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Transfer of tannin characteristics from grape skins or seeds to wine-like solutions and their impact on potential astringency

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ABSTRACT

A simulated maceration was carried out with skins or seeds of Aglianico, Merlot and Cabernet Sauvignon grape cultivars in order to study the entity of the transfer of tannins from grape to wine. The structural characteristics of skins and seeds extracts (native tannins) and corresponding wine-like solutions (macerated tannins) were analysed by phloroglucinolysis. Their potential astringency was evaluated according to the reactivity towards salivary proteins by the Saliva Precipitation Index (SPI). From grape to wine-like solution, the transfer of structural characteristics varied differently for skins and seeds. A significant influence of extraction method and grape variety was also stated, principally as regard the percentage of prodelphinidins (%P) for skins, and of galloylation (%G) for seeds. For some parameters that were able to differentiate grapes through the maceration process, a cultivar effect emerged. Reactivity of tannins also differed, but not for seeds. For Aglianico native and macerated tannins, the highest in SPIs, seeds monomers and oligomers were quantified by HPLC/MS. Principal Component Analysis (PCA) allowed to reveal the structural characteristics mainly associated with the reactivity of tannins towards salivary proteins. The proanthocyanidins content and the %G increased potential astringency, while it was reduced by the %P.

1. Introduction

Monomeric, oligomeric and polymeric flavan-3-ols (condensed tannins or proanthocyanidins, PAs) play a relevant role in the sensory characteristics of red wines, because they contribute to wine bitterness and astringency (Cheynier et al., 2006; Vidal et al., 2003). They are located in grape skins and seeds and are extracted during the fermentative maceration process in red winemaking. As anthocyanins that are situated in the vacuoles of skin cells, skin PAs can rapidly diffuse into the must-wine when cell walls are broken (Kennedy, 2008). As regard the extraction of PAs from seeds, it is slower and needs of increasing ethanol concentrations in order to extract the monomeric flavan-3ols found in the outer seed coat and endosperm, and the PAs localized in the brown hull (Thorngate & Singleton, 1994). Despite seeds generally presented a high content of flavanols respect to skins, skins are believed to mainly contribute to the flavanol composition of wine because of the rapid extraction (Busse-Valverde et al., 2010; Peyrot des Gachons &

Abbreviations: B1, (−)-epicatechin-(4β-8)-(+)-catechin; B2, (−)-epicatechin-(4β-8)-(−)-epicatechin; B3, (+)-catechin-(4α-8)-(+)-catechin; B4, (+)-catechin-(4α-8)-(−)-epicatechin; C, (+)-catechin; C1, (−)-epicatechin-(4β-8)-(−)-epicatechin-(4β-8)-(−)-epicatechin; DOCG, Denominazione di Origine Controllata e Garantita; EC, (−)-epicatechin; eC, (+)-catechin in extension units; ECG, (−)-epicatechin-3-O-gallate; eEC, (−)-epicatechin in extension units; eECG, (−)-epicatechin-3-O-gallate in extension units; eEGC, (−)-epigallocatechin in extension unit; EGC, (−)-epigallocatechin; GA, gallic acid; GAE, gallic acid equivalent; mDP, mean degree of polymerization; MSd, macerated seeds; MSk, macerated skins; NSd, native seeds; NSk, native skins; PAs, proanthocyanidins; SDS-PAGE, sodium dodecyl sulfate gel electrophoresis; SPI, Saliva Precipitation Index; tC, (+)-catechin in terminal units; tEC, (−)-epicatechin in terminal units; tECG, (−)-epicatechin-3-O-gallate in terminal units; tEGC, (−)-epigallocatechin in terminal units; %G, percentage of galloylation; %P, percentage of prodelphinidin.

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Kennedy, 2003). However, other studies supported the opposite (Kovac, Alonso, Bourzeix, & Revilla, 1992; Nicolini, Mattivi, & Malossini, 1998). Nevertheless, the PAs extraction is influenced by many factors such as temperature, contact time (Pinelo, Arnous, & Meyer, 2006), their concentration, the type of PAs (Hanlin, Hrmova, Harbertson, & Downey, 2010), and the adsorption affinity for cell wall material, either in situ within the skin cells or by contact with suspended flesh material (Bindon, Smith, & Kennedy, 2010).

Different availability for extraction into wine but also different flavanol composition characterizes grape tissues. The analytical method using acid depolymerization with nucleophilic agent greatly contributed to the determination of the subunit composition and mean degree of polymerization (mDP) of proanthocyanidins and to the evaluation of the extent of the PAs extraction during maceration (Kennedy & Jones, 2001; Peyrot des Gachons & Kennedy, 2003). Skins PAs differed from seeds for the presence of prodelphinidins, higher mDP, and lower proportion of galloylated subunits (Vidal et al., 2003). The difference in structural characteristics between skins and seeds is associated with different sensory perceptions, mainly astringency. This tactile sensation derives from the formation of a protein-tannin complex which precipitates and causes the shrinking, drawing and puckering of the epithelium (ASTM, 2004). According to the wheel of taste (Gawel, Oberholster, & Francis, 2000), astringency can be defined by 33 different sub-qualities. In particular, for the increasing chain length of the tannin molecule, the astringency attributes “drying”, “chalky”, “adhesive”, and “pucker” were mainly correlated with skins tannins. Seeds tannins were found to be coarser than skins, but were also characterized by terms as “drying” and “chalkiness” due to the correlation with a higher degree of galloylation (Vidal et al., 2003).

The propensity of tannins to complex with macromolecules, such as salivary proteins, is due to the presence on the molecule of both hydrophobic aromatic rings and hydrophilic hydroxyl groups which allow them to bind simultaneously to acceptor sites of peptide bonds (Murray, Williamson, Lilley, & Haslam, 1994). The aggregation of polyphenols with salivary proteins seems to be firstly mediated by hydrophobic forces; successively, hydrogen bonding has been postulated to provide strong and directional bonding that stabilizes the complex. The stability of these complexes depends on tannin concentration, dimension and on the number of free phenolic groups (Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007). The binding between salivary proteins and tannins and the precipitation of the formed complex depend also on the structural characteristics of PAs (Haslam, 1974). The mechanism of proteins/tannins binding and precipitation is at the basis of the Saliva Precipitation Index (SPI). Briefly, the SPI consists of a binding reaction between wine or grape solution with human saliva at 37 °C for 5 min, and allows estimation of the percentage of proteins that have been precipitated by tannins. Since a good correlation ($R = 0.97$) was found between SPI and red wine astringency, this index represents an indirect measure of astringency (Rinaldi, Gambuti, & Moio, 2012a).

The aim of this work is the evaluation of the extent of the tannin transfer from solid parts of grape (skins and seeds) into wine-like solutions as regard: i) structural characteristics of PAs by phloroglucinolysis; and ii) potential astringency as reactivity of PAs towards salivary proteins by SPI. Native skins and seeds tannins were extracted from Aglianico, Merlot, and Cabernet Sauvignon grape cultivars (*Vitis vinifera* L. cv). A simulation of the maceration process has been carried out separately for skin and seed grape tissues to obtain macerated skins and seeds tannins. A comparison between native and macerated tannins was made to study grape contribution to the composition and potential astringency of wine-like solutions.

2. Material and methods

2.1. Chemicals

Phloroglucinol, (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-*O*-gallate (ECG), procyanidin B1 [(−)-epicatechin-(4β-8)-(+)-catechin], procyanidin B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin], and gallic acid (GA) were supplied from Sigma–Aldrich (Saint Quentin Fallavier, France). All solvents (HPLC grade) were purchased from Prolabo-VWR (Fontenays sous Bois, France). Procyanidin B3 [(+)-catechin-(4α-8)-(+)-catechin] and procyanidin B4 [(+)-catechin-(4α-8)-(−)-epicatechin] and trimer (C1) [(−)-epicatechin-(4β-8)-(−)-epicatechin-(4β-8)-(−)-epicatechin] were obtained from Polyphenols Biotech (Villenave d'Ornon, France).

2.2. Native and macerated tannins

2.2.1. Grapes

V. vinifera L. cv. Aglianico, Merlot and Cabernet Sauvignon derived from the vineyards of “Cantine del Taburno” located in Taburno DOCG area (Foglianise, BN, Italy). The Merlot and Cabernet Sauvignon grapes were harvested in their technological maturity in September 2010, while Aglianico in October 2010.

Berry sampling was done choosing 10 vines per variety. Groups of five to six berries from different parts of the cluster and from different clusters on the same vine were sampled randomly. From a total of 3 Kg of berries, two subsamples (200 g) were prepared for duplicate analysis.

2.2.2. Skins and seeds native (NSk and NSd) tannins

Seeds and skins were removed by hand from grapes and separated, lyophilised for 2 days and stored at −20 °C. The frozen seeds or skins were finally ground in a ball grinder. Five grams of the obtained seed and skin powder was extracted with acetone/water (80:20, v/v) and with methanol/water (60:40, v/v) using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor with automatic pressure control at 25 °C, as described by Ćurko et al. (2014). All extracts were combined and evaporated under reduced pressure at 30 °C to remove organic solvents; the residue was dissolved in water and lyophilised to obtain native tannin extracts.

Native tannins samples were obtained from the dissolution of NSk and NSd tannins at a concentration of 1 g/L in model wine solution (ethanol 12%; pH = 3.2; tartaric acid = 5 g/L). The choice of this concentration was taken because the macerated tannins measured in the MSk and MSd by buthanol-chloride method (Bate-Smith, 1954) were statistically the same (mean values of 1.31 ± 0.14 g/L for skins, and 1.00 ± 0.18 g/L for seeds; p-value = 0.0793).

2.2.3. Skins and seeds macerated (MSk and MSd) tannins

The simulated maceration was obtained with a selective extraction method, specifically designed to mimic the winemaking process as described by Mattivi, Zulian, Nicolini, and Valenti (2002). A hydroalcoholic solution consisting of EtOH:H_2O (12:88 v/v), containing 100 mg/L of SO_2 and 5 g/L of tartaric acid, pH = 3.2, was used for the separate maceration of skins and seeds.

Two hundreds g of berries were randomly sampled, counted and weighed. The skins were manually separated from the seeds of each berry, while the pulp was discarded. Most of the pulp on the inner face of the berry skins was gently removed with the aid of an end-flattened spatula, trying to preserve the skin integrity. The skins were immediately immersed, one by one to avoid oxidations, in a 500 mL Erlenmeyer conical glass flask (narrow neck) containing