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The role of wine polysaccharides on salivary protein-tannin interaction: A molecular approach

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ABSTRACT

Polysaccharides are described to inhibit aggregation between food polyphenols and salivary proteins (SP) and may hence lead to astringency modulation. In this work, the effect of two wine polysaccharides (arabinogalactan proteins-AGPs and rhamnogalacturonan II- RGII) on SP-polyphenol interaction was evaluated.

In general, both polysaccharides were effective to inhibit or reduce SP-polyphenol interaction and aggregation. They can act by two different mechanisms (ternary or competitive) depending on the SP-tannin pair. In the case of salivary P-B peptide, AGPs and RGII seem to act by a ternary mechanism, in which they surround this complex, enhancing its solubility. Concerning acidic proline-rich proteins (aPRPs), it was possible to observe both mechanisms, depending on the tannin and the polysaccharide involved.

Overall, this work point out for a specific property of wine polysaccharides important to modulate this and other beverages and food astringency perception.

1. Introduction

Astringency is usually defined as the array of tactile sensations felt in the mouth including shrinking, puckering and tightening of the oral surface (ASTM, 1989). Although the physicochemical mechanism of astringency is not completely understood, it is generally accepted that it is directly correlated with the capacity of food tannins to interact with salivary proteins (SP), resulting in the formation of protein-tannin aggregates in the mouth (de Freitas & Mateus, 2012).

Tannins are divided into two classes according to their structure: condensed tannins (also known as proanthocyanidins) which are polymers of catechins, and hydrolyzable tannins which are monosaccharide esters of glucose connected to at least one gallic (gallotannins) or ellagic (ellagic tannins) acid (de Freitas & Mateus, 2012).

Over the years, condensed tannins have been the tannins mostly associated with astringency. Although some human sensory experiments revealed that ellagitannins can have an impact on astringency, there is not much information about the interaction of hydrolyzable tannins and SP (Glabasnia & Hofmann, 2006; Lipinska, Klewicka, & Sojka, 2014).

The major SP families include proline-rich proteins (PRPs), histatins, statherin, cystatins and P-B peptide (Messana, Inzitari, Fanali, Cabras, & Castagnola, 2008). PRPs are one of the most studied families due to their high ability to interact with tannins (Pascal, Poncet-Legrand, Cabane, & Vernhet, 2008). They are characterized by a high content in proline residues (25–42%) and are usually divided in three classes: basic PRPs (bPRPs) have mainly basic residues, glycosylated PRPs (gPRPs) are bPRPs with carbohydrates in their structure, and acidic PRPs (aPRPs) that are rich in aspartic and glutamic acid residues (Manconi et al., 2016). Despite of its high amount in proline residues, salivary P-B peptide is not usually included into the PRP family, due to its similarities with statherin (Inzitari et al., 2006). This peptide has been recently described to strongly interact with tannins (Soares et al., 2016).

It has been known that astringency is affected by several factors. Polysaccharides have been shown to inhibit tannin-protein aggregation, and may hence lead to astringency modulation (de Freitas, Carvalho, & Mateus, 2003; Soares, Mateus, & de Freitas, 2012a; Watrelot, Schulz, & Kennedy, 2017). Two mechanisms were proposed to explain this inhibitory effect: (I) competition between polysaccharides

Abbreviations: AS, acidic saliva; AGPs, arabinogalactan proteins; aPRPs, acidic proline-rich proteins; bPRPs, basic proline-rich proteins; GC, gas chromatography; gPRPs, glycosylated proline-rich proteins; HPLC, high performance liquid chromatography; PRPs, proline-rich proteins; PNG, punicalagin; RG II, rhamnogalacturonan II; SP, salivary proteins; STD-NMR, saturation-transfer difference nuclear magnetic resonance; TMS, trimethylsilyl

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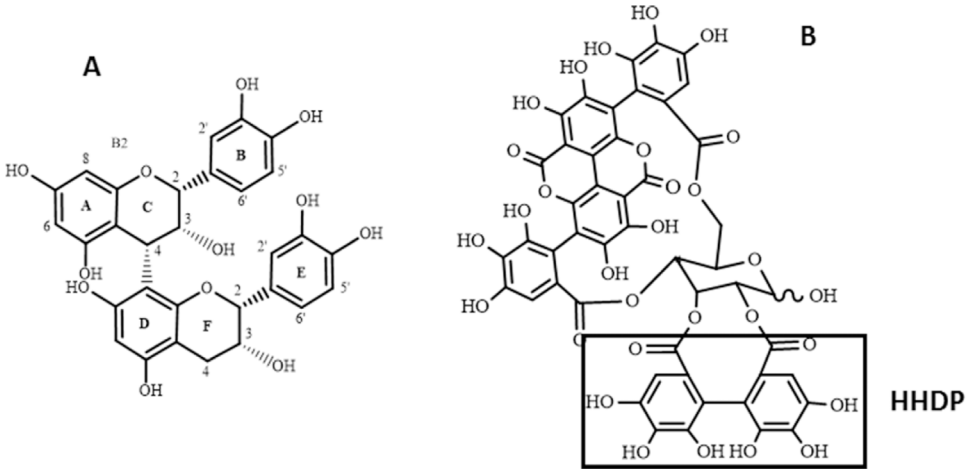

Fig. 1. Structures of procyanidin B2 (A) and PNG (B) with the evidence of the hexahydroxydiphenoyl (HHDP) moiety of PNG.

and SP toward tannins; or, (II) formation of a ternary complex protein – polyphenol – polysaccharides, with enhanced solubility in an aqueous medium (Mateus, Carvalho, Luis, & de Freitas, 2004). Polysaccharides are present in red wine being the major high-molecular weight components of wines. They have been associated to the mouth-feel perception, modifying the sensory properties of wine (Riou, Vernhet, Doco, & Moutounet, 2002; Vidal, Francis et al., 2014). The major wine polysaccharides are rhamnogalacturonan type II (RGII) and Polysaccharides Rich in Arabinose and Galactose (PRAGs) consisting of arabinogalactan-proteins (AGPs) that derive from grape cell walls, and mannoproteins from yeast cell walls (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003).

In this study, the effect of two wine polysaccharides (AGPs and RGII) on the interaction between hydrolyzable and condensed tannins toward two SP families (aPRPs and P-B peptide) was investigated. Punicalagin (PNG) and procyanidin dimer B2 (Fig. 1) were chosen as model tannins, since the first one is commonly used as a hydrolyzable tannin model and is easily found in pomegranate (Lu, Ding, & Yuan, 2008), while the second one is one of the most abundant procyanidin dimers in red wine (de Freitas & Mateus, 2012). On the other hand, aPRPs and P-B peptide have been previously reported as the SP that most interact with tannins both *in vitro* and *in vivo* studies (Brandao, Soares, Mateus, & de Freitas, 2014b; Soares, Mateus, & de Freitas, 2012b).

With this purpose, High Performance Liquid Chromatography (HPLC) analysis and nephelometry measurements were used as the main techniques, complemented with Saturation-Transfer Difference (STD)-NMR.

The aim of this work was to go deeper on understanding the influence of polysaccharides naturally present in wine on the interaction between SP and tannins. This could be an important information to winemaking industry which could use these polysaccharides to modulate astringency.

2. Material and methods

2.1. Isolation and characterization of SP

A pool of saliva was collected from several healthy volunteers and treated as previously reported (Brandao, Soares, Mateus, & de Freitas, 2014a). The different SP families were then isolated by semi-preparative HPLC (details in Supplementary material). This study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of Medical School of University of Porto (EK84032011).

2.2. Isolation of procyanidin dimer B2 and punicalagin

Procyanidin dimer B2 was isolated from grape seed fractions (*Vitis vinifera*), while PNG was isolated from pomegranate (details in Supplementary material).

2.3. Isolation and characterization of polysaccharide fractions

2.3.1. Isolation of RGII and AGPs

RGII fraction was isolated from wine, following the procedure reported elsewhere (Pellerin et al., 1997). Briefly, wine samples were dealcoholized and fractionated by adsorption chromatography (polystyrene/divinyl benzene resin), obtaining two different fractions. The first fraction (A) was not retained on the column and was eluted with water, while the second one (B) was eluted with 20% ethanol. Then, size exclusion chromatography was conducted to purify fraction B. Elution was performed on a Superdex-75 HR column (60 × 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 × 4.0 cm) (equilibrated at 1 mL min^{-1} with 30 mM ammonium formate, pH = 5.8), an Intelligent pump 301 (FLOM, France) and a rheodyne injector with a 2 mL loop. Elution of complex polysaccharides were followed using a refractive index detector (RI 101) (Shodex Showa Denko, Japan). RGII fraction was eluted between 65 and 75 min, and then was freeze-dried, redissolved in water and freeze dried again for three times to remove the ammonium salt.

AGPs fraction was isolated from wine sample fractions after anion-exchange and/or size-exclusion chromatographic steps. A final fraction containing a mixture of AGPs and mannoproteins was purified by affinity chromatography, using mannoproteins' ability to bind to Concanavalin A lectin (Vidal, Williams et al., 2003). This mixture was loaded on a Concanavalin A-Sepharose (Pharmacia XH 26/40) column equilibrated with 50 mM sodium acetate buffer pH 5.6 containing 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $MnCl_2$. AGPs were the first fraction to be eluted with the same buffer. The desorption of the mannoproteins (second fraction) was carried out using two bed volumes of the previous buffer containing 100 mM of methyl-α-D-mannopyrannoside. AGPs fraction was dialyzed extensively against water to remove all salts and freeze-dried.

Both RGII and AGPs fractions were then analyzed in terms of their neutral and acidic sugar composition.

2.3.2. Neutral and acidic sugar composition

Neutral sugar composition was determined as alditol acetates after trifluoroacetic acid hydrolysis (75 min at 120 °C), reduction and acetylation, as described in the literature (Apolinar-Valiente et al., 2013; Ducasse, Williams, Meudec, Cheynier, & Doco, 2010). The resulting alditol acetate derivatives were quantified by gas chromatography (GC)