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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 ~23° 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 $l\,cm^{-1}\,mol^{-1}$ 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