterhalter, & Hermosín-Gutiérrez, 2007; Sarni-Manchado, Fulcrand, Souquet, Cheynier, & Moutounet, 1996). Although Grenache noir grapes are widely used in wine making worldwide, little information is available on the individual polyphenol composition of the different components of Grenache berries (i.e., rachises, skins, seeds, and pulp). However, the polyphenol constituents in the various cell types of Grenache Noir grapes are expected to be similar to those reported for other red *V. vinifera* varieties (Adams, 2006).

Caffeic acid, a hydroxycinnamic acid, and its tartrate ester have been previously reported in Grenache noir grapes and wines (De Beer et al., 2004; Landrault et al., 2001) but was not present in measurable amounts in these samples. Low concentrations of galloylated flavan-3-ols, including epicatechin gallate, epigallocatechin gallate, and epigallocatechin have also been reported in Grenache noir grape seeds but were not observed in quantifiable levels herein (Guendez et al., 2005). The anthocyanin, cyanidin 3-*O*-glucoside has been previously identified in Grenache grape skins and wine, but concentrations were typically more than 50% lower than for the other anthocyanins present (Sarni-Manchado et al., 1996) and this anthocyanin was not present in all wines (Landrault et al., 2001). Although an authentic cyanidin 3-*O*-glucoside standard was available for confirmation, this compound could not be identified in these samples. Significant amounts of the flavonols, quercetin 3-*O*-glucurononide, myricetin 3-*O*-glucoside, and isorhamnetin 3-*O*-glucoside, have been identified in Grenache grapes and wines but were not present in measurable amounts herein (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosin-Gutiérrez, 2007; Rentzsch et al., 2007).

Polymeric polyphenols (tannins) were not measured in this study. They are typically the most abundant class of polyphenols in grape skins and seeds and in finished red wines (Adams, 2006). Due to the large number of possible tannin structures in grapes, quantitation of individual tannin components is difficult; however, methods for monitoring different classes of tannins are available (De Beer et al., 2004). Such methods could be employed in a mass balance approach as used here to better understand processing effects on tannin composition in post-fermentation products during red winemaking.

3.2.3. Component balance of polyphenols during Grenache noir winemaking

Concentrations of individual polyphenols were determined in each of the winemaking products and co-products and were reported on a mg kg^{-1} fresh weight basis (Table 2). From these concentrations and the total masses of each product or co-product produced during winemaking, the changes in polyphenol mass for each pre- or post-fermentation sample could be monitored throughout the winemaking process (Table 2). No monomeric polyphenols could be quantified in the berry pulp tissue, so this data is not included in Table 2.

3.2.4. Gallic acid

In fresh grapes, gallic acid was predominantly present in the seeds with approximately 20% of the total amount being present in the rachises. Grape seeds have been shown to be a major source of gallic acid in other grape varieties also (Yilmaz & Toledo, 2004). Rachises were efficiently removed during winemaking and did not make up a significant portion of the pomace mass (Fig. 2); as a result, rachises did not represent a significant source of gallic acid in the pomace (Table 2). However, in contrast to the fresh grape skins, pomace skins contained significant amounts of gallic acid, possibly due to hydrolysis of galloylated flavan-3-ols and procyanidin oligomers and polymers during crushing and fermentation resulting in release of free gallic acid from grape tissues (De Freitas et al., 1998; Núñez et al., 2006; Veluri et al., 2006). Sorption of gallic acid released from seed tissues onto skins may have also occurred. Release of gallic acid from galloylated polyphenols in seeds and skins during fermentation is consistent with a ~200% increase in total gallic acid mass in the post-fermentation products relative to the whole grapes (i.e., calculated from the sum of gallic acid mass in rachises, press wine and pomace relative to mass in whole grapes). No galloylated polyphenols were measured in this study however, and further work is necessary to follow changes in the galloylated precursors during winemaking.

During the secondary malolactice fermentation, very little hydrolysis of galloylated precursors was observed since the total mass of gallic recovered in the finished wine and lees nearly identical to that in the initial press wine. However, lees appeared to sorb approximately 10% of the total gallic acid from the press wine.

3.2.5. Flavan-3-ols

The flavan-3-ols were present in high concentrations in grapes and pomace and constituted the largest monomeric polyphenol component by mass (Table 2). Both (+)-catechin and (−)-epicatechin were present in whole grapes, with more than half of the total mass being present in the seeds, about 30% in the skins, and 10–19% in the rachises, consistent with literature reports in other grape varieties (Adams, 2006; Waterhouse, 2002). Total recoveries of catechin and epicatechin at the end of the primary alcoholic fermentation were 92.5% and 72.7%, respectively, compared to the amount in the whole grapes prior to fermentation; approximately 45% of the original flavan-3-ol mass was retained in the pomace (Table 2). Losses during winemaking are likely due to oxidation and polymerization reactions during fermentation. Jensen et al. (2008), observed flavan-3-ol recoveries of 50–60% in finished Grenache wine compared to the original amount in the grapes, similar to our results, however, they did not measure flavan-3-ol content of the pomace and so processing effects on overall losses and changes in levels of these compounds could not be evaluated.

During secondary malolactic fermentation and pressing, very little change in total mass of the flavan-3-ols was observed (Table 2). However, approximately 15–23% of the mass of catechin and epicatechin were sorbed onto the lees resulting in overall concentrations in the lees that were approximately three times that of the finished wine (Table 2). Bindon, Smith, Holt, H, & Kennedy (2010) recently observed that flesh cell wall material suspended in solution can bind large molecular weight proanthocyanidins, however, they did not report binding of monomeric flavan-3-ols. Other studies have shown that yeast lees can sorb small amounts of monomeric polyphenols with some differences in sorption observed for different yeast strains (Mazauric & Salmon, 2006; Rizzo et al., 2006).

3.2.6. Anthocyanins

In whole grapes, anthocyanins were only found in skins, with malvidin 3-*O*-glucoside being the most abundant anthocyanin, consistent with the literature reports in Grenache noir and other varieties (Adams, 2006; Landrault et al., 2001; Rentzsch et al., 2007). The total amounts (by mass) of all measured anthocyanins decreased by 57–100% during alcoholic fermentation (determined from sum of mass in press wine and pomace relative to mass in the whole grapes). Anthocyanins are highly labile and readily undergo hydrolysis, oxidation, and polymerization reactions, which may account at least partially for losses of these compounds (Jackson, 2008; Kennedy, 2008). Losses of delphinidin and petunidin were greater than for malvidin, consistent with their greater susceptibility to oxidation (Cheynier, Souquet, Kontek, & Moutounet, 1994).

Pomace contained only ~10% of the original mass of malvidin glucosides present in the whole grapes, however, absolute concentrations of malvidin 3-*O*-glucoside in pomace remained high (Table 2). Interestingly, pomace rachises appeared to sorb the anthocyanins during fermentation. Jensen et al. (2008) observed anthocyanin recoveries in Grenache wine of ~30%, similar to our result. However, these authors did not measure the anthocyanin content of the other fermentation products so that the overall fate of the anthocyanins during processing could not be evaluated.

During secondary malolactic fermentation, the total amount of malvidin 3-*O*-glucoside and peonidin 3-*O*-glucoside remained constant (Table 2; 99% recovery in finished wine and lees relative to press wine). However, the total amount of malvidin 3-acetylglucoside and petunidin 3-*O*-glucoside in the final wine decreased by 15 and 30%, respectively, compared to the amount in the press wine. Again this is consistent with the greater lability of these anthocyanins. As was observed for the flavan-3-ols, lees sorbed up to 30% of the mass of the anthocyanins, resulting in anthocyanin concentrations in the lees being several times greater than in the final wine (Table 2). Sorption of anthocyanins by yeast lees has been previously observed (Mazauric & Salmon, 2006; Rizzo et al., 2006).

3.2.7. Flavonols

Quercetin 3-*O*-glucoside was found only in the skins of whole grapes. At the end of the alcoholic fermentation, 98.5% of the total quercetin 3-*O*-glucoside initially present in the grapes was accounted for in the pomace and press wine; approximately 25% of the total mass was retained in the pomace (Table 2). The aglycone, quercetin, was not detected in whole grapes or pomace, however, this compound was found in low concentrations in the press wine (Table 2). The aglycone may have been released from hydrolysis of the quercetin glycosides in the grapes during fermentation as previously suggested by Rentzsch et al. (2007).

During malolactic fermentation a loss of ~10% of the total amount of quercetin 3-*O*-glucoside in the press wine was observed (Table 2). This coincided with a significant increase in total quercetin aglycone in the finished wine and lees. However, the amount of free quercetin was greater than could be obtained from the quercetin 3-*O*-glucoside alone, therefore hydrolysis of other quercetin glycosides probably also occurred. Hernández, Estrella, Carlavilla, Martín-Álvarez, and Moreno-Arribas (2006) did not observe hydrolysis of flavonol glycosides and increases in aglycone levels during malolactic fermentation of Tempranillo grapes, however their malolactic conditions were not fully specified with respect to time, temperature and lactic acid bacterial strain used. In a later study, Hernández et al. (2007) observed that some bacterial strains can result in a significant increase in quercetin aglycone levels during malolactic fermentation. In particular, increases in quercetin aglycone levels were greatest for the two *Oenococcus oeni* strains studied (Oe-18 and Oe-159). The observed increase in quercetin aglycone in this study is consistent with use of *O. oeni* cultures for the malolactic fermentation.

As was observed for the other polyphenols, the lees sorbed significant amounts of both the quercetin glucoside and the aglycone. Previous studies have shown that various metabolically active or dead enological microrganism species, including *Saccharomyces cerevisiae*, can sorb or even transport grape phenolic compounds including quercetin 3-*O*-glucoside (Rizzo et al., 2006). The sorption and uptake of phenolic compounds by lees resulted in absolute concentrations in the lees that were greater than observed in the finished wine, making this winery waste a potentially valuable source of these health protective compounds.

4. Conclusion

Using a component balance approach we are able to quantitatively monitor changes in polyphenol composition in all pre- and post-fermentation products and better understand the physical (*i.e*., sorption) and chemical (*i.e*., hydrolysis, oxidation, etc.) mechanisms that impact final polyphenol concentrations. Understanding of the disposition and fate of key constituents throughout the winemaking process enables a better understanding of basic reactions (i.e. transport, oxidation, hydrolysis, chemical modification, concentration, etc.,) in the skins, seeds, pulp, and stems. This information allows for the discovery of changes that may not have been understood previously (e.g., changes in gallic acid levels coming from the hydrolysis of galloylated compounds), and lays the foundation for manipulating processes to create pomaces, lees, and wines with improved quality or expanded functionality.

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References

Adams, D. O. (2006). Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture, 57*, 249–256.
Bindon, K. A., Smith, P. A., Holt, H., & Kennedy, J. A. (2010). Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. *Journal of Agricultural and Food Chemistry, 58*, 10736–10746.
Birt, D. I., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics, 90*, 157–177.
Bocevska, M., & Stevcevska, V. (1997). Quality evaluation of anthocyanin extract obtained from wine grape pomace. *Food Technology and Biotechnology, 35*, 139–143.
Botella, C., Diaz, A., De Ory, I., Webb, C., & Blandino, A. (2009). Xylanase and pectinase production by Aspergillus awamori on grape pomace in solid state fermentation. *Process Biochemistry, 44*, 949–1062.
Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (1996). *Principles and practices of winemaking*. New York, NY, USA: Chapman and Hall.
Braga, F. G., Lencart e Silva, F. A., & Alves, A. (2002). Recovery of winery by-products in the Douro demarcated region: production of calcium tartrate and grape pigments. *American Journal of Enology and Viticulture, 53*, 41–45.
Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdy, S. G., Arija, I., et al. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science, 87*, 307–316.
Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosin-Gutiérrez, I. H. (2007). Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *Journal of Agricultural and Food Chemistry, 55*, 992–1002.
Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2010). Flavonol profiles of *Vitis vinifera* white grape cultivars. *Journal of Food Composition and Analysis, 23*, 699–705.

Cheynier, V., Souquet, J. M., Kontek, A., & Moutounet, M. (1994). Anthocyanin degradation in oxidizing grape musts. *Journal of the Science of Food and Agriculture, 66*, 283–288.
De Beer, D., Harbertson, J. F., Kilmartin, P. A., Roginsky, V., Barsukova, T., Adams, D. O., et al. (2004). Phenolics: a comparison of diverse analytical methods. *American Journal of Enology and Viticulture, 55*, 389–400.
De Freitas, V. A. P., Glories, Y., Bourgeois, G., & Vitry, C. (1998). Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. *Phytochemistry, 49*, 1435–1441.
Downey, M. O., Dokoozlian, N. K., & Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture, 57*, 257–268.
El-Shami, S. M., El-Mallah, M. H., & Mohammed, S. S. (1992). Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing. *Grasas Aceites, 43*, 157–160.
Famuyiwa, O., & Ough, C. S. (1982). Grape pomace: possibilities as animal feed. *American Journal of Enology and Viticulture, 33*, 44–46.
Fernández de Simón, B., Hernández, T., & Estrella, I. (1993). Phenolic composition of white grapes (Var. Airén) Changes during ripening. *Food Chemistry, 47*, 47–52.
Ferrer, J., Páez, G., Mármol, G., Ramones, E., Chandler, C., Marín, M., et al. (2001). Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology, 76*, 39–44.
Formica, J. V., & Regelson, W. (1995). Review of the biology of quercetin and related bioflavonoids. *Food and Chemical Toxicology, 33*, 1061–1080.
Ginjom, I. R., D'Arcy, B. R., Caffin, N. A., & Gidley, M. J. (2010). Phenolic contents and antioxidant activities of major Australian red wines throughout the winemaking process. *Journal of Agricultural and Food Chemistry, 58*, 10133–10142.
Guendez, R., Kallithraka, S., Makris, D. P., & Kefalas, P. (2005). Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: correlation with antiradical activity. *Food Chemistry, 89*, 1–9.
Hang, Y. D., & Woodams, E. E. (1985). Grapes a novel substrate for microbial production of citric acid. *Biotechnology Letters, 7*, 253–254.
Hang, Y. D., Lee, C. Y., & Woodams, E. E. (1986). Solid-state fermentation of grape pomace for ethanol production. *Biotechnology Letters, 1*, 53–56.
Hernández, T., Estrella, I., Carlavilla, D., Martín-Álvarez, P. J., & Moreno-Arribas, M. V. (2006). Phenolic compounds in red wine subjected to industrial malolactic fermentation and ageing on lees. *Analytica Chimica Acta, 563*, 116–125.
Hernández, T., Estrella, I., Pérez-Gordo, M., Alegría, E. G., Tenorio, C., Ruiz-Larrrea, F., et al. (2007). Contribution of malolactic fermentation by *Oenococcus oeni* and *Lactobacillus plantarum* to the changes in the nonanthocyanin polyphenolic composition of red wine. *Journal of Agricultural and Food Chemistry, 55*, 5260–5266.
Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., et al. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven countries Study. *Archives of Internal Medicine, 155*, 381–386.
Jackson, D. I., & Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality—a review. *American Journal of Enology and Viticulture, 44*, 409–430.
Jackson, R. S. (2008). Chemical constituents of grapes and wines. In *Wine science: Principles and applications* (3rd ed.). (pp. 270–299). San Diego, CA, USA: Academic Press.
Jensen, J. S., Demiray, S., Egebo, M., & Meyer, A. S. (2008). Prediction of wine color attributes from the phenolic profiles of red grapes (*Vitis vinifera*). *Journal of Agricultural and Food Chemistry, 56*, 1106–1115.
Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry, 52*, 4360–4436.
Kelm, M. A., Johnson, J. C., Robbins, R. J., Hammerstone, J. F., & Schmitz, H. H. (2006). High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *Journal of Agricultural and Food Chemistry, 54*, 1571–1576.
Kennedy, J. A. (2008). Grape and wine phenolics: observations and recent findings. *Ciencia e investigación agraria, 35*, 107–120.
Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G., & Teissedre, P. L. (2001). Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. *Journal of Agricultural and Food Chemistry, 49*, 3341–3348.
Llobera, A., & Cañellas, J. (2008). Antioxidant activity and dietary fibre of Prensal Blanc white grape (*Vitis vinifera*) by-products. *International Journal of Food Science & Technology, 43*, 1953–1959.
Lu, Y., & Foo, L. Y. (1998). The polyphenol constituents of grape pomace. *Phytochemistry, 47*, 1137–1140.
Mazauric, J. P., & Salmon, J. M. (2006). Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees. *Journal of Agricultural and Food Chemistry, 54*, 3876–3881.
Núñez, V., Gómez-Cordovés, C., Bartolomé, B., Hong, Y. J., & Mitchell, A. E. (2006). Non-galloylated and galloylated proanthocyanidin oligomers in grape seeds from Vitus vinifera L. cv. Graciano, Tempranillo and Cabernet Sauvignon. *Journal of the Science of Food and Agriculture, 86*, 915–921.
Ough, C. S., & Amerine, M. A. (1988). *Methods for analysis of musts and wines*. New York, NY, USA: Wiley-Interscience.
Peña-Neira, A., Cáceres, A., & Pastenes, C. (2007). Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): effect of cluster thinning and vineyard yield. *Food Science and Technology International, 13*, 153–159.
Pérez-Jiménez, J., Serrano, J., Tabernero, M., Arranz, S., Díaz-Rubio, M. E., García-Diz, L., et al. (2009). Bioavailability of phenolic antioxidants associated with dietary fiber: plasma antioxidant capacity after acute and long-term intake in humans. *Plant Foods for Human Nutrition, 64*, 102–107.
Pinelo, M., Laurie, F., & Waterhouse, A. L. (2006). A simple method to separate red wine nonpolymeric and polymeric phenols by solid phase extraction. *Journal of Agricultural and Food Chemistry, 54*, 2839–2844.
Rentzsch, M., Schwarz, M., Winterhalter, P., & Hermosín-Gutiérrez, I. (2007). Formation of hydroxyphenyl-pyranoanthocyanins in Grenache wines: precursor levels and evolution during aging. *Journal of Agricultural and Food Chemistry, 55*, 4883–4888.
Rizzo, M., Ventrice, D., Varone, M. A., Sidari, R., & Caridi, A. (2006). HPLC determination of phenolics adsorbed on yeasts. *Journal of Pharmaceutical and Biomedical Analysis, 42*, 46–55.
Rohn, S., Rawel, H. M., & Kroll, J. (2004). Antioxidant activity of protein-bound quercetin. *Journal of Agricultural and Food Chemistry, 52*, 4725–4729.
Romeyer, F. M., Macheix, J. J., Goiffon, J. P., Reminiac, C. C., & Sapis, J. C. (1983). The browning capacity of grapes. 3. Changes and importance of hydroxycinnamic acid-tartaric acid esters during development and maturation of the fruit. *Journal of Agricultural and Food Chemistry, 31*, 346–349.
Sacchi, K. L., Bisson, L. F., & Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture, 56*, 197–206.
Sarni-Manchado, P., Fulcrand, H., Souquet, J. M., Cheynier, V., & Moutounet, M. (1996). Stability and color of unreported wine anthocyanin-derived pigments. *Journal of Food Science, 61*, 938–941.
Saura-Calixto, F. D. (1998). Antioxidant dietary fiber product: a new concept and a potential food ingredient. *Journal of Agricultural and Food Chemistry, 46*, 4303–4306.
Saura-Calixto, F. D. (2003). Antioxidant dietary fibre. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 2*, 223–226.
Siddiqui, I. M., Raisuddin, S., & Shukla, Y. (2005). Protactive effects of black tea extract on testosterone induced oxidative damage in prostate. *Cancer Letters, 227*, 125–132.
Silva, M. L., & Malcata, F. X. (1999). Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 208*, 134–143.
Singleton, V. L., & Esau, P. (1969). *Phenolic substances in grapes and wine, and their significance*. New York, NY, USA: Academic Press.
Singleton, V. L., Salgues, M., Zaya, J., & Trousdale, E. (1985). Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *American Journal of Enology and Viticulture, 36*, 50–56.
Threlfall, R. T., Morris, J. R., Howard, L. R., Brownmiller, C. R., & Walker, T. L. (2006). Pressing effects on yield, quality, and nutraceutical content of juice, seeds, and skins from black beauty and sunbelt grapes. *Journal of Food Science, 70*, S167–S171.
Torres, J. L., Varela, B., García, M. T., Carrilla, J., Matito, C., Centelles, J. J., et al. (2002). Valorization of grape (*Vitis vinifera*) byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *Journal of Agricultural and Food Chemistry, 50*, 7548–7555.
Veluri, R., Singh, R. P., Liu, Z., Thompson, J. A., Agarwal, R., & Agarwal, C. (2006). Fractionation of grape seed extract and identification of GA as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. *Carcinogenesis, 27*, 1445–1453.
Waterhouse, A. L. (2002). Wine phenolics. *Annals of the New York Academy of Sciences, 957*, 21–36.
Yilmaz, Y., & Toledo, R. T. (2004). Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal of Agricultural and Food Chemistry, 52*, 255–260.