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Effect of flavonols on wine astringency and their interaction with human saliva

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ABSTRACT

The addition of external phenolic compounds to wines in order to improve their sensory quality is an established winemaking practice. This study was aimed at evaluating the effect of the addition of quercetin 3-*O*-glucoside on the astringency and bitterness of wines. Sensory results showed that the addition of this flavonol to wines results in an increase in astringency and bitterness.

Additionally, flavonol-human salivary protein interactions were studied using fluorescence spectroscopy, dynamic light scattering and molecular dynamic simulations (MD).

The apparent Stern-Volmer (K_{svApp}) and the apparent bimolecular quenching constants (k_{qApp}) were calculated from fluorescence spectra. The K_{svApp} was $12620 \pm 390\ M^{-1}$, and the apparent biomolecular constant was $3.94 \times 10^{12}\ M^{-1}\ s^{-1}$, which suggests that a complex was formed between the human salivary proteins and quercetin 3-*O*-glucoside.

MD simulations showed that the quercetin 3-*O*-glucoside molecules have the ability to bind to the $IB9_{37}$ model peptide.

1. Introduction

Exogenous phenolic compounds are often added to must or wines in order to mask faults and to improve the sensory quality of these beverages by, for example, modifying the mouth-feel or stabilizing the colour. For this purpose, many extracts and isolated compounds obtained from different sources have been tested (Alcalde-Eon et al., 2014; Aleixandre-Tudo et al., 2013; Cliff, Stanich, Edwards, & Saucier, 2012; Harbertson, Parpinello, Heymann, & Downey, 2012). In this way, it has been stated that flavonols are of crucial importance to the colour quality of red wine because they act as good co-pigments (Escribano-Bailón & Santos-Buelga, 2012; Gomez-Míguez, González-Manzano, Escribano-Bailón, Heredia, & Santos-Buelga, 2006). Recently, the addition of extracts from white wine by-products containing glycosylated flavonols has been suggested as a good practice to stabilize red wine colour (Gordillo et al., 2014; Jara-Palacios et al., 2014). Additionally, flavonols are of great interest for the food industry because of their well-known beneficial effects, for example, antioxidant properties, anticancer activities, and their roles in reducing inflammation and reducing the risk of chronic disease. Therefore, the addition of these compounds to beverages and food may also be an interesting practice. Nevertheless, the subject of possible changes in the mouth-feel perception due to exogenous addition of flavonols is not adequately addressed in these studies.

The determination of the relationship between phenolic structures and the sensory perceptions elicited by them is one of the top current challenges in food chemistry and, in particular, in oenology research. This goal is closely related to the mechanisms implicated in the astringency perception, which remain unclear. It has been established that the phenolic-salivary protein interaction is not the only mechanism involved in astringency. Some sensory receptors may also participate in this oral perception (Carpenter, 2013; Gibbins & Carpenter, 2013). Recently, it has been stated that the chemosensory detection of astringent phenols together with the stimulation of trigeminal mechanosensors create the entire sensation of astringency (Schobel et al., 2014). The simultaneous activation of both chemosensory and

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mechanosensory receptors could be the reason for some synergisms of astringency shown by certain phenolic compounds (Ferrer-Gallego, Hernandez-Hierro, Rivas-Gonzalo, & Escribano-Bailon, 2014).

A combination of sensory analysis and analytical techniques is necessary for developing a proper understanding of the astringency phenomenon. Over the years, astringent properties of many phenolic compounds have been described. However, little research has been reported in relation to the astringency of wine flavonols (Hufnagel & Hofmann, 2008). These compounds are present in numerous fruits and beverages, grapes and wines being one of their most important sources in our diet (Makris, Kallithraka, & Kefalas, 2006).

Red wine and tea contain up to 45 mg/L of flavonols, but the richest sources are onions, curly kale, leeks, broccoli and blueberries (Manch, Scalbert, Morand, Rémésy, & Jiménez, 2004). Flavonols are present as aglycones, and 3-*O*-glucosides are their most common conjugated forms. Quercetin and its conjugated derivatives are frequently the main flavonols in both red and white grapes. In red varieties, they can represent up to 87% of the total flavonol content, while in white varieties, they can form up to 97% of the total flavonol content (Mattivi, Guzzon, Vrhovsek, Stefanini, & Velasco, 2006).

Other beverages such as cider, tea and beer also contain flavonols that may contribute to the mouth-feel sensation. Global flavonol content in apple juice ranges from 5 to 16 mg/L, which represents, on average, only 1–3% of the total phenolic concentration. Apple juice presents very low levels of quercetin glycosides (Ramírez-Ambrosi et al., 2015), and their astringency has been mainly attributed to the hydroxycinnamic acids (Mangas, Rodríguez, Suárez, Picinelli, & Dapena, 1999). However, flavonol content has been associated with mouth-drying astringency in blackcurrant juices (Laaksonena, Mäkiläa, Tahvonenb, Kallioa, & Yang, 2013) and black tea. The flavonol glycosides were found to induce a mouth-drying and mouth-coating sensation at very low threshold concentrations. Particularly, the threshold of quercetin 3-glucoside has been established as 0.65 μM, its concentration in black tea being around 6 μM (Scharbert & Hofmann, 2005).

Flavonols also seem to have some influence on the astringency perception in legumes (Troszynska et al., 2011).

In this work, the addition of quercetin 3-*O*-glucoside in wines has been evaluated in terms of astringency and bitterness. For this, two wines, seven sensory attributes, five different concentrations and two sensory evaluation times were considered.

To obtain further insights into the flavonol-human salivary protein interactions, experiments between quercetin 3-*O*-glucoside and saliva were performed using fluorescence spectroscopy, dynamic light scattering (DLS) and molecular dynamics simulations (MD).

This study combines analytical techniques and sensory analysis, in order to understand the molecular mechanism of the flavonol-salivary protein interaction and its relationship with the sensory perception of wine. To our knowledge, this is the first time that sensory analysis of flavonols and their molecular interactions with saliva have been studied.

2. Materials and methods

All solvents were of HPLC grade, and all chemicals were of analytical reagent grade. Quercetin 3-*O*-glucoside was supplied by Sigma-Aldrich (St. Louis, MO, USA).

Fluorescence and DLS measurements were performed in 0.1 M acetate buffer (12% ethanol) at pH 5.0. At this pH, salivary proteins have been shown to interact strongly with phenolic compounds (Soares, Mateus, & De Freitas, 2007).

2.1. Sensory assessment

A sensory panel was composed of 10 panellists previously trained in the quantitative and qualitative description of astringency. They were aged from 23 to 60 years old. Eight of them were professional tasters, and the others were students of Oenology. The panellists attended four preliminary training sessions in order to be familiarized with the sensory attributes and to standardize the use of terms and the scale. All of them had previously participated in analogous sensory tests, and the consistency of the trained panel was determined during this time (Ferrer-Gallego et al., 2014; Ferrer-Gallego et al., 2015a, 2015b).

The intensity of astringency, bitterness, velvety, dryness, dynamic, greenness, roughness and persistence of astringency were evaluated according to Gawel, Oberholster, and Francis (2000). Tests were carried out using a red wine and a white wine, both selected for their low astringency. Nineteen wines from different Spanish regions were previously evaluated for this purpose. The selected red wine was a young wine elaborated with 'Tempranillo' (2012) from Rioja D.O.C. The selected white wine was a young wine elaborated with 'Macabeo' (2012) from Vino de la Tierra de Castilla I.G.P.

Tests were carried out in individual booths in a professional tasting room at 20 °C. The addition of five different concentrations (0.25, 0.5, 1, 1.5 and 2 g L^{-1}) of quercetin 3-*O*-glucoside was evaluated in both wines. In the case of the red wine, the sensory analysis was also performed three months after storage at 4 °C.

A labelled magnitude scale (LMS) was used for rating the intensity of each attribute. This scale is characterized by a quasi-logarithmic spacing of its verbal labels from 0 ("barely detectable" oral sensation) to 100 ("strongest imaginable" oral sensation) (Fig. 1) (Green et al., 1996). Samples were tested in 25-mL screw neck glass bottles and were randomly served. Tasters took 5 mL of the sample and 15 s and after this, rated the intensity for each attribute. This protocol was repeated two times per sample to assign the final value in the mentioned scale. They rinsed with deionized water and waited for 2 min between samples.

2.2. Saliva collection

A saliva pool was collected according to Soares et al. (Soares, Mateus, & de Freitas, 2012), from healthy, non-smoking volunteers aged from 22 to 32 years. All panellists held saliva inside their mouth for 10 min and spit it all at once; they had previously avoided food and beverages for at least 1 h before collection. The standardized collection time was approximately 2 p.m. to reduce variability linked to circadian rhythms (Messana, Inzitari, Fanali, Cabras, & Castagnola, 2008). Saliva was treated with 10% of trifluoroacetic acid (final concentration 0.1%) and centrifuged. Finally, supernatant was used for fluorescence and DLS analyses.

2.3. Fluorescence spectroscopy

A quercetin 3-*O*-glucoside solution (0.25 mM) was prepared in 0.1 M acetate buffer (12% ethanol) at pH 5.0. Then, 100 μL of saliva was added to different volumes of this stock solution (from 5 to 100 μL). All solutions were brought up to a final volume of 200 μL by addition of acetate buffer. The final flavonol concentrations were 0, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 μM. All samples were mixed in a vortex for 10 s. Spectra were recorded at a controlled temperature 24 ± 0.1 °C. A Varian Cary Eclipse spectrofluorometer equipped with a "single cell peltier accessory" in 1-cm quartz cuvettes, with a slit width of 10 nm, scan rate of 600 nm/min, and data range of 1 nm was used. The excitation wavelength was set to 282 nm, and the emission spectrum was recorded from 300 to 500 nm. The assay was performed in triplicate.