



Short communication

Influence of extraction methodology on grape composition values

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ABSTRACT

This work demonstrated similarities and differences in quantifying many grape quality components (>45 compounds) that were extracted from berries by three distinct preparations, before being analysed by eight spectrophotometric and HPLC methods. All sample extraction methods were appropriate for qualitative results only. Different extraction procedures showed altered component composition in 'Pinot noir' berries, possibly due to the localisation of the compounds of interest within the grape and how those compounds were extracted from the berry. Sample extraction is an often-overlooked part of berry evaluations, but this study illustrates that it should be carefully considered prior to berry component analysis for its influence upon measurements.

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1. Introduction

There is no uniform or official extraction procedure for small berry (principally grape and wine) research. This inconsistency often contributes to misunderstanding when researchers compare their results to other sets of data in the literature. Important grape quality components are distributed throughout the grape berry and extraction methods are selective in the compounds that they make available for measurement, especially when different treatments are contrasted (Fragoso, Mestres, Busto, & Guasch, 2010; Lee & Schreiner, 2010).

Recently, we demonstrated similarities and differences amongst 'Pinot noir' juice samples and exhaustively extracted (entire berry) samples from a grapevine nutrient study (Lee & Schreiner, 2010). Juice samples were significantly lower in ammonia, total free amino acids, and yeast assimilable nitrogen (YAN) compared to exhaustively extracted samples. Individual free amino acid content values were also altered. Juice from berries is the common sample form analysed by wineries for their harvest and fermentation addition decisions, although that extraction method may underestimate YAN (Bell & Henschke, 2005; Lee & Schreiner, 2010) and lead to an over-addition of YAN supplements.

There are numerous grape components that are important to grape and wine quality; many have been well reviewed by others (Bell & Henschke, 2005; Cheynier, 2005; Conde et al., 2007). Grape phenolics are crucial quality factors that ultimately play roles in premium wine appearance and mouthfeel (Cheynier, 2005; Conde et al., 2007). Sugars and organic acids are important for alcoholic fermentation and also contribute to organoleptic properties (Conde et al., 2007; Torija et al., 2003). Grape nitrogen (N) compounds are vital nutrients for yeast/bacteria to finish alcoholic/malolactic fermentations and to develop the desired flavours (Bell & Henschke, 2005; Conde et al., 2007). Different grape compounds are localised in different parts of the grape berry, moreover, as they are structurally diverse, complete extraction requires multiple processing methods. Quantities are often cultivar dependent, altered by growing season, maturity level, environment, etc. (Conde et al., 2007; Fragoso et al., 2010). Though a single extraction procedure might not be suitable to examine every grape quality compound of interest, only a few research groups have examined the influence extraction technique has upon measurements (Fragoso et al., 2010; Hunter, Visser, & De Villers, 1991; Khanal, Howard, & Prior, 2009; Lee & Schreiner, 2010; Mane et al., 2007; Spigno, Tramelli, & De Faveri, 2007).

Extraction solvent, extraction temperature, extraction duration, sample particle size after pulverisation, number of re-extractions, sample to solvent ratio, and the like, influence what ultimately can be extracted from the berry (Hunter et al., 1991; Karvela, Makris, Kalogeropoulos, Karathanos, & Kefalas, 2009; Kim & Verpoorte, 2010; Lee & Schreiner, 2010; Mane et al., 2007; Spigno

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et al., 2007). The intention of this study, however, was to determine how three commonly used sample preparations affected measurements of grape quality components (>45) commonly reported and monitored by the research community.

2. Materials and methods

2.1. Plant material

Details of the grape berries used in this study are in Lee and Schreiner (2010). Briefly, 'Pinot noir' berries were from vines planted in 2003 at Lewis-Brown Research Farm (Oregon State University, Corvallis, OR, USA), and were sampled in 2007 at commercial ripeness (composite berry samples reached $\sim 23^\circ$ Brix). Vines were self-rooted 'Pinot noir' clone FPS (Foundation Plant Services) 91, Pommard. All berry samples were pooled then randomly grouped into 50 berries prior to sample preparation, except the samples that would be homogenised. Homogenised samples required 100 berries to cover the blender blade correctly. Harvested grapes were stored at -80°C until extraction.

2.2. Reagents, chemicals and standards

All reagents, chemicals and standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), unless mentioned otherwise. Chemicals for free amino acid in-line derivatization prior to HPLC injection were purchased from Agilent Technologies Inc. (Palo Alto, CA, USA). Methylcellulose (12–18 cP) was purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Malvidin-3-glucoside (mvd-glu) was purchased from Polyphenols Laboratories AS (Sandnes, Norway). Liquid nitrogen (N) was obtained from Norco Inc (Nampa, ID, USA). Only analytical and high performance liquid chromatography (HPLC) grade chemicals, solvents and water were used.

2.3. Sample extraction procedures

Grape extractions were carried out by three approaches prior to chemical analyses, in triplicates. For the first group, thawed berries (~ 1 h at room temperature) were pureed using a hand blender for 3 min, which macerated the skin, pulp and seeds. In a typical winery quality control lab, purees would be centrifuged and the supernatants collected for analyses (Lee, Keller, Rennaker, & Martin, 2009; Lee & Schreiner, 2010; personal communication, anonymous). But, for uniformity in this study the solid to liquid ratio was held constant with a known weight (~ 20 g) of berry puree and extraction water (final volume 25 ml). Puree/water mixtures were centrifuged for 10 min at 4000 rpm, before the supernatants were collected. This was repeated two additional times (total three times). Extraction of pureed berries will be referred to as homogenates.

For the second sample extraction, berries were first fractionated into two portions (FA – skin and pulp fraction; FB – seeds fraction) as described in detail previously (Lee & Martin, 2009; Lee & Schreiner, 2010). Then, frozen berries were fractionated using a razor blade, then immediately placed in liquid nitrogen (LN_2), excess LN_2 was evaporated off, and fractions were then stored at -80°C until extraction. FA was LN_2 powdered (using an IKA M20 Universal mill; IKA works Inc., Wilmington, NC, USA) and extracted with acidified methanol (0.1% formic acid; total three times), and FB whole seeds were also extracted (total three times) as previously described in detail (Lee & Martin, 2009). Acidified methanol was evaporated using a RapidVap Vacuum Evaporation System (Labconco Corp., Kansas City, MO, USA) and re-dissolved in 25 ml of

water. Values obtained for the two fractions were summed, which will be referred to as fractionated extracts.

Third sample extraction was LN_2 powdering of entire berries (Lee & Finn, 2007) which were then extracted following the same procedure as that for fractionated extracts (Lee & Schreiner, 2010). Products from this preparation will be referred to as whole berry extracts. All aqueous extract forms from each of the three sample methods were kept at -80°C until comprehensive chemical analysis.

2.4. Chemical analyses

Analysis procedures did not alter from previously published works listed below, and were performed in duplicates. All three sample extracts were subjected to the following analyses:

- (1) Total anthocyanin (TACY) determination by the pH differential method (Lee, Durst, & Wrolstad, 2005; Lee & Martin, 2009). Absorbances were taken at 520 and 700 nm. Values were expressed as mg mvd-glu/100 g, and calculated using extinction coefficient $28,000\text{ l cm}^{-1}\text{ mol}^{-1}$ and molecular weight of 493.3 g mol^{-1} . A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) was used for this analysis and all other spectrophotometric methods listed below.
- (2) Total phenolics (TP) by Folin-Ciocalteu method (Lee & Martin, 2009; Waterhouse, 2002). Absorbance was measured at 765 nm. Values were expressed as mg gallic acid/100 g.
- (3) Total tannins (TT) by methylcellulose precipitation method (Lee & Martin, 2009; Sarneckis et al., 2006). Absorbance was measured at 280 nm. Values were expressed as mg epicatechin/100 g.
- (4) Simple sugars (glucose and fructose) and organic acids (tartaric acid and malic acid) were determined using an isocratic mobile phase method by HPLC/DAD/RID as described in Lee et al. (2009). An Agilent 1100 HPLC system was used for this analysis and all other HPLC methods listed below. Standards of each sugar and organic acid were used for identification and quantification. Values from this analysis were expressed as g/100 g.
- (5) Ammonia was determined by an enzymatic assay (Sigma ammonia assay kit; Lee & Schreiner, 2010; Lee et al., 2009). Free amino acids were analysed via a HPLC/DAD by in-line derivatization by *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) as previously described (Lee & Schreiner, 2010; Lee et al., 2009). Ammonia and primary amino acids were summed and YAN (yeast assimilable nitrogen) content was obtained. All nitrogen containing compound values were expressed as mg of N/100 g.
- (6) Individual anthocyanins (monitored at 520 nm) and other polyphenolics (monitored at 280, 320, and 370 nm) analyses were as conducted previously described (Lee & Finn, 2007; Lee & Martin, 2009) using a HPLC/DAD and MS when needed, and identified as reported (Lee & Finn, 2007; Lee & Martin, 2009). Two mobile phase systems were utilised (Lee & Finn, 2007) to analyse anthocyanins and other polyphenolics. Individual anthocyanins were quantified as mvd-glu, expressed as mg/100 g. Phenolic acids were quantified as mg of caffeic acid/100 g, flavanols as mg of catechin/100 g, and flavonol-glycosides as mg of quercetin-rutinoside/100 g. Polyphenolics other than anthocyanins will be referred to as polyphenolics for conciseness in this paper.

Since the analytical conditions were not altered from what was formerly published, the methods are not thoroughly described here. The specific settings regarding column information, mobile

phase program, injection volumes, syringe filter information, etc. can be found in the references above.

2.5. Statistical analysis

Statistica for Windows version 7.1 was used (StatSoft, Inc., Tulsa, OK, USA). Differences amongst the component results obtained were tested using one-way analysis of variances (ANOVA) and the Tukey HSD (Honest Significant Difference; $\alpha = 0.05$).

3. Results and discussion

As pointed out in Section 2, berries that were homogenised and then water extracted will be referred to as homogenates. Berries that were fractionated and extracted will be referred to as fractionated extracts. Whole berries that were exhaustively extracted will be referred to as whole berry extracts. Fractionation and extraction of separate portions were conducted, since the research community typically fractionates prior to extraction to segregate sources of skin proanthocyanidins from seed proanthocyanidins, though they are difficult to distinguish analytically, they contribute distinct quality attributes to finished wines (Lee, Kennedy, Devlin, Redhead, & Rennaker, 2008). Powdering of whole berry prior to extraction remains a customary method for comprehensive analyses (Lee & Finn, 2007).

All results from simple phenolic spectrophotometer methods (TACY, TP, and TT), individual and total sugars and organic acids are listed in Table 1. TACY and TP were significantly higher in fractionated extracts and whole berry extracts compared to homogenates, due to the increased extraction of phenolics from skin portions. Whole berry extraction increased the yield of seed portions and was higher in TP and TT, as expected, since the seed fractions were completely powdered in contrast to homogenates and fractionated extracts. It is possible that some of the phenolic compounds degraded during the thawing of the berries, prior to

homogenisation, which contributed to the lower levels of TACY, TP, and TT. Identical sugars and organic acids were found in each of the three extracts. Individual sugars and total sugar levels were significantly higher in fractionated and whole berry extracts, compared to homogenates. Tartaric acid was higher in homogenates compared to fractionated extracts and whole berry extracts, which could be due to the extraction solvent employed (water vs. methanol), changes that occurred during frozen storage, and differences in compound extractability (Hunter et al., 1991). Malic acid was higher in whole berry extracts compared to homogenates. Total organic acids were higher in homogenates, compared to fractionated extracts. Hunter et al. (1991) have shown significantly lower levels of tartaric acid and malic acid in -20°C frozen prior (to being juiced) 'Cabernet Sauvignon' grapes compared to freshly juiced grapes.

Individual anthocyanin concentrations and proportions are in Table 2 and Fig. 1A. The anthocyanin profile of 'Pinot noir' grapes has been well established, and five anthocyanins were found in all three extracts, as reported earlier (Lee & Martin, 2009). The principal 'Pinot noir' anthocyanin was mvd-glu. The three minor anthocyanins (in *Italic*), in order of most to least altered in the homogenates; (mvd-glu > ped-glu > cyd-glu > ptd-glu > dpd-glu (abbreviations listed in Table 2) they remained in the same order for fractionated and whole berry extracts (mvd-glu > ped-glu > ptd-glu > dpd-glu > cyd-glu). Fractionated and whole berry extracts were significantly higher in all five anthocyanins and total anthocyanins compared to homogenated samples, again due to more efficient extraction from the skin portion of the grape berry (Mane et al., 2007), probably aided by smaller particle sizes from LN_2 powdering the anthocyanin containing fraction. Quantifications of grape anthocyanins are not appropriate after homogenisation of samples, but fractionated extracts and whole berry extracts are appropriate for individual anthocyanin quantification. Homogenates and whole berry extracts were 82% lower in total anthocyanins compared to fractionated extracts and whole berry extracts.

Table 1

Total anthocyanins (TACY), total phenolics (TP), total tannins (TT), glucose, fructose, total sugar, tartaric acid, malic acid, and total organic acid for each extraction preparation method. Values in parenthesis indicate standard errors. Units are indicated in the measurements column of the table.

Measurements	Sample preparation methods		
	Homogenates	Fractionated extracts	Whole berry extracts
TACY (mg/100 g)	10.6 (0.3)a	51.9 (2.1)b	55.6 (1.0)b
TP (mg/100 g)	129.9 (4.2)a	226.6 (2.3)b	373.7 (3.3)c
TT (mg/100 g)	104.8 (6.8)a	115.2 (6.5)a	230.0 (14.3)b
Glucose (g/100 g)	7.74 (0.01)a	8.70 (0.12)b	8.96 (0.06)b
Fructose (g/100 g)	7.75 (0.06)a	8.33 (0.13)b	8.51 (0.05)b
Total simple sugars (g/100 g)	15.49 (0.07)a	17.03 (0.24)b	17.48 (0.11)b
Tartaric acid (g/100 g)	0.33 (0.02)b	0.16 (0)a	0.20 (0.01)a
Malic acid (g/100 g)	0.30 (0.01)a	0.33 (0.01)ab	0.38 (0.01)b
Total organic acid (g/100 g)	0.63 (0.02)b	0.50 (0.01)a	0.57 (0.02)ab

Means followed by a different letter within each row are significantly different (Tukey's HSD) amongst the three sample extraction methods evaluated.

Table 2

Individual and total anthocyanin results obtained by HPLC. All values were expressed as mg of mvd-glu/100 g. Values in parenthesis indicate standard errors. 'Pinot noir' grapes contain five anthocyanins and are listed in the order of elution (Lee and Martin, 2009).

Compounds	Abbreviations	Sample preparation methods		
		Homogenates	Fractionated extracts	Whole berry extracts
Delphinidin-glucoside	dpd-glu	0.42 (0)a	5.81 (0.24)b	6.64 (0.26)b
Cyanidin-glucoside	cyd-glu	0.75 (0.01)a	2.65 (0.05)b	2.99 (0.12)c
Petunidin-glucoside	ptd-glu	0.67 (0.04)a	7.10 (0.28)b	7.85 (0.24)b
Peonidin-glucoside	ped-glu	6.50 (0.03)a	29.79 (0.44)b	30.80 (1.23)b
Malvidin-glucoside	mvd-glu	12.78 (0.37)a	72.27 (3.26)b	73.75 (1.61)b
Total anthocyanin		21.1 (0.44)a	117.62 (4.18)b	122.04 (3.35)b

Means followed by a different letter within each row are significantly different (Tukey's HSD) amongst the three sample extraction method used.

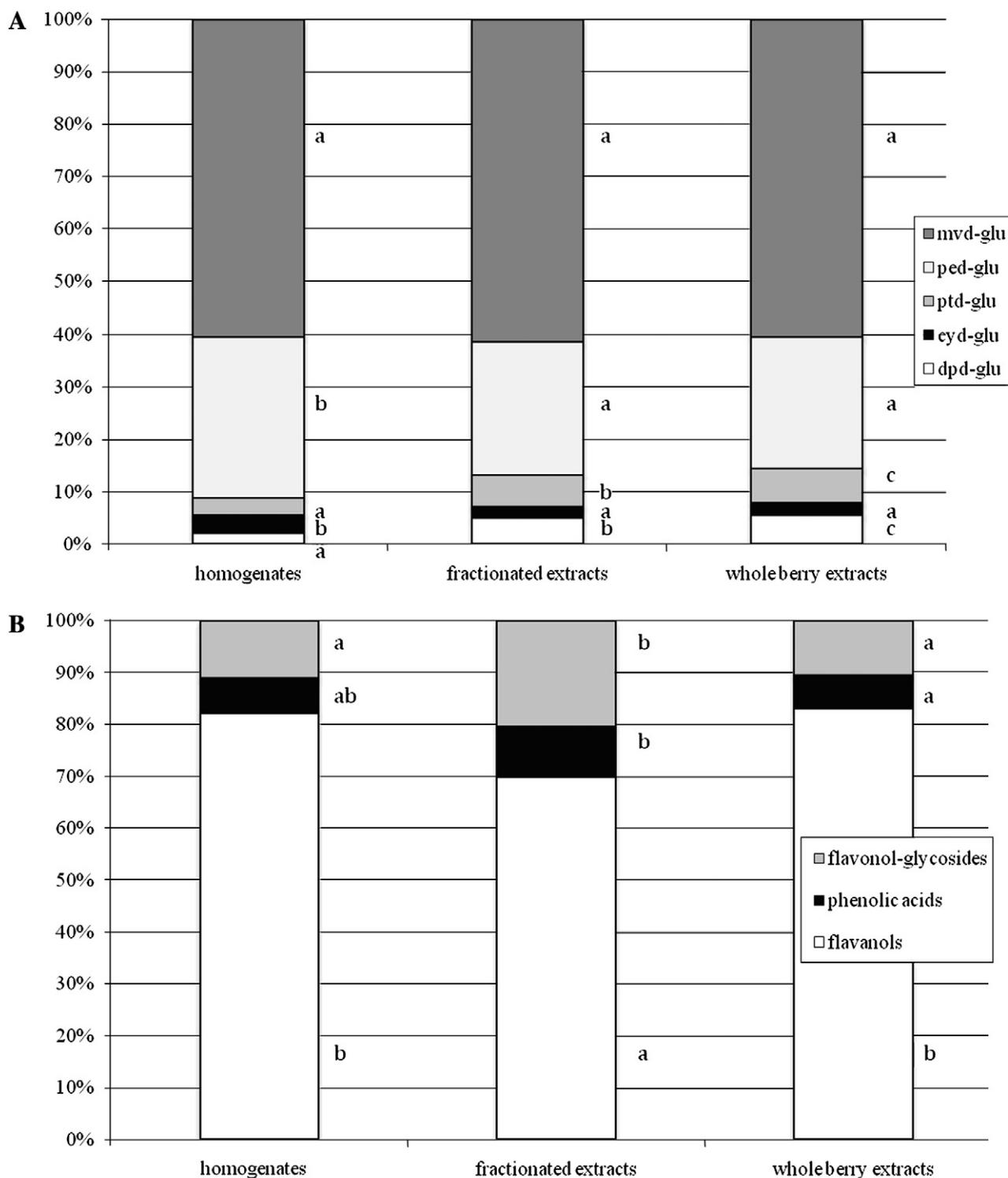


Fig. 1. Percent proportions of individual anthocyanins (A) and polyphenolics (B). A different letter indicates significant difference (Tukey's HSD) amongst the three sample extraction method used.

Homogenisation altered the proportion of the individual anthocyanins as well (Fig. 1A). Although % mvd-glu was not altered, % ped-glu and % cyd-glu were significantly higher in homogenates compared to fractionated and whole berry extracts. For anthocyanin quantification, homogenisation is not appropriate for berries where anthocyanins are mainly concentrated in the skin of the berry.

Individual polyphenolics are reported in Table 3. All polyphenolics have been identified and were reported earlier (Lee & Martin, 2009). Fifteen polyphenolics were identified in all three extracts. Amounts for each of the six flavanols were higher in whole berry extracts compared to homogenates, which was probably due to the efficient extraction of the skin and seed flavanols (Mane et al., 2007). Flavanol concentrations from fractionated extracts

Table 3

Individual polyphenolics results obtained by HPLC. All units were mg/100 g. All identifications were conducted as previously reported in Lee and Martin (2009). Values in parenthesis indicate standard errors.

Compounds	λ_{\max} (nm)	Sample preparation methods		
		Homogenates	Fractionated extracts	Whole berry extracts
Gallocatechin	280	t	t	t
Proanthocyanidin	280	2.59 (0.42)a	1.09 (0.14)a	7.09 (0.92)b
Catechin	280	6.88 (0.75)a	12.04 (0.87)a	38.99 (3.00)b
Proanthocyanidin	280	1.17 (0.16)a	1.98 (0.10)a	6.14 (0.71)b
Epicatechin	280	4.35 (0.53)a	8.52 (0.42)b	24.45 (0.91)c
(epi)Catechin-epicatechin gallate or epicatechin gallate-(epi)catechin	280	1.32 (0.10)a	1.45 (0.43)a	5.39 (0.11)b
Epicatechin gallate	280	0.52 (0.07)a	1.66 (0.20)a	7.06 (0.57)b
Total flavanol		17.37 (1.65)a	26.74 (1.78)a	89.13 (6.01)b
Protocatechuic acid	320	1.24 (0.12)b	0.20 (0.03)a	1.33 (0.07)b
Caftaric acid	320	0.10 (0.02)a	2.64 (0.40)b	4.27 (0.06)c
Coutaric acid	320	0.07 (0)a	0.75 (0.08)b	1.14 (0.02)c
Fertaric acid	320	0.03 (0.02)a	0.08 (0.01)b	0.13 (0)c
Total phenolic acid		1.43 (0.12)a	3.66 (0.53)b	6.88 (0.15)c
Quercetin-galactoside	370	0.31 (0.03)a	0.96 (0.15)b	1.43 (0.02)c
Quercetin-glucuronide	370	1.37 (0.14)a	4.63 (0.75)b	6.70 (0.17)c
Kaempferol-glucoside	370	0.31 (0.03)a	1.01 (0.23)b	1.53 (0.02)b
Isorhamnetin-glucoside	370	0.34 (0.02)a	1.22 (0.17)b	1.60 (0.02)b
Total flavonol-glycoside		2.33 (0.23)a	7.82 (1.29)b	11.26 (0.23)c
Total polyphenolics		21.13 (1.65)a	38.22 (2.09)b	107.26 (5.93)c

t, present at trace levels. Means followed by a different letter within each row are significantly different (Tukey's HSD) amongst the three sample extraction method used.

Table 4

Comparison of ammonia, individual free amino acids, and YAN values. All N-containing compounds were expressed as mg N/100 g. All identifications were described previously (Lee et al., 2009; Lee and Schreiner, 2010). Values in parenthesis indicate standard errors.

Compounds		Sample preparation methods		
		Homogenates	Fractionated extracts	Whole berry extracts
Ammonia		0.03 (0)a	0.04 (0)b	0.03 (0)ab
Aspartic acid	ASP	0.41 (0.01)a	0.38 (0.01)a	0.42 (0.01)a
Glutamic acid	GLU	0.58 (0.01)a	0.67 (0.05)a	0.70 (0.02)a
Asparagine	ASN	0.15 (0.01)b	0.09 (0.01)a	0.21 (0.01)c
Serine	SER	0.62 (0.01)a	0.65 (0.01)ab	0.69 (0)b
Glutamine	GLN	1.43 (0.05)a	1.48 (0.09)a	1.50 (0.05)a
Histidine	HIS	0.52 (0.01)c	0.31 (0.01)a	0.45 (0.01)b
Glycine	GLY	0.13 (0)b	0.08 (0.01)a	0.16 (0.01)c
Threonine	THR	0.83 (0.03)a	1.03 (0.03)b	0.97 (0.01)b
Citrulline	CIT	0.05 (0)a	0.12 (0.02)ab	0.15 (0.03)b
Arginine	ARG	8.17 (0.19)a	8.74 (0.40)a	8.16 (0.12)a
Alanine	ALA	1.41 (0.05)a	1.80 (0.13)b	1.62 (0.01)ab
γ -Aminobutyric acid	GABA	1.56 (0.01)a	1.53 (0.06)a	1.68 (0.01)a
Tyrosine	TYR	0.13 (0.01)a	0.15 (0.01)a	0.19 (0)b
Valine	VAL	0.47 (0.01)a	0.51 (0.02)ab	0.56 (0.01)b
Methionine	MET	0.07 (0)a	0.08 (0)a	0.08 (0)a
Tryptophan	TRP	0.20 (0.01)a	0.21 (0)a	0.27 (0.01)b
Phenylalanine	PHE	0.14 (0.01)a	0.16 (0.01)a	0.17 (0)a
Isoleucine	ILE	0.30 (0.01)a	0.34 (0.01)b	0.36 (0.01)b
Leucine	LEU	0.55 (0.01)a	0.63 (0.02)b	0.66 (0.01)b
Lysine	LYS	0.16 (0.01)b	0.11 (0)a	0.15 (0)b
Hydroxyproline	HYP	1.19 (0.02)a	1.31 (0)b	1.21 (0.01)a
Proline	PRO	2.03 (0.04)a	2.62 (0.09)b	2.49 (0.07)b
Total free amino acid		21.13 (0.43)a	23.00 (0.92)a	22.85 (0.02)a
YAN		17.94 (0.40)a	19.11 (0.83)a	19.19 (0.05)a

Means followed by a different letter within each row are significantly different (Tukey's HSD) amongst the three sample extraction methods used.

were not different from homogenates, except for epicatechin. Gallocatechin was found in trace levels in all three samples. Catechin and epicatechin were the main flavanols in all three extracts. Phenolic acids were highest in whole berry extracts, followed by fractionated extracts, and lastly by homogenates. Caftaric acid was the main phenolic acid found in fractionated and whole berry extracts, as reported earlier (Lee & Martin, 2009), but not the main phenolic acid measured in homogenates (protocatechuic acid). All four flavonol-glycosides were higher in fractionated extracts, as well as whole berry extracts, compared to homogenates. As flavonol-gly-

cosides are present mainly in the skin (Tarara, Lee, Spayd, & Scagel, 2008), more efficient extraction of skin fractions naturally increased the flavonol-glycosides found in the resulting extracts. Total polyphenolics were 5.1–2.8 times higher in whole berry extracts compared to homogenates and fractionated extracts, respectively.

Homogenates and whole berry extracts had both higher proportions of flavanols (Fig. 1B) than the fractionated extracts. Whole berry extracts had lower % phenolic acids and % flavonol-glycosides, compared to fractionated extracts. The percent proportions

for each of the three phenolic classes were not significantly different between homogenates and whole berry extracts.

Individual free amino acids, ammonia, YAN and abbreviations are reported in Table 4. Twenty-two free amino acids were found in 'Pinot noir' berries, 21 free amino acids were previously found (Lee & Martin, 2009; Lee & Schreiner, 2010) plus one additional free amino acid (GABA). Total free amino acids, seven individual amino acids (ASP, GLU, GLN, ARG, GABA, MET, PHE), and YAN were not significantly different amongst the three samples. ASN, HIS, GLY, and LYS were significantly higher in homogenates and whole berry extracts compared to fractionated extracts. ARG remained the main free amino acid found in all extracts, as reported for control 'Pinot noir' berries in Lee and Schreiner (2010). Relative concentrations of free amino acid, varying by extraction procedure, have been noted in Lee and Schreiner (2010). Though there was no significant difference found in total free amino acid and YAN amongst the three extracts, study conditions could change provided the field treatment altered N containing compound localisation within the berry, again reported in Lee and Schreiner (2010).

As mentioned before, the results here show that winemakers, industry wine chemists and researchers should consider how different sample preparations effect grape analysis yields. This then influences composition and content values, which ultimately affect the winemaking approach; how a grape cultivar's compounds can contribute to a wine, complimentary fermentation conditions, the winemaker's desired outcome, etc (Lee et al., 2008).

4. Conclusion

This work demonstrated that measured amounts of expected quality constituents found in 'Pinot noir' berries were altered by the sample preparation method utilised prior to chemical analyses. Exhaustive extraction of whole berry is more appropriate for phenolic analysis compared to homogenisation or seeds extracted intact. All three preparations might be appropriate for total free amino acid and YAN determination, but showed differing individual free amino acid and ammonia concentrations. Total sugars and organic acid levels were affected by extraction procedure as well.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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