Tetrahedron 71 (2015) 2999–3006

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Compositional and sensory characterization of grape proanthocyanidins and oak wood ellagitannin

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ARTICLE INFO

*Article history:*
Received 9 December 2014
Accepted 4 February 2015
Available online 11 February 2015

*Keywords:*
Astringency
Proanthocyanidin
Ellagitannin
Purification
Grape
Wine

ABSTRACT

Aimed at increasing our knowledge on the astringency of sensory-active non-volatile compounds migrating principally from grapes and from oak wood into the wine, grape extracts and an aqueous ethanolic extract from oak wood chips were used for their key taste compounds. Monomeric/oligomeric and polymeric proanthocyanidin fraction of seed and skin extracts were obtained from grapes. Ellagitannins were extracted and purified from oak wood. Compositional characterization, purity and sensory evaluation of grape extracts were performed by liquid chromatography/mass spectrometry and sensory analysis, respectively. Purification of ellagitannin extract at 93.4% was realized by successive fractionations on Toyopearl TSK HW-40 (F) and on C-18 column. At the same concentration, ellagitannin fraction was perceived rather mellow, seed and skin monomeric/oligomeric fraction was characterized slight astringent, polymeric seed fraction was identified as tannic whereas polymeric skin fraction was appreciated rather mellow.



## 1. Introduction

'In-mouth' sensory properties of red wines, encompass multiple interacting sensations such as acidity, sweetness, bitterness, retronasal aroma perception (flavour), viscosity, warmth, and astringency. Among the sensations, astringency is probably the most enigmatic one: it is a tactile sensation with a trigeminal (irritation) component.[1] It has been described as an oral sensation, which causes the drying, roughing and puckering of the mouth epithelia and a complete terminology has been developed to describe this complex sensation in red wines.[2] The term astringent is derived from the latin for 'binding', and is associated with the ability of certain chemicals to bind and precipitate salivary mucoproteins that normally lubricate the tissues of the mouth.

The overwhelming majority of studies on astringency, support the notion that astringency is primarily a tactile sensation.[3] It is described as a tactile sensation for three reasons: first, the sensation can be produced on non-gustatory tissues such as the upper lip and gum;[3] second, certain lubricating rinses can offset the sensation of astringency;[3] and third, no sensory adaptation is observed for astringent stimuli.[4]

It has been classically postulated that astringency results from the cross-linking of polyphenols with glycoproteins[3] found between and above the epidermal cells of the mucosal tissue in the mouth,[1] and/or from the binding and subsequent precipitation of salivary proteins by polyphenols.[5] The polyphenol–protein interaction results in a saliva with poorer lubricating properties and greater friction between mouth surfaces. The increased friction ultimately activates the mechano-receptors in the mouth leading to the perception of astringency.[6]

However, more recently it has been evidenced that the quantity of the non-bound, 'free' astringent stimulus in the saliva liquid might be more closely related to the sensory perception of astringency than the amount complexed or precipitated by proteins.[7] It is therefore questionable as to whether oral perception of astringency is related to the complexation and/or precipitation of salivary proteins. Additionally, a recent research[8] suggest that changes in friction or lubricity are not a necessary condition for astringency. None of the astringent solutions produced a change in sensory friction, and only tannins produced a small increase in the instrumental friction of the saliva/astringent mixtures. Suggesting that an oral phenomenon other than a decrease in salivary lubricity is likely to cause astringency.

Astringency being one of the most important red wine attributes it is close related to its overall quality, high quality level wine has a balanced level of astringency. Astringency is more

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http://dx.doi.org/10.1016/j.tet.2015.02.018
0040-4020/

related to non-volatile wine compounds. Among non-volatile molecules, polyphenols, metal salts, acids, and dehydrating agents mainly engender astringency.[1] Wine phenolic compounds and especially tannins have been widely related to astringency perception.[9–13] Astringency, global intensity and persistence are positively correlated to the total polyphenol content, TPI.[14] Although the real existence of this relationship has been already confirmed by complete reconstitution experiments[15,16] the contribution of the different phenolic families to the sensory perception of astringency has not been well established. Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG), and (−)-epigallocatechin (EGC). Skin proanthocyanidins differ from those found in seeds, in that skin tannins include prodelphinidins (EGC), have a higher degree of polymerization and a lower proportion of galloylated subunits. Regarding grape proanthocyanidins, several variables, such as total concentration, mean polymerization degree (mDP),[11,12] their subunit composition and their distribution are highly correlated with astringency perception.[17] Monomers are more bitter than astringent, whereas the reverse is true for large molecular weight derivatives. Oral monomers astringency is significantly lower comparing to this of dimers or trimers, which did not differ significantly.[18] In skin extracts a positive correlation between B3 concentration and astringency intensity has been observed.[19]

With regard to the gustatory properties of the ellagitannins, only one research team estimated the thresholds of bitterness and astringency of oak wood ellagitanins.[20] Ellagitannins (hydrolyzable tannins), impart an oral sensation described as astringent at relatively low threshold concentrations spanning from 0.2 to 6.3 μmol/L by means of the half-mouth test in bottled water (pH 4.5). The test of half-tongue consists in placing a drop of the solution containing the studied compound on one side of the tongue whereas pure water is applied to the other side of the tongue like witness. Then, the judges must move their tongue in their palate during 15 s in order to identify if there is a difference in feeling between the two sides of the tongue. From an oenological point of view, one of the major limitations of the test half-tongue is the absence of contact between the ellagitannins and the entire oral cavity since astringency is a sensation that can be produced on non-gustatory tissues such as the upper lip and gum.[3] Moreover, they determined the recognition threshold concentrations of ellagitannins in bottled water (pH 4.5), conditions that are different to a wine media. Ellagitannin monomers, vescalagin and castalagin under these conditions exhibit very strong astringency and no bitterness. The dimers roburin A and D are less astringent than vescalagin but two times more bitter. Glycosidic monomers (grandinin and roburin E) are five times more astringent than vescalagin and three times more bitter. These observations confirm the need for evaluating ellagitannin astringency under conditions close to those of tasting wine.

Being aware of the importance of gaining knowledge about the real impact of proanthocyanidins and ellagitannins on the in-mouth sensory properties of wines in order to provide further insights into wine sensory perception; knowing that up to now, comprehensive investigations on the correlation of the composition of red grape polymers and oligomers as well as of oak wood ellagitannins with its sensory impact are rather fragmentary. The objectives of the present study were, therefore, (i) to fractionate the oligomers/polymers isolated from grape seeds and skins, (ii) to extract and purify ellagitannins from oak wood, (iii) to perform a compositional analysis on the oligomer/polymer fractions after hydrolytic depolymerization, (iv) to determinate total ellagitannin concentration and ellagitannin composition and finally (v) to investigate the impact of astringency by means of human sensory analysis.

## 2. Results

### 2.1. Proanthocyanidin composition

Ten oligomeric and polymeric proanthocyanidin fractions were obtained from grape seed and skin of Cabernet Sauvignon and Merlot variety. Acid-catalyzed depolymerization in the presence of phloroglucinol was performed in skins and seed tannin extracts in order to obtain information about the proanthocyanidin subunit composition. The percentage of galloylation (%*G*), the percentage of prodelphinidins (%*P*), as well as the mean degree of polymerization (mDP) of both seed and skin tannin extracts are presented in Table 1. Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin, (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate, and (−)-epigallocatechin. Skin proanthocyanidins differ from those found in seeds in that skin tannins include prodelphinidins and have a higher degree of polymerization and a lower proportion of galloylated subunits.[19,21,22] Indeed, independent variety, skin and seed proanthocyanidin profile differed by their low amounts of galloylated derivatives and higher mDP (Table 1). The percentage of galloylation (epicatechin gallate subunits) in the oligomeric seed fraction of both varieties is higher than in the polymeric. The mean value of prodelphinidins percentage ((−)-epigallocatechin subunits) was greater in the poymeric skin fractions of M variety, in CS variety the differences between these two fractions were less important.

**Table 1**
Structural characteristics and composition of seed and skin tannin extract

| | CS | mDP | | | | %*G* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD |
| Seed extracts* | V1 CS | 3.8 | 0.2 | 11.7 | 0.0 | 50.1 | 2.3 | 4.4 | 0.0 |
| | V2 CS | 3.9 | 0.1 | 18.1 | 0.1 | 46.5 | 1.7 | 13.2 | 0.1 |
| | V3 CS | 7.2 | 0.2 | 8.8 | 0.1 | 51.3 | 4.9 | 11.6 | 0.2 |
| | V4 CS | 8.8 | 0.3 | 19.3 | 0.7 | 11.6 | 2.5 | 11.5 | 0.3 |
| | V5 CS | 4.1 | 0.4 | 15.9 | 1.4 | 16.1 | 5.9 | 4.0 | 0.6 |
| | Mean value | *5.6* | *0.2* | *14.7* | *0.5* | *35.1* | *3.4* | *8.9* | *0.2* |

| | mDP | | | | | %*G* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD |
| Seed extracts* | V1 M | 3.5 | 0.0 | 9.0 | 0.3 | 48.7 | 2.1 | 1.3 | 0.0 |
| | V2 M | 2.8 | 0.1 | 6.1 | 0.0 | 34.2 | 1.5 | 1.6 | 0.0 |
| | V3 M | 3.3 | 0.2 | 10.9 | 0.2 | 46.5 | 3.1 | 5.9 | 0.4 |
| | V4 M | 2.3 | 0.3 | 12.9 | 0.5 | 17 | 1.2 | 5.4 | 0.3 |
| | V5 M | 2.0 | 0.4 | 9.6 | 0.9 | 20.3 | 3.1 | 10.9 | 2.2 |
| | Mean value | *2.8* | *0.2* | *9.7* | *0.4* | *33.3* | *2.2* | *5.0* | *0.6* |

| | CS | mDP | | | | %*G* | | | | %*P* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD |
| Skin extracts* | V1 CS | 29.4 | 2.1 | 43.1 | 2.3 | 2.2 | 0.0 | 1.5 | 0.0 | 18.2 | 1.5 | 21.5 | 1.3 |
| | V2 CS | 15.7 | 1.4 | 27.2 | 1.5 | 2.5 | 0.1 | 1.5 | 0.1 | 19.4 | 1.1 | 19.4 | 1.1 |
| | V3 CS | 22.8 | 1.9 | 48.8 | 3.7 | 9.4 | 0.2 | 8.4 | 0.6 | 4.3 | 0.6 | 8.2 | 0.6 |
| | V4 CS | 7.8 | 0.9 | 57.7 | 2.0 | 4.2 | 0.7 | 9.0 | 0.5 | 11.9 | 0.5 | 4.3 | 0.5 |
| | V5 CS | 31.9 | 2.9 | 50.5 | 3.9 | 3.5 | 0.4 | 3.6 | 0.3 | 2.5 | 0.2 | 16.5 | 1.0 |
| | Mean value | *21.5* | *2.2* | *45.5* | *2.3* | *4.4* | *0.3* | *4.8* | *0.3* | *11.3* | *0.8* | *14.0* | *0.9* |

| | mDP | | | | | %*G* | | | | %*P* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD | *F*1 | SD | *F*2 | SD |
| Skin extracts* | V1 M | 4.3 | 0.2 | 15.4 | 0.2 | 1.3 | 0.1 | 0.7 | 0.1 | 4.0 | 1.1 | 36.0 | 2.1 |
| | V2 M | 13.1 | 0.9 | 17.0 | 0.9 | 1.4 | 0.0 | 2.0 | 0.0 | 7.8 | 1.0 | 27.5 | 1.3 |
| | V3 M | 22.4 | 0.8 | 23.0 | 0.8 | 1.8 | 0.2 | 1.4 | 0.2 | 2.9 | 0.2 | 5.1 | 0.7 |
| | V4 M | 24.2 | 1.0 | 26.7 | 1.0 | 2.3 | 0.3 | 1.8 | 0.2 | 2.7 | 0.1 | 12.5 | 0.9 |
| | V5 M | 11.7 | 0.7 | 20.8 | 0.7 | 0.2 | 0.0 | 0.5 | 0.0 | 0.6 | 0.0 | 20.8 | 1.1 |
| | Mean value | *15.1* | *0.7* | *20.6* | *0.7* | *1.4* | *0.1* | *1.3* | *0.1* | *3.6* | *0.5* | *20.4* | *1.2* |

V, Vineyard; CS, Cabernet Sauvignon; M, Merlot; mDP, mean degree of polymerization; %*G*, percentage of galloylation; %*P*, percentage of prodelphinidins; *F*1, oligomeric fraction; *F*2, polymeric fraction; SD, standard deviation.
*ANOVA to compare data, values with different letters within each row are significantly different (Tukey's test, $p<0.05$).