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Measurement of the interaction between mucin and oenological tannins by Surface Plasmon Resonance (SPR); relationship with astringency

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ABSTRACT

The interaction between stomach porcine mucin and 3 oenological tannins (extract of ellagitannins from oak, extract of gallotannins from gall nuts and extract of proanthocyanidins from grape seeds) was measured by Surface Plasmon Resonance (SPR). These tannins were analysed and their astringency was determined using the Astringency Index method and by tasting. The interaction constants were determined using a Biacore SPR device (1:1 Langmuir binding model). The results indicate that the ellagitannins are more astringent than gallotannins and those, in turn, are more astringent than seed proanthocyanidins if the richness of the commercial extracts is considered. The astringency index of these tannins had high correlation and regression coefficients with their kinetic and thermodynamic dissociation constants. This data support a hypothesis that astringency depends not only on the thermodynamic tendency to form the complex between tannins and salivary proteins but also probably on the time required to dissociate the complex.

1. Introduction

Astringency has been defined by the American Society for Testing and Materials in 1989 as a complex of sensations of dryness and roughness in the epithelium caused by exposure to substances such as tannins and alum (Llaudy et al., 2004). Excessive astringency is usually considered a negative sensation in wine, however it is widely acknowledged that high-quality red wines have a balanced level of astringency, with the renowned oenologist Emile Peynaud asserting that the harmony, balance and elegance of astringency are signs of great red wines (Peynaud & Blouin, 1980). Peynaud also observed that excessive astringency in wine affects its balance and quality, but on the contrary, a wine lacking in astringency appears flat, bland and without interest.

Wine astringency is caused by the capacity of some phenolic compounds to bind salivary proteins, producing drying and puckering sensations in the mouth (Gawel, 1998; Llaudy et al., 2004). Naturally occurring proanthocyanidins, also called condensed tannins, are mainly responsible for the astringency of red wines. However, aging wine in oak barrels and using oenological tannins can also provide a certain amount of hydrolysable tannins (gallotannins and ellagitannins) (Chira et al., 2015; Navarro et al., 2016). Both, condensed and hydrolysable tannins contribute to wine astringency. It has been reported that hydrolysable tannins (pentagalloylglucose), which are very nonpolar, precipitates by forming a hydrophobic coat around the protein, whereas the much more polar proanthocyanidins (EC_{16}-C) forms hydrogen-bonded cross-links between protein molecules (Hagerman, Rice, & Ritchard, 1998). It has also been described that the enthalpy of the interaction between proteins and proanthocyanidins, associated with hydrophobic interaction and hydrogen bonding, tends to decrease with the age of the wine (McRae, Falconer, & Kennedy, 2010) which would explain why the astringency of wines decreases over time.

Proanthocyanidins are released from the solid parts of the grape (skins and seeds) during the maceration process of red winemaking (Ribéreau-Gayon, Glories, Maujean, & Dubordieu, 2000). The composition of proanthocyanidins depends heavily on their origin. Seed proanthocyanidins are polymers composed of (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-gallate (Prieur, Rigaud, Cheynier, & Moutounet, 1994). Skin proanthocyanidins are composed of the same monomers but also contain (−)-epigallocatechin, and the proportion of (−)-epicatechin-3-gallate is much lower (Souquet, Cheynier, Brossaud,

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& Moutounet, 1996). In addition, seed proanthocyanidins have a lower degree of polymerization (mDP) than skin proanthocyanidins (Cheynier, Prieur, Guyot, Rigaud, & Moutounet, 1997). Consequently, skins release procyanidins and prodelphinidins with a higher mDP, whereas seeds mainly release procyanidins with a higher proportion of galloylation and a lower mDP.

Gallotannins are formed by the esterification of gallic acid with the hydroxyl group of a polyol carbohydrate such as glucose (Hagerman et al., 1998). Gallotannins are mixtures of polygalloyl glucoses or polygalloylquinic acid esters with a number of galloyl moieties per molecule ranging from 2 to 12 depending on their plant source (Sylla et al., 2015).

The chemical structure of ellagitannins consists of an open-chain glucose esterified at positions 4 and 6 by a hexahydroxydiphenoyl unit (HHDP) and a nonahydroxyterphenoyl unit (NHTP) esterified at positions 2, 3 and 5 with a C-glycosidic bond between the carbon of the glucose and position 2 of the trihydroxyphenoyl unit (Quideau et al., 2004).

Objective assessment of red wine astringency is usually estimated by tasting. This method, however, needs a group of expert wine tasters and even then there is an inherent subjectivity associated with the process (Valentova, Skrvánková, Panovskä, & Pokorny, 2002). The perception of astringency also increases with repeated exposure and this can lead to fatigue of the tasters which could alter the result of the tasting (Colonna, Adams, & Noble, 2004). In addition, the perception of astringency varies widely among individuals (Fischer, Boulton, & Noble, 1994), which makes its objective assessment extremely complicated. For these reasons, there is a great interest in having objective methods to assess astringency.

There are many studies about the use of protein-tannin interactions to investigate astringency mechanisms. Some of these studies use non-salivary proteins such as gelatin (Glories, 1984), ovalbumin (Llaudy et al., 2004), bovine serum albumin (Serafini, Maiani, & Ferro-Luzzi, 1997; Soares, Mateus, & De Freitas, 2007) or polyproline (McRae et al., 2010). with the aim of improved understanding of the mechanisms involved and also as analytical methods for measuring the intensity of astringency. However, these proteins have structures with limited structural similarity to salivary proteins, which leads to challenges in determining meaningful conclusions. Several studies of protein-tannin interactions using salivary proteins have been reported, using strategies such as nephelometry (De Freitas & Mateus, 2001), viscosity evaluation (Prinz & Lucas, 2000), PAGE-SDS (Gambuti, Rinaldi, Pessina, & Moio, 2006), HPLC (Schwarz & Hofmann, 2008), dynamic light scattering (DLS) (Pascal, Poncet-Legrand, Cabane, & Vernhet, 2008), FT-MIR spectroscopy (Simoes-Costa, Costa-Sobral, Delgadillo, Cerdeira, & Rudnitskaya, 2015), ESI-MS (Canon et al., 2009), NMR (Cala, Fabre, Fouquet, Dufour, & Pianeta, 2010) and microcalorimetry (McRae et al., 2010). More recently, Atomic Force Microscopy (AFM) (Ma, Lee, Liang, & Zhou, 2016) and Surface Plasmon Resonance (SPR) (Rafaela et al., 2012; Watrelot et al., 2016; Guerreiro, Teixeira, De Freitas, Sales, & Sutherland, 2017) have been also used for measuring salivary proteins-tannin interactions.

The phenomenon of Surface Plasmon Resonance occurs due to charge-density oscillation that exists at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric material (Homola, Yee, & Gauglitz, 1999). The resonance is a result of energy and momentum being transformed from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the metal from the reflected light. The SPR signal is a direct measure of the angle of minimum reflected intensity and is monitored by a fixed array of light-sensitive diodes covering the whole wedge of reflected light. The angle of incidence which results in plasmon formation depends on the thickness of the metal, including the other material adhered to it. When a molecule is fixed to the surface of the metal and another molecule is brought into contact with this surface, the molecular interaction between them can be investigated in real time by measuring the variation of this angle (Akimoto, Sasaki, Ikebukuro, & Karube, 2000). Biacore systems exploit SPR and allow not only investigation of the molecular interactions specificity but also the kinetic and thermodynamic constants of their interaction.

The use of SPR for studying the interactions between proteins and tannins has the drawback that can alter the stoichiometry of the interactions that take place in a solution but has the advantage that it allows determining accurately the thermodynamic and kinetic constants of the interaction.

The aim of this work was to study the interaction between mucin and different oenological tannins by Surface Plasmon Resonance (SPR), in order to better understand the mechanism of astringency. Mucin was selected instead of other salivary proteins because it is the protein present at the highest concentration in unstimulated whole human saliva, constituting 20–30% of the total protein (Van Nieuw Amerongen, Bolscher, & Veerman, 2004). Mucins are highly glycosylated (80%) extracellular glycoproteins with molecular weights ranging from 0.5 to 20 MDa. The protein core (20%) is composed by a central glycosylated region mainly composed by a large number of tandem repeats of serine, threonine and proline (STP repeats). Mucin also has peptide chains, linked to the carboxy-terminals, and sometimes interspersed between the STP-repeats, with a lower glycosylation and with a high proportion of cysteine (> 10%). These cysteine rich regions are involved in the formation of mucin polymers via disulfide bond formation (van Nieuw Amerongen et al., 2004). Mucin from porcine stomach was used as a model of salivary protein in these experiments because it was commercially available and has a similar structure to human salivary mucin (Bansil & Turner, 2006).

The first objective was to determine the kinetic and thermodynamic interaction constants of each type of tannin with mucin. We have preferred to immobilize mucin in in the metallic surface and not tannins because this possibility is more similar to the real behavior. When we taste a red wine we introduce tannins inside the palate where mucin is present. The second objective was to determine whether a relationship exists between these constants and the sensory assessment of astringency elicited by these tannins.

2. Material and methods

2.1. Chemicals

Methanol, ethanol, formic acid, acetic acid and hydrochloric acid were of HPLC-grade and were purchased from Panreac (Barcelona, Spain). Methylcellulose, phloroglucinol, ascorbic acid, sodium acetate, ammonium acetate, sodium hydroxide, tannic acid, mucin from stomach porcine type III, Polyvinylpyrrolidone (average molecular weight 10,000; PVP10), phosphate buffered saline, TWEEN 20, HEPES and albumin from chicken egg were purchased from Sigma Aldrich (Madrid, Spain). (−)-epicatechin (+95%) was purchased from Extrasynthese (Genay, France). All ellagitannin standards (castalagin, vescalagin, grandinin, roburinA, roburin B, roburin C, roburin D and roburin E) were provided by ADERA (Pessac, France).

2.2. Oenological tannins

Three different oenological tannins were used in this study: a proanthocyanidin from grape seeds (Protanpépin, AEB Iberica SA, Castellbisbal, Barcelona, Spain), an ellagitannin from oak (Ellagitan Chêne, AEB Iberica SA, Castellbisbal, Barcelona, Spain) and a gallotannin from Chinese natural gall nuts (Tannic acid, Sigma–Aldrich, St Louis, MO, USA).

2.3. Tannin analysis by precipitation with methyl-cellulose

All oenological tannin extracts were analyzed using the methyl-