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A saliva molecular imprinted localized surface plasmon resonance biosensor for wine astringency estimation

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ABSTRACT

Wine astringency was evaluated based on the interaction of two complex matrices (red wine and saliva) by combining localized surface plasmon resonance (LSPR) and molecular imprinted polymers (MIP) at gold nanodisks as an alternative to sensorial analysis. The main objective of the work was to simulate wine astringency inside the mouth by mimicking this biological system. The LSPR/MIP sensor provided a linear response for astringency expressed in pentagalloyl glucose (PGG) units in concentrations ranging from 1 to 140 μmol/L. The sensor was also applied to wine samples correlating well with sensorial analysis obtained by a trained panel. The correlation of astringency and wine composition was also evaluated showing that anthocyanins may have an important role, not only for pigmentation but also in astringency.

1. Introduction

Polyphenols are among the most abundant compounds in the human diet. Colour, smell and taste, amongst other sensorial characteristics of food products, result directly from the presence of polyphenols or from their collective action such as astringency. Astringency is generally accepted to result from the interaction between polyphenols present in food matrixes and salivary mouth proteins, decreasing mouth lubrication and leading to dryness, puckering and constriction of the tissues. The interactions between polyphenol and protein are characterized by the formation of complexes that might extend to larger complexes and finally lead to precipitation. However, the initial complexation between polyphenols and proteins seems more closely associated to astringency than the later precipitation (Obreque-Slier, Lopez-Solis, Pena-Neira, & Zamora-Marin, 2010).

Astringency is a sensorial parameter that is especially important for wine production, determining the product quality, and for that reason requires an accurate control. Wine polyphenols comprise an enormous family of compounds such as anthocyanins, condensed tannins extracted from grapes and stable/more complex derived compounds that generally result from oxidation, degradation and even precipitation (between condensed tannins/anthocyanins) during wine aging (He et al., 2012). Not all polyphenols in wines come from the grapes; some compounds like hydrolysable tannins (e.g. pentagalloyl glucose) might be added during wine production or result from their migration along the contact between wine and oak barrels (Sarneckis et al., 2006). Astringency of wine polyphenols has also been associated with their individual characteristics, such as concentration, polymerization degree, molecular weight and number of galloyl substituents of polyphenols (Batesmit, 1973; Hagerman & Robbins, 1987; Kawamoto, Nakatsubo, & Murakami, 1995).

Wine is a complex matrix due to the variety of polyphenols directly extracted from grapes, as well as due to their ability to undergo numerous interactions and chemical reactions changing the overall polyphenol profile over time. Wine astringency evaluation is therefore a tough task and even with the use of sensorial analysis the results are subjective, time-consuming and expensive (Vidal et al., 2004). Regardless of the disadvantages, sensorial panels with several elements remain the most common approach to evaluate astringency. These sensorial panels are normally composed of professionals that can also be trained with several standard solutions prior to wine astringency evaluation. Despite that,

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salivary protein production also varies with the time of the day, food consumption, diet, circadian rhythms, age, gender, several disease states and pharmacological agents (Dodds, Johnson, & Yeh, 2005). Therefore, the development of more effective methods to assess astringency and understand the mechanisms of action between polyphenols and proteins is of great importance.

Different methods have been applied to study astringency at the molecular level based on precipitation studies between specific proteins (gelatin, BSA, mucins,...) or salivary proteins and individual or mixture of polyphenols, thus evaluating the protein fraction before and after precipitation. The direct and/or indirect quantification of polyphenols has also been used as an alternative way to estimate astringency (Caceres-Mella et al., 2013; Guerreiro, Sutherland, De Freitas, & Sales, 2013). However, astringency does not always correlate well with precipitation assay or polyphenol content.

Other techniques such as SDS-PAGE (Rinaldi, Gambuti, & Moio, 2012), spectrophotometry (Simoes Costa, Costa Sobral, Delgadillo, Cerdeira, & Rudnitskaya, 2015), fluorescence (Fia, Dinnella, Bertuccioli, & Monteleone, 2009), nephelometry (Monteleone, Condelli, Dinnella, & Bertuccioli, 2004), NMR (Furlan, Jobin, Pianet, Dufburc, & Gean, 2015), DLS (Pascal, Poncet-Legrand, Cabane, & Vernhet, 2008) and mass spectrometry (Careri, Corradini, Elviri, Nicoletti, & Zagnoni, 2004) have also been used to study proteins and polyphenols interactions with and without the focus on astringency. Generally, the methods used to assess astringency also compare the new methods with sensorial analysis performed by a trained panel. The emergence of new technologies allowed the study of these interactions, not just at the molecular level, but likewise at the nanoscale through nanotechnology such as localized surface plasmon resonance (LSPR) (Guerreiro et al., 2014).

Considering the complexity of astringency mechanisms and variation in astringency estimation by different approaches, these effects suggest that for each class of polyphenol the astringency phenomenon may be different, while the synergetic effects play an important role. Therefore, the methods that consider the global mechanism at the molecular/atomic level seem to be the most suitable for astringency estimation in beverages.

Recently, LSPR sensors were reported for the detection and quantification of biomolecules, via specific interactions due to their high sensitivity, good reproducibility, real-time responses and potential for label-free detection. LSPR's are collective oscillations of conduction electrons at confined metal nanostructures formed from metals such as Au, Ag, Al and are typically probed by interaction with light. The highly sensitive dependence of the resonance energy on the refractive index (RI) surrounding the metal nanostructure means that biomolecules adsorption on the metal surroundings causes the shift of the LSPR peak wavelength, with larger RI changes giving large shifts. Generally, antibodies are used as biomolecular recognition elements immobilized at the surface of a sensor. Nevertheless, new artificial antibodies also known as molecular imprinted polymers (MIPs) have been developed to improve the stability of sensory devices. The design of molecular imprinted materials involves the copolymerization of monomers and cross-linker elements in the presence of the target molecule. After polymerization the target molecule is removed by an appropriate method from the polymeric matrix, leaving imprinting sites with complementary shape, size and functional chemistry to the target molecule, which leads to selectivity on rebinding. The integration of MIPs technology with plasmonic sensors results in a combination of a high sensitivity biosensor method coupled with robustness, stability and reusability features.

The main purpose of this work was to develop a sensor device combining LSPR and MIPs to evaluate wine astringency at the molecular/atomic level, compare sensorial and analytical wine astringency and then correlate astringency with wine composition. We used salivary proteins as target molecules and aqueous compatible monomers cross-linked in a polymer network with specific binding sites at gold nanodisks for salivary proteins through a surface imprinting process. The interactions between polyphenol and protein used salivary proteins and pentagalloyl glucose (PGG) for sensor calibration, followed by real wine samples analysis and astringency estimation.

2. Experimental

2.1. Apparatus

QCM-D measurements were performed with the Q-sense E4 system (Q-sense AB) using the 7th, 9th and 11th overtones recording both frequency and dissipation. AT-cut quartz crystals with a fundamental frequency of 5 MHz were purchased from Q-sense AB (Sweden) with gold electrodes.

SPR measurements were performed with a Biacore X system from Biacore AB (Sweden) and gold SPR chips were purchased from GE Healthcare.

Prior to any measurement, both QCM crystals and SPR chips were cleaned by basic piranha solution consisting of MilliQ water (MQ), ammonia 25% and hydrogen peroxide 30% (5:1:1) at 75 °C for 5 min, followed by rinsing with MQ and blow dried with nitrogen. In order to finalize the cleaning procedure, the substrates were treated with 10 min of UV/ozone (Kern & Puotinen, 1970).

LSPR measurements were performed in a Shimadzu UV–Vis–NIR spectrophotometer UV-360 using a flow injection cell and wavelength ranging from 400 to 900 nm. Extinction spectra were collected in the absorbance mode.

Wine characterization assays were performed in an UV–vis spectrophotometer Thermo Scientific Evolution Array UV–vis spectrophotometer, a HACH 2100 N turbidimeter equipped with a 100 × 12 mm cell adaptor, and a Thermo® Scientific HPLC with a Thermo® Scientific Spectra System P4000 pump on a 250 × 4.6 mm i.d. reversed-phase C18 column (Merck®, Darmstadt) at 25 °C; detection was carried out between 200 and 800 nm using a Thermo® Scientific Spectra System UV8000 diode array detector; 20 µl of each sample was injected using an autosampler Thermo® Scientific Spectra System AS3000.

2.2. Reagents and solutions

All chemicals were of analytical grade. Buffers were prepared with MQ, additional filtering (0.2 µm pore filter) and sonication was required for both QCM-D and SPR experiments. The used buffers were: 10 mM PBS (Phosphate buffered saline) pH 7, PBS with 5% Ethanol and 50 mM ammonium bicarbonate (AMBIC) supplied by Sigma-Aldrich. PGG standard solutions were prepared in PBS 5% ethanol.

The fabrication of gold nanodisks involved the use of polymethylmethacrylate Mw 495,000, 4% anisole (PMMA) purchased from Micro resist technology GmbH, poly(diallyldimethylammonium chloride) (PDDA), poly(sodium-4-styrenesulfonate) (PSS) purchased from Sigma-Aldrich, polyammonium chloride (PAX) purchased from KemiraMiljø and polystyrene (PS) colloidal particles acquired from Invitrogen.

Gold (flat or nanodisks) surface modification included 16-mercaptohexadecanoic acid, 99% from Assemblon, *N*-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) purchased from Aldrich.

The imprinted process required thiophenecarboxylic acid (TPCA), methacrylic acid (MAA), (vinylbenzyl)trimethylammonium