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Degradation kinetics of grape skin and seed proanthocyanidins in a model wine system

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ABSTRACT

Catechin (monomer), purified grape skin proanthocyanidin (polymer), and purified grape seed proanthocyanidin underwent monitored accelerated oxidation under continuous oxygenation and UV light, at a constant 20 °C. Compounds were dissolved in model wine solutions with (and without) catechol. Solutions were examined and then contrasted by absorbance measurements, phloroglucinolysis, and subsequent HPLC analysis. Oxidation of these monomers and polymers revealed significant colour changes (measurable increase in colour density). The presence of catechol increased the half-life of catechin, but the opposite was observed for total skin and seed proanthocyanidins. Skin and seed proanthocyanidin degradation half-life decreased with the addition of catechol. In general, based on second order rate reactions, total subunits of seed proanthocyanidin solutions degraded faster than that of skin proanthocyanidin solutions. As expected, there were decreases of measurable phenolics in both monomer and polymer solutions. Under the study conditions, flavanol monomer and polymer oxidation was chiefly dependant upon initial solution concentration.

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1. Introduction

Numerous research reports have shown (Cheynier, 2005, references therein; Jorgensen, Marin, & Kennedy, 2004; Lee, Kennedy, Devlin, Redhead, & Rennaker, 2008), phenolics' importance to red grapes and to the resulting wines made from them. Wine proanthocyanidins (also referred to as tannins, condensed tannins, or flavanol polymers) and colour are important red wine quality factors that can be manipulated by grape growing conditions and wine-making practices. Proanthocyanidins are a high-interest research topic as they play important roles in red wine by stabilizing colour and enhancing mouth-feel, which are critiqued qualities of premium wines (Cheynier, 2005, references therein; Bajec & Pickering, 2008, references therein).

Grape proanthocyanidins are the main group of phenolics found in the skin, seed, and stems of grapes (Cheynier, 2005; Jorgensen et al., 2004; Souquet, Labarbe, Le Guerneve, Cheynier, & Moutounet, 2000; Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002). Once proanthocyanidins have been extracted from their natural source, they undergo a number of reactions, and oxidation is one of them (Cheynier, 2005; Cheynier et al., 2006; Fulcrand, Duenas, Salas, & Cheynier, 2006; Wang, Zhou, & Wen, 2006; Waterhouse & Laurie, 2006). The significance of wine phenolic oxidation and its mechanisms have been well reviewed by others (Fulcrand et al., 2006; Waterhouse & Laurie, 2006). Consumers expect particular organoleptic properties in certain foods (e.g. tea, chocolate, wine, coffee, etc.) that result from oxidised proanthocyanidins (Bajec & Pickering, 2008; Cheynier, 2005; Cheynier et al., 2006; Manach, Wiliiamson, Morand, Scalbert, & Remesy, 2005; Santos-Buelga & Scalbert, 2000; Vidal et al., 2004; Wang et al., 2006). Sensory properties and evaluation of these compounds are ongoing, as purification and analytical methods to identify these compounds become more accessible (Vidal et al., 2002, 2003, 2004).

Any examination of proanthocyanidins is complex and as these compounds alter their structure from their native forms, analysis becomes even more challenging (De Freitas, Glories, & Laguerre, 1998; Es-Safi, Cheynier, & Moutounet, 2003a; Fulcrand et al., 2006; Vidal et al., 2004). Few studies to date have examined the kinetics of grape proanthocyanidins (De Freitas et al., 1998; Jorgensen et al., 2004; Vidal et al., 2002) and due to the intricate nature of wine matrix and the compounds of interest, the topic remains one for further exploration.

The objective of this experiment was to determine the kinetic parameters of catechin, skin proanthocyanidin, and seed proanthocyanidin in model wine system during continuous oxygenation

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and UV light exposure, with and without the presence of catechol (a monomer). This study was initiated to better understand the evolution of polyphenolics.

2. Materials and methods

2.1. Purification of skin and seed proanthocyanidins

Grape samples (*Vitis vinifera* cv. Pinot noir) were harvested from OSU experimental vineyard (Alpine, OR, USA) two weeks prior to véraison (mid-August) in 2006. Berries were collected at what Bogs et al. (2005), considered maximum proanthocyanidin containing stage: slightly larger than pea size, hard, and green with no pink colouration in any of the berries. The immature clusters were picked, placed in a cooler filled with ice, and transported to laboratory (within two hours of picking). Upon arrival at the laboratory the berries were immediately fractionated into skins and seeds. These samples were used for fractionation, extraction, and purification of skin and seed proanthocyanidins; the details were as described in Kennedy and Jones (2001). Resulting purified freeze-dried skin and seed proanthocyanidins were stored at −80 °C for further experiments.

2.2. Reagents, chemicals, standards, and gases

All chemicals (ammonium formate, formic acid, ethanol, catechin, catechol, phloroglucinol, acetic acid, acetonitrile, etc.) used in this study were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and chemicals for this investigation were analytical and high performance liquid chromatography (HPLC) grade. Oxygen (extra dry) and nitrogen (ultra high purity) gases were purchased from Norco Inc. (Nampa, ID, USA).

2.3. Proanthocyanidin oxidation system and sample preparation

All monomers and polymers were dissolved in model wine solutions (total 25 ml) consisting of the following: 20 mM ammonium formate, 14% ethanol (v/v), adjusted to pH 3.5 with formic acid. Model solutions of skin proanthocyanidins were dissolved at a concentration of 2 g/l, without and with catechol (two equal molar). Seed proanthocyanidin and catechin concentrations were determined based on the catechol amount required for skin proanthocyanidin, so the two equal molar ratio remained the same. A microscale photochemical reactor assembly with quartz well (Ace Glass Inc., Vineland, NJ, USA) was used to carry out the controlled oxidation experiment, as originally designed by Penn and Orr (1989). A PS-1 model UV lamp (5.5 W, 5.4 cm, 115 V/60 Hz, lamp current 18 mA, Ultra-Violet Products, Ltd., Upland, CA, USA) was placed in the reactor. Oxygen gas was continuously bubbled through a gas-washing bottle, fitted with a gas diffusion tube (both obtained from Ace Glass Inc.) containing the model wine solution (made as described previously), into the reaction mixture. Reaction mixtures were maintained at 20 °C by a circulating water bath (Isotemp 3013, Fisher Scientific Inc., Pittsburgh, PA, USA), pumping chilled water through double walled assembly for the duration of each reaction.

The different phenolic solutions (six total combinations; catechin, catechin + catechol, skin proanthocyanidin, skin proanthocyanidin + catechol, seed proanthocyanidin, and seed proanthocyanidin + catechol) were oxidised in the photochemical reaction assembly under continuous oxygenation and UV light as described in the previous section. Samples for oxidation measurements were taken during each treatment at eight intervals over the 15,360 s period (samples taken at time = 0, 240, 480, 960, 1920, 3840, 7680, and 15,360 s). Two aliquots from each time point sample were immediately dried under a gentle nitrogen stream with a N-evap 112 nitrogen evaporator (Organomation Associates Inc., Berlin, MA, USA) with the water bath temperature set to 40 °C. One dried sample from each time point later underwent phloroglucinolysis (as described), while the other was retained for monomer corrections. These steps did not deviate from the previously described methods (Kennedy & Jones, 2001; Lee et al., 2008).

2.4. Absorbance measurements, phloroglucinolysis, and HPLC analysis

Absorbances at 280, 420, and 520 nm were conducted using a SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). Absorbances were auto-corrected for a one-cm pathlength cell value.

Purified skin and seed proanthocyanidins, along with the oxidisation reaction samples were subjected to phloroglucinolysis (acid-catalysis in the presence of excess phloroglucinol), prior to HPLC injection (Kennedy & Jones, 2001). A HP1100 system equipped with a DAD was used to analyse the samples (Agilent Technologies Inc., Palo Alto, CA, USA). Analysis was performed as previously described by Kennedy and Jones (2001) with the exception of the analytical column employed (two sequentially connected Onyx Monolithic C_{18} columns; Phenomenex Inc., Torrance, CA, USA); the identical procedure with alterations can be found in Lee et al. (2008). Peaks were monitored at 280, 320, and 520 nm. Injection volume was 20 μl. All peaks were identified based on retention time, spectra, and known previous identifications (Jorgensen et al., 2004; Lee et al., 2008). All values were expressed as catechin. Mean degrees of polymerization (mDP), yield conversion, % molar proportion, and galloylation rate calculations have been well documented in previously published work (Kennedy & Jones, 2001; Kennedy & Taylor, 2003; Lee et al., 2008).

3. Results and discussion

3.1. Purified skin and seed proanthocyanidin: starting material composition

Composition summary of the purified proanthocyanidin fractions are listed in Table 1. Freeze-dried skin proanthocyanidins had an estimated total molecular weight of 5797 g/mol, an estimated average subunit molecular weight of 295 g/mol, 19.6 mDP, and a 95.6% conversion yield. Freeze-dried seed proanthocyanidins had an estimated total molecular weight of 3247 g/mol, an estimated average subunit molecular weight of 309 g/mol, 10.5 mDP, and a 87.0% conversion yield. Skin proanthocyanidins were higher in molecular weight, mDP, and conversion yield compared to the purified seed proanthocyanidins, as noted in previous studies (Jorgensen et al., 2004; Kennedy & Taylor, 2003). Phloroglucinolysis of the skin proanthocyanidins yielded (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG) extension subunits, and C terminal subunits. Phloroglucinolysis of the seed proanthocyanidins yielded C, EC, ECG as extension subunits and C, EC, and ECG as terminal subunits. Based on molar proportion, the main extension subunits in skin proanthocyanidins were EC (62.0%) and EGC (28.6%). The main extension subunits in seed proanthocyanidins were EC (71.4%) and ECG (11.1%), again based on % molar proportion. The composition of the 'Pinot noir' skin and seed proanthocyanidins did not diverge from previous reports (Jorgensen et al., 2004; Kennedy & Taylor, 2003).

3.2. Spectrophotometric measurements

Colour density ($A_{420} + A_{520}$) of all solution mixtures increased with time (Fig. 1). Skin proanthocyanidin + catechol mixture exhib-