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Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium

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ABSTRACT

A study of proanthocyanidin and anthocyanin composition and concentrations in seed and skin extracts of two Croatian native red grape cultivars (Plavac mali and Babić) by HPLC–UV-Fluo/MS analysis was conducted in this work. A sensory analysis of extracts astringency and bitterness intensity was also performed. In the seeds, Babić showed generally higher concentrations of proanthocyanidins, while in the skins, Plavac mali showed higher concentrations of proanthocyanidins and anthocyanins. Babić proanthocyanidin seed fractions, greater in polymer size and percentage of galloylation, were perceived to be significantly more astringent. Babić proanthocyanidin skin fractions, greater in polymer size and lower in percentage of galloylation and prodelphinidins, were perceived to be significantly bitterer, but only in polymeric fractions. A positive correlation was found between the degree of polymerization, the percentage of galloylation and astringency intensity in the seeds. A negative correlation was found between the percentage of prodelphinidins and bitterness intensity in the skins.

1. Introduction

Proanthocyanidins (tannins) and anthocyanins are important polyphenolic constituents of red grapes that are extracted into must during wine-making and contribute to the chemical and sensory properties of wine (Singleton & Noble, 1976). Proanthocyanidins are polymers consisting of flavan-3-ol sub-units primarily responsible for astringent and bitter characteristics of wine. In grape berry, they are located in seeds and skins, but their content and structure differs according to the location of tissues. Seed proanthocyanidins contain only (epi)catechin sub-units forming procyanidins (Prieur, Rigaud, Cheynier, & Moutounet, 1994), while skin proanthocyanidins also include (epi)gallocatechin sub-units, forming prodelphinidins as well (Souquet, Cheynier, Brossaud, & Moutounet, 1996). Furthermore, skins proanthocyanidins are more highly polymerized and contain a lower proportion of galloylated sub-units (Prieur et al., 1994; Souquet et al., 1996). Anthocyanins are the main pigments of red grapes located in skins and primarily responsible for the colour of red wine.

Astringency is a tactile sensation described as drying, roughing or puckering mouth-feel that results from the interaction of proanthocyanidins with salivary proteins, causing a loss in the lubrication power of saliva (Gawel, 1998). Bitterness is a taste perceived by taste receptors on the tongue. Sensory perceptions of astringency and bitterness intensity are greatly affected by proanthocyanidin composition. A positive relationship was found between astringency intensity and proanthocyanidins concentration (Arnold & Noble, 1978; Robichaud & Noble, 1990). Furthermore, polymer size was found to be the most discriminatory structural variable affecting astringency intensity, and also positively correlated with astringency perception (Arnold, Noble, & Singleton, 1980; Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003). Increased galloylation could be responsible for increased "coarseness", while trihydroxylation of B-ring could decrease "coarseness" (Vidal et al., 2003). Flavan-3-ol monomers were found to be more bitter than astringent (Arnold et al., 1980; Peleg et al., 1999), where epicatechin showed greater maximum intensity and persistence of bitterness and astringency than catechin.

Plavac mali and Babić (*Vitis vinifera* L.) are Croatian red native grapevine cultivars, both originating from Dalmatia vine-growing region. Recently, it has been shown that Plavac mali is a progeny of Zinfandel (syn. Primitivo) and Dobričić (Maletić et al., 2004); and also, the possibility of a parent-offspring relationship between Babić and Dobričić has been proposed (Zdunić et al., 2008).

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Polyphenolic characterization based on anthocyanins and proanthocyanidins determination was conducted in various grape cultivars due to the overall importance of these compounds to wine quality. However, only few authors studied the flavonoids composition of Plavac mali and Babić grapes (Budić-Leto, Vrhovšek, Gajdoš Kljusurić, & Lovrić, 2009; Katalinić et al., 2010) and none of these studies provided information of grape proanthocyanidin structural composition and their sensory properties. The aim of this work was to study the proanthocyanidin and anthocyanin composition of Plavac mali and Babić grape seed and skin extracts, as well as their sensory perception of astringency and bitterness intensity in a wine model medium in order to investigate their polyphenolic varietal differences.

2. Materials and methods

2.1. Chemicals

Deionized water was purified with the Milli-Q water system (Millipore Corp., Bedford, MA, USA). The acetone, methanol, ethanol, chloroform, ethyl acetate and acetonitrile were HPLC grade and were purchased from Fisher Chemical (Loughborough, Leicestershire, UK). The Folin Ciocalteu's reagent, gallic acid, sodium carbonate, sodium bisulphite, sodium acetate, phloroglucinol, L-ascorbic acid, hydrochloric acid, formic acid, acetic acid, (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG), B1 [(−)-epicatechin-(4β-8)-(+)-catechin], B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin] were purchased from Sigma Aldrich (Saint Louis, USA). The malvidin-3-*O*-glucoside-chloride (Mv) was purchased from Extrasynthese (Genay, France). B3 [(+)-catechin-(4α-8)-(+)-catechin], B4 [(+)-catechin-(4α-8)-(−)-epicatechin], C1 [(-)-epicatechin-(4α-8)-(-)-epicatechin-(4α-8)-(-)-epicatechin] and B2G [(−)-epicatechin-(4β-8)-(−)-epicatechin-3-*O*-gallate] were synthesised by the Laboratory of Organic Chemistry and Organometallic, Université Bordeaux 1 (Tarascou et al., 2006).

2.2. Grape samples

The research was conducted on two Croatian native red grape cultivars, grown in Dalmatia (Croatia southern vine-growing region). The grape samples of Plavac mali were collected from the Central and Southern Dalmatia sub-region, while the samples of Babić were collected from the Northern Dalmatia sub-region. Both sub-region are classified in C_3 viticultural climatic zone (Winkler, Cook, Kliewer, & Lider, 1974), with yearly insolation up to 2700 h for the first and around 2500 for the latter, and annual precipitations of around 730–1050 mm in both sub-regions. The Plavac mali sampling area included the slopped vineyards of Dingač (up to 250 m above sea level) on the Pelješac peninsula (V_1), Bol (up to 50 m above sea level) on the island of Brač (V_2) and the vineyards of Kaštela (14 m above sea level) (V_3). Babić sampling included the Primošten vineyards of Bucavac (up to 40 m above sea level) (V_4), Šljinovac (100 m above sea level) (V_5) and Plošnjak (299 m above sea level) (V_6). The grapes were harvested in their technological maturity in October 2010, since Plavac mali and Babić are late-ripening cultivars, due to their long vegetative cycle. The physicochemical characteristics at technological maturity amounted: reducing sugars 232.3 ± 5.7 g/l in Plavac mali and 235.2 ± 3.8 g/l in Babić; total acidity 5.5 ± 0.1 g/l and 5.5 ± 0.2 g/l in Plavac mali and Babić respectively, with pH = 3.4 ± 0.1 in the first and 3.6 ± 0.1 in the latter. The quantity of 4 kg of randomly selected grapes was used for the study. The seeds and skins were immediately manually separated from the pulp; freeze-dried and stored at −20 °C before the subsequent analysis.

2.3. Extracts preparation

2.3.1. Extraction of proanthocyanidins

A portion containing 5 g of lyophilized seeds and skins powder was extracted using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor with automatic pressure control at 25 °C. Extraction was performed by repeating eight extraction cycles with acetone/water (80:20, v/v) and four cycles with methanol/water (60:40, v/v) (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2008). Organic solvents were further evaporated under reduced pressure at 30 °C, and the residue was dissolved in water and freeze-dried in order to obtain a crude seed and skin tannin extract. The procedure was conducted in triplicate.

2.3.2. Extraction of anthocyanins

A portion containing 1 g of lyophilized skins powder was extracted using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor under the same conditions as previously mentioned. Extraction was performed by repeating six extraction cycles with acidified methanol (0.1% HCl) (Lorrain, Chira, & Teissedre, 2011). The solvent was evaporated under reduced pressure at 30 °C, and the residue was dissolved in water and freeze-dried in order to obtain an anthocyanins extract. The procedure was conducted in triplicate.

2.4. Spectrophotometric polyphenolic characterization

Seed and skin crude tannin extracts were dissolved at the concentrations of 2 g/l and 6 g/l in wine model solution (12% ethanol, 4.5 g/l tartaric acid, pH = 3.5) in order to determine the total polyphenolic content (TPC) by the Folin Ciocalteu method (Singleton & Rossi, 1965). The quantity of 0.25 g/l for seeds and 1 g/l for skin crude tannin extracts was prepared in order to determine the total tannin content (TT) by acidic hydrolysis (Ribéreau-Gayon & Stonestreet, 1966). Anthocyanins skin extracts were dissolved at the concentrations of 10 g/l and total anthocyanins were determined using the bisulphite bleaching method (Ribéreau-Gayon & Stonestreet, 1965).

2.5. Proanthocyanidins characterization

2.5.1. Fractionation of proanthocyanidins

The crude tannin extract was quantitatively dissolved in 250 ml of water/ethanol (95:5, v/v). In order to purify the extracts, liquid–liquid extraction with 250 ml of chloroform was repeated three times. Further, to separate low from high molecular weight tannins, liquid–liquid extraction with 250 ml of ethyl acetate was repeated three times. Both fractions, the ethyl acetate fraction of monomeric/oligomeric procyanidins (F I.) and the aqueous fraction of polymeric proanthocyanidins (F II.) were evaporated under reduced pressure at 30 °C, freeze-dried and analysed by HPLC-UV-Fluo/MS.

2.5.2. HPLC-UV-Fluo/MS analysis of monomeric and oligomeric flavan-3-ols

Seeds and skins monomeric/oligomeric procyanidins fractions were dissolved in methanol/water (50:50, v/v) and filtered through a 0.45 μm nylon filter prior to injection. The concentrations injected were 1 g/l and 6 g/l, for seeds and skins, respectively. HPLC analysis was performed on a Thermo-Finnigan Surveyor Plus HPLC system equipped with an autosampler (Autosampler Plus), a ternary pump (MS Pump Plus), a diode array detector (PDA Plus Detector) coupled with a Finnigan Xcalibur data system and a fluorescence detector (FL Plus Detector) coupled to a ChromQuest data system. Separation was performed on a reversed phase Agilent Nucleosil C18 (250 mm × 4 mm, 5 μm). Water/formic acid (solvent