Chem. Senses 37: 191–198, 2012

doi:10.1093/chemse/bjr092
Advance Access publication November 15, 2011

Effect of Condensed Tannins Addition on the Astringency of Red Wines

Susana Soares, André Sousa, Nuno Mateus and Victor de Freitas

Chemistry Investigation Center, Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

Correspondence to be sent to: Victor de Freitas, Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal. e-mail: vfreitas@fc.up.pt

Accepted August 11, 2011

Abstract

Astringency has been defined as a group of sensations involving dryness, tightening, and shrinking of the oral surface. It has been accepted that astringency is due to the tannin-induced interaction and/or precipitation of the salivary proline-rich proteins (PRPs) in the oral cavity, as a result of the ingestion of food products rich in tannins, for example, red wine. The sensory evaluation of astringency is difficult, and the existence of fast and reliable methods to its study in vitro is scarce. So, in this work, the astringency of red wine supplemented with oligomeric procyanidins (condensed tannins), and the salivary proteins (SP) involved in its development were evaluated by high-performance liquid chromatography analysis of human saliva after its interaction with red wine and by sensorial evaluation. The results show that for low concentration of tannins, the decrease of acidic PRPs and statherin is correlated with astringency intensity, with these families having a high relative complexation and precipitation toward condensed tannins comparatively to the other SP. However, for higher concentrations of tannins, the relative astringency between wines seems to correlate's to the glycosylated PRPs changes. This work shows for the first time that the several families of SP could be involved in different stages of the astringency development.

Key words: human saliva, oligomeric procyanidins, proline-rich proteins, sensory analysis

Introduction

The astringency perception on the human palate has been defined as a complex group of sensations involving dryness, tightening and shrinking of the oral surface, and puckering sensations of the oral cavity (ASFTTO Materials 1989). Astringency sensation results from the ingestion of some food products rich in tannins, namely tea and red wine. Since 1954, when Bate-Smith (1954) proposed that astringency results from the interaction of tannins with salivary proteins (SP) in the mouth, it has been generally accepted and supported by recent literature (De Freitas and Mateus 2001; Kallithraka et al. 2001; Mateus et al. 2004; Hofmann et al. 2006; Preys et al. 2006) that astringency is due to the tannin-induced interaction and/or precipitation of salivary proline-rich proteins (PRPs) in the oral cavity. Although many research groups and literature support this mechanism for astringency development, astringency is a very complex sensory experience, and actually, the possible mechanisms for its development are controversially discussed by the scientific community. Schwarz and Hofmann (2008) proposed that astringency sensory perception is related to the ''nonbound free'' astringent stimulus present in saliva and also suggested the involvement of laminin receptor in its development; others suggested that modifications of the viscous and lubrication properties of saliva are important, either by the precipitation of several proteins (Rossetti et al. 2008) and increased friction (Green 1993) or by interaction with glycosylated PRPs (gPRPs) modifying their rheological properties (Pascal et al. 2008).

SP have been grouped into 6 structurally related major classes namely, histatins, basic PRPs (bPRPs), acidic PRPs (aPRPs), gPRPs, statherin, and cystatins (Oppenheim et al. 1971, 1988; Bennick 1982; Shomers et al. 1982; Hay et al. 1988; Schlesinger et al. 1989; Helmerhorst and Oppenheim 2007). All these peptides, except bPRPs, have well-defined important biological functions in saliva, including maintenance of ionic calcium concentration (aPRP and statherin), antimicrobial action (histatins and cystatins), or protection of oral tissues against degradation by proteolytic activity (cystatins). For bPRPs, it has been proposed (Mehansho et al. 1983, 1985; Lu and Bennick 1998) that one of their functions is to bind tannins, preventing their toxic effects in the gastrointestinal tract.

One well-known beverage rich in tannins is red wine. Red wine quality takes into account the fine balance between traditional parameters, such as acidity, sugar, color, bitterness, and astringency, some of which are related to anthocyanins and tannins. Anthocyanins and tannins are polyphenolic compounds responsible for the color and astringency attributes, respectively.

The composition of wines in tannins from grapes (condensed tannins) changes drastically between wines depending on variety, wine-making procedures, region, and among other factors. These aspects influence importantly the wines sensorial characteristics, mainly the flavor. So, it is of fundamental importance to understand the effect of tannins on wine astringency, in order to not compromise the overall wine quality. The sensory evaluation of astringency is difficult, time consuming, and expensive. Moreover, it is often wrongly associated with bitterness, being sometimes difficult for a panel to distinguish different astringency attributes (Peleg et al. 1999), and the existence of fast and reliable methods to study the astringency in vitro is scarce. So, in this work, the astringency of red wine supplemented with oligomeric procyanidins (OPC, condensed tannins isolated from grape seeds), and the SP involved in its development were evaluated by chromatographic analysis of human saliva after its interaction with red wine and also by sensorial evaluation by a trained panel. This approach also allowed to study the effect of the red wine matrix in the reactivity of condensed tannins toward human SP.

Materials and methods

Reagents

All reagents used were of analytical grade or better. Acetonitrile (ACN) and hydrochloric acid were purchased from Panreac Quimica; acetic acid (HOAc) was purchased from Carlo Erba Reagents; Folin-Ciocalteu, sodium acetate, and trifluoroacetic acid (TFA) were purchased from Fluka Biochemica; ethanol was purchased from AGA, Álcool e Géneros Alimentares, SA.; ethyl acetate was purchased from Valente e Ribeiro, Lda; chloroform was purchased from Pronalab, José M. Vaz Pereira, Lda; sodium hydroxide was purchased from Laboratório Maialab, Lda; sodium carbonate was purchased from Sigma-Aldrich; and tartaric acid was purchased from Aldrich.

Isolation of OPC from grape seeds

OPC (condensed tannins) were extracted from *Vitis vinifera* grape seeds with an ethanol/water/chloroform solution (1:1:2, v/v/v), and the chloroform phase, containing chlorophylls and lipids, was rejected. Then, the hydroalcoholic phase was extracted with ethyl acetate. The organic solvent was removed using a rotary evaporator (30 °C) yielding a residue (OPC) that corresponds to catechin monomers and procyanidin oligomers (Darné and Madero 1979; De Freitas et al. 1998). This residue was characterized by high-performance liquid chromatography (HPLC) regarding the composition in procyanidins, accordingly to De Freitas and Glories (1999). Briefly, 2 Lichrospher (C18) ODS (250 × 4.6 mm id) columns placed in line were used for all analysis. The chromatograms were monitored at 280 nm using an ultraviolet (UV) detector. The elution system consisted of 2 solvents, A: 2.5% HOAc and B: 80% ACN + 20% A. The gradient applied was linear from 7% to 20% (eluent B) in 90 min at a flow rate of 1.0 mL·min^{-1}. The procyanidins identified and quantified were: (+)-catechin (56.0 mg·g^{-1}), (–)-epicatechin (50.0 mg·g^{-1}), dimers B1–B3 (270.0 mg·g^{-1}), B2 (138.0 mg·g^{-1}), B4 (38.0 mg·g^{-1}), B5 (26.0 mg·g^{-1}), B2-gallate (151.0 mg·g^{-1}), and epicatechin gallate (16.0 mg·g^{-1}). Total of catechins and procyanindins correspond to 745.0 mg·g^{-1} and remaining 255.0 correspond to others high molecular weight (MW) OPC (255.0 mg·g^{-1}).

Red wine supplementation with grape seed OPC

A red wine (*V. vinifera*, *Touriga nacional*, and *T. Franca* cv.) from the Douro demarked region was provided by Lavradores de Feitoria, S. A. This wine has been determined to have 0.993 ± 0.006 g catechin equivalents·L^{-1} of condensed tannins, determined based on the Folin-Ciocalteu method described by Singleton and Rossi (1965). Red wine was also characterized by HPLC regarding the composition in procyanidins, accordingly to De Freitas and Glories (1999), as described above. The procyanidins identified and quantified were: (+)-catechin (2.51 ± 0.11 mg·L^{-1}), (–)-epicatechin (1.57 ± 0.02 mg·L^{-1}), dimers B1–B3 (109.63 ± 10.37 mg·L^{-1}), B2 (50.40 ± 5.86 mg·L^{-1}), B4 (3.38 ± 0.70 mg·L^{-1}), B5 (20.95 ± 0.63 mg·L^{-1}), B6 (10.33 ± 0.63 mg·L^{-1}), B2-gallate (42.64 ± 2.35 mg·L^{-1}), and epicatechin gallate (2.35 ± 0.21 mg·L^{-1}). The total quantity of procyanidins is 243.76 mg·L^{-1}. Different red wines with different concentration in OPC were prepared by adding increasing quantities of OPC extract to the selected wine (control wine): 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g·L^{-1}. The resulting wines were bottled in triplicate (triplicates of 50 mL) and kept in the dark.

Human saliva collection

Saliva was collected from 6 healthy nonsmoking volunteers, and 2 mL of saliva from each volunteer were used to make a saliva pool (whole saliva, WS). Collection time was standardized at 2 PM in order to reduce concentration variability connected to circadian rhythms of secretion (Messana et al. 2004). The saliva pool was mixed with 10% TFA (final concentration 0.1%), mixed and centrifuged at 8000 × *g* for 5 min. After the centrifugation, the supernatant (acidic saliva, AS) was separated from the precipitate and used for the following experiments.

Protein–tannin interaction

The proteins in AS sample were analyzed by HPLC before and after the interaction with increasing volumes of red

wines enriched with different concentration of OPC. The control condition was a mixture of AS (150 μL) and acetate buffer 0.1 M, 12% ethanol, and pH 5.0 (50 μL) in order to maintain the final volume constant (200 μL) between the several tested volumes of red wine. The control condition was done in buffer with 12% ethanol to simulate a model wine and at pH 5.0 because it is the average pH between wine (3.4) and saliva (7.0). The first experiments were made with increasing volumes (10, 20, 30, 40, and 50 μL) of red wine supplemented with 1.5 $g \cdot L^{-1}$ of OPC, and acetate buffer was added to make the final volume 200 μL. Each volume tested of red wine was an independent experiment. After shaking, the mixture reacted at room temperature for 5 min and then was centrifuged (8000 × *g*, 5 min). The reaction time (5 min) was established as the minimal time to have stable interactions and good reproducible results. The supernatant was analyzed by HPLC. The precipitate resultant from some experiments was resolubilized in 110 μL of eluent A used for the HPLC analysis (see below) and analyzed by HPLC. The same experiments were done for the other red wines supplemented with different concentrations of OPC (control, 0.5, 1.0, 2.0, 2.5, and 3.0 $g \cdot L^{-1}$), using 10 or 20 μL of wines in the reaction with saliva.

Negative control experiments were made with OPC dissolved in acetate buffer. OPC solutions were made in increasing concentrations (the same concentrations referred above), and 20 μL of these solutions were used to react with saliva in the same experimental conditions described above.

HPLC analysis

Ninety microliters of each solution was injected on an HPLC Lachrom system (L-7100) equipped with a Vydac C8 column, with 5 μm particle diameter (column dimensions 150 × 2.1 mm); detection was carried out at 214 nm, using a UV-Vis detector (L-7420). The HPLC solvents were (eluent A) 0.2% aqueous TFA and (eluent B) 0.2% TFA in ACN/water 80/20 (v/v). The gradient applied was linear from 10% to 40% (eluent B) in 60 min, at a flow rate of 0.30 $mL \cdot min^{-1}$. After this program, the column was washed with 100% eluent B for 20 min in order to elute S-type cystatins and other late-eluting proteins. After washing, the column was stabilized with the initial conditions (Messana et al. 2004; Soares et al. 2011).

Tannin-specific activity

The tannin-specific activity (TSA) toward WS and AS was determined by nephelometry as described by De Freitas and Mateus (2001). This method is based on the characteristic property of tannins to interact and precipitate proteins. The red wines supplemented with condensed tannins were diluted 50 times with filtered (0.45 μm) model solution (12% ethanol, 5.0 $g \cdot L^{-1}$ tartaric acid, pH 3.20). About, 4.0 mL of this solution were transferred to a test tube and mixed with 50 μL of WS or AS. The test tube was kept in the dark for 30 min, and after this time, the maximum turbidity was measured in a turbidimeter HACH 2100 N adapted for cells of 100 × 12 mm. The TSA is expressed in turbidity units NTU/mL of wine and is determined by the following expression, where 0.08 corresponds to the dilution factor of wine:

$$\text{Turbidity}(\text{NTU}/\text{mL}) = \frac{\text{Turbidity}_{\text{sample}}}{0.08}.$$

Sensory evaluation

Red wines (control and supplemented wines with OPC) were rated for astringency by a 6-member trained sensory panel. The wines were presented to the panel members in glass cups (at room temperature, 20 °C) in random order. The panel members were asked to rate the intensity of the perceived astringency for each sample in each series on a 1–7 score scale. Water was used for mouth rinsing between consecutive samples.

Data and statistical analysis

Values are expressed as the arithmetic means ± standard deviation. Statistical significance of the difference between the TSA of WS and AS was evaluated by *t*-test unparametric. Differences were considered to be statistically significant when $P < 0.05$. Statistical significance of the difference between the perceived astringency by the sensory panel was evaluated by one-way analysis of variance, followed by the Bonferroni test. Differences were considered to be statistically significant when $P < 0.1$.

Results

The reactivity of red wines supplemented with condensed tannins (OPC) toward SP was evaluated by chromatographic analysis of human saliva after its interaction with red wine and also by sensorial analysis.

The initial acidic treatment of saliva with TFA is used to precipitate several high MW SP (such as α-amylases, mucins, carbonic anhydrase, and lactoferrin) and to preserve sample protein composition because TFA partially inhibits intrinsic protease activity (Messana et al. 2004). However, peptides and proteins like histatins, bPRPs and aPRPs, statherin, cystatins, and defensins are soluble in AS solution and may be directly analyzed by Reverse Phase HPLC, as previously described (Messana et al. 2004; Soares et al. 2011).

The HPLC chromatogram of this AS solution at 214 nm is presented in Figure 1, and the profile was similar to the one previously described in the literature (Messana et al. 2004; Soares et al. 2011). The top of the figure shows the distribution of the different families of SP along the chromatogram that were established previously by proteomic approaches, namely ESI-MS and MALDI-TOF/TOF (Messana et al. 2004; Soares et al. 2011).

The HPLC chromatogram of the AS solution is roughly divided into 4 salivary peptide family regions: The first Region 1

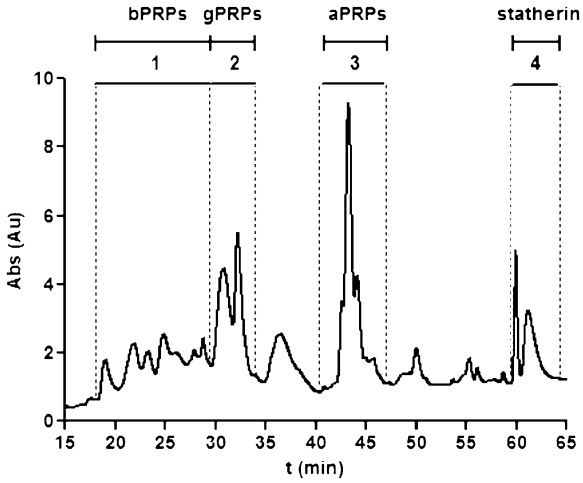

Figure 1 Typical Reverse Phase HPLC profile detected at 214 nm of the AS solution of human saliva. The dotted lines and numbers show the ranges and the main SP family assigned to each HPLC peptide region.

comprises proteins that belong to the classes of bPRPs and histatins. The bPRPs identified in this region include IB-8b, IB-8c, IB-9, IB-4, and P-J; and the histatins include histatins 3, 5, 7, 8, and 9. Region 2 comprises mainly a gPRPs, the bPRP3. Region 3 corresponds entirely to aPRPs, namely PRP1 and PRP3, and the last Region 4 has phosphorylated and nonphosphorylated forms of statherin and peptide P-B (Soares et al. 2011).

The peak between regions 2 and 3 has not been previously assigned to any family of SP, and in this work, it has shown a weak reactivity toward OPC. So, this peak was not considered in the results analysis and discussion.

A red wine enriched with increasing concentrations of OPC was used in order to compare the reactivity of the several SP families toward wine tannins directly in a wine matrix. The wines were mixed with AS, and the insoluble aggregates were removed by centrifugation. The supernatant was analyzed by HPLC.

The first experiments were done with the red wine supplemented with 1.5 $g{\cdot}L^{-1}$ of OPC, which is the middle concentration of OPC used. These experiments were done with increasing volumes of red wine from 10–50 μL in order to study if the interaction with the different families of SP was affected by the wine volume. Some examples of the HPLC profiles of AS solution before and after the interaction with different volumes of this red wine are presented in Figure 2A. The percentage of area decreases for each HPLC region after the AS solution interaction with increasing volumes of red wine supplemented with 1.5 $g{\cdot}L^{-1}$ of OPC are summarized in Figure 2B. It was also perceptible that the amount of precipitate increased with the volume of red wine added to AS.

The results displayed in Figure 2 show that the HPLC profile of the AS solution and, therefore, the area of each different family of proteins are interestingly affected in a different way by the interaction with increasing volumes of red wine supplemented with 1.5 $g{\cdot}L^{-1}$ of OPC. Although the areas of HPLC regions 3 (aPRPs) and 4 (statherin) decline significantly (to 50% and 20%, respectively) with the lowest red wine volume added (10 μL), the area of the other regions remains relatively constant (Figure 2B). Indeed, only the highest volume added (50 μL) decrease the bPRPs to about 40% and gPRPs to 30%.

Volumes of 10 and 20 μL were chosen for further experiments with the other wines supplemented with different concentrations of OPC. These volumes were chosen because they correspond to the lowest volumes that affected differently the several regions of the chromatogram and allowed comparing the reactivity of several wines toward different families of SP. Indeed, for volumes of wine of 30 μL and higher, statherin and aPRPs precipitated almost completely (Figure 2B).

Figure 3 presents the HPLC profile of AS solution before and after the interaction with 10 μL of red wine supplemented with increasing concentrations of OPC (1.0, 2.0, and 3.0 $g{\cdot}L^{-1}$). Only some chromatograms representative of the overall tendency are presented.

From the results presented in Figure 3, it is possible to observe that for the same volume of wine (10 μL) added to AS solution, the resulting decrease of SP is markedly different. The addition of 10 μL of red wine supplemented with 3.0 $g{\cdot}L^{-1}$ of OPC, results in an important decrease of almost all regions. On the other hand, for the red wine supplemented with 1.0 $g{\cdot}L^{-1}$ of OPC, only the areas of aPRPs and statherin decrease importantly. These experiments were done with 2 different volumes of wine (10 and 20 μL), and the decrease of percentage area is summarized in Figure 4.

The results presented in Figure 4 clearly indicate that the interaction of SP present in the AS solution with red wine is affected by the volume of red wine used and also by the quantity of condensed tannins present in the red wines. While the aPRPs and statherin are significantly affected for the lowest volume used (Figure 4A) and with the increasing in the concentration of condensed tannins in wines, bPRPs are practically not precipitated. Increasing the volume of red wine to 20 μL leads to a significant decrease of gPRPs and a slight decrease of bPRPs.

The area of statherin is reduced below 20% with addition of control red wine (without OPC supplementation). This means that the tannins present in the control red wine are enough to complex and precipitate statherin in the concentration that it is present in saliva, and the presence of higher quantities of condensed tannins leads to a total depletion of this protein.

For aPRPs, the behavior is similar: 20 μL of control red wine is enough to reduce its area to 20%. However, for the experiments with 10 μL, it is possible to observe that the area of aPRPs is reduced importantly for wine enriched in OPC.

For gPRPs, only the experiments with 20 μL showed a significant decrease of their area. Indeed, increasing the volume to 20 μL and increasing the concentration of condensed

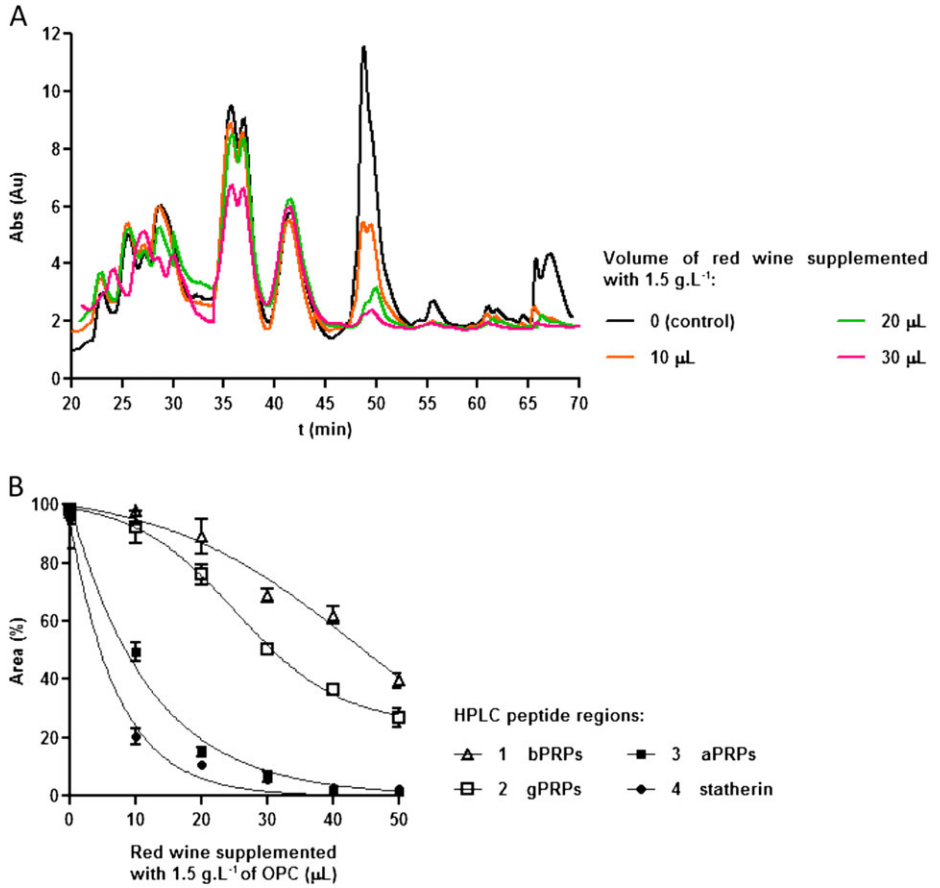

Figure 2 (**A**) HPLC profile detected at 214 nm of the AS solution before (control) and after the interaction with increasing volumes of red wine supplemented with 1.5 g·L^{-1} of OPC (condensed tannins). (**B**) Percentages of area decrease of each HPLC salivary peptide region after the interaction of AS solution with

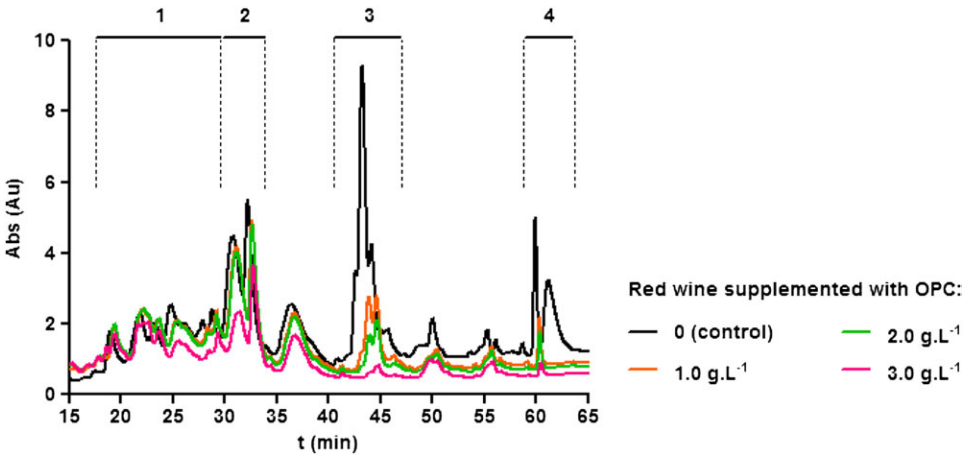

Figure 3 Reverse Phase HPLC profile detected at 214 nm of the AS solution before (control) and after the interaction with 10 µL of each red wine supplemented with different concentrations of OPC. This figure appears in color in the online version of *Chemical Senses*.

tannins lead to a decrease of their area up to 20%. For bPRPs, neither increasing the volume nor increasing the concentrations used, reduce significantly its area.

In order to study the contribution of OPC added to supplemented wines in the SP precipitation, blank experiments were done in which the interaction with SP was made using OPC solutions made in acetate buffer (pH = 5.0), with the

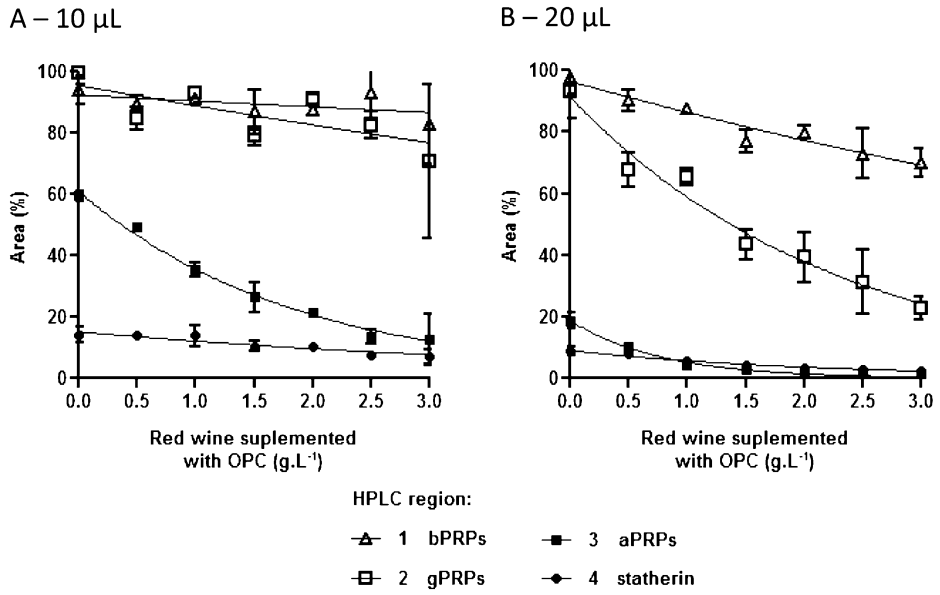

Figure 4 Percentages of area decrease of each HPLC peptide regions after the interaction of AS solution with (**A**) 10 and (**B**) 20 µL of red wine supplemented with increasing concentrations of OPC. Values are expressed as the arithmetic means of 3 experiments ± standard deviation.

same range of concentrations used previously (Figure 5). From these results, it is possible to observe that aPRP and statherin families decrease significantly along with the concentration of OPC as it was observed in red wines (Figure 4B, 20 µL), whereas gPRP are practically not affected by OPC in blank experiments. The concentration of OPC (catechin and dimers) in original wines (244 mg·L^{-1}, see the experimental section) probably do not explain the high precipitation observed in wines (Figure 4B, 20 µL). Indeed, in Figure 5, for the concentration around 244 mg·L^{-1}, only a small percentage of SP precipitates. This means that in original wine, already exists other components (e.g., tannin-like structures) that interact strongly with SP. However, the slight increase of tannins concentration in wines with OPC addition has an important contribution to discriminate wines concerning their ability to precipitate SP.

It is important to refer that the mixture of AS solution with red wine always resulted in the formation of insoluble precipitates that perceptibly increased along with the added volume of wine and also with the concentration in OPC.

The HPLC analysis of the precipitates resulting from the interaction of 10 or 20 µL of red wine supplemented with 0.5, 2.0, and 3.0 g·L^{-1} of OPC with the AS are shown in Figure 6.

It is possible to observe that several SP families are effectively precipitated by wine tannins. Indeed, in the HPLC chromatogram of the solubilized precipitate, aPRPs (region 3) and statherin (region 4) appear in the precipitate formed by the addition of 10 µL of red wine supplemented with 0.5 g·L^{-1} of condensed tannins. Increasing the concentration in condensed tannins (red wine supplemented with 2.0 g·L^{-1} of OPC) also increases the percentage of those proteins in the precipitate. The difference between the percentages of those

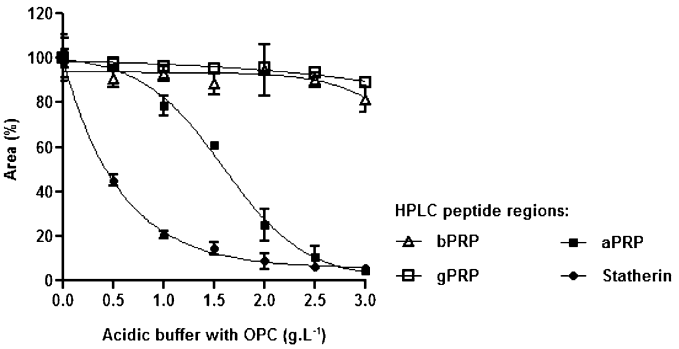

Figure 5 Percentages of area decrease of each HPLC peptide regions after the interaction of AS solution with 20 µL of OPC solutions in acetate buffer matrix. Values are expressed as the arithmetic means of 3 experiments ± standard deviation.

proteins in the precipitates obtained with 10 µL of wines supplemented with 0.5 and 2.0 g·L^{-1} of OPC is not very high because the lowest concentration of condensed tannins leads to depletion of almost all these 2 families of proteins.

For the highest volume of the more concentrated wine (20 µL of red wine supplemented with 3.0 g·L^{-1} of OPC), it is possible to observe the appearance of other proteins belonging to gPRPs in the precipitate. However, regarding bPRPs, small peaks start to be detected in the insoluble aggregates resulting from the interaction with 20 µL of red wine supplemented with 3.0 g·L^{-1} of OPC. The appearance of these groups of proteins in the precipitate is in agreement with their disappearance in the respective supernatant described previously. In general, the first proteins to be precipitated by condensed tannins are aPRPs and statherin, followed by gPRPs.

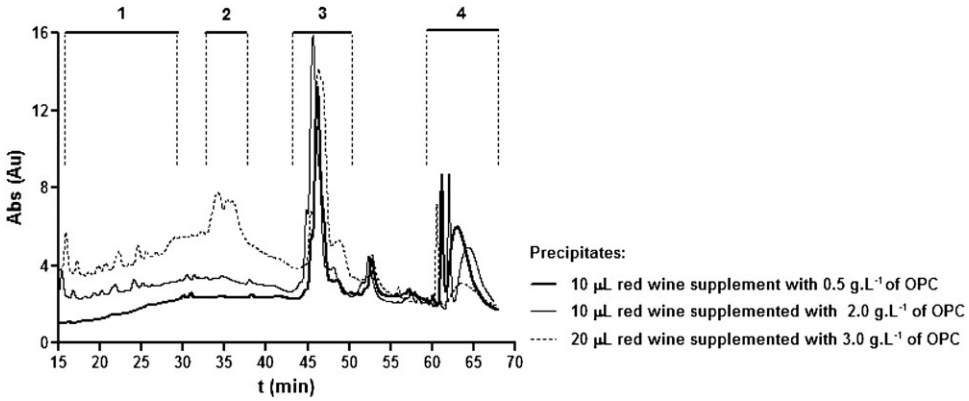

Figure 6 Reverse Phase HPLC profile detected at 214 nm of the precipitates resultant from the interaction of 10 or 20 µL of red wine supplemented with 0.5, 2.0, and 3.0 g·L^{-1} of OPC with the AS.

Table 1 TSA of 50 µL of WS and AS toward red wines supplemented with several concentrations of OPC

Concentration of OPC, g·L^{-1}	0	0.5	1.0	1.5	2.0	2.5	3.0
TSA, NTU·mL^{-1}							
WS	66.6 ± 0.7	83.8 ± 7.3	90.5 ± 1.4	95.0 ± 3.1	86.6 ± 1.5^{a}	103.7 ± 3.2	114.2 ± 4.4
AS	77.8 ± 2.9	98.8 ± 6.5	103.8 ± 0.3	119.4 ± 7.1	97.5 ± 10.7^{a}	129.7 ± 11.7	141.3 ± 9.4
Astringency rating	1.2 ± 0.4^{b}	2.3 ± 1.4^{b,c}	3.0 ± 0.0^{c,d}	4.0 ± 0.0^{c,d,e}	4.8 ± 1.6^{e,f}	6.0 ± 0.0^{f,g}	6.7 ± 0.8^{g}

For the same concentration of OPC, the results of WS and AS are significantly different ($P < 0.05$), except for the 2.0 g·L^{-1} concentration (^{a}, these results are statistically equal). Astringency rating from the sensorial evaluation. The orders with different letters are significantly different ($P < 0.1$).

Overall, these results seem to indicate that statherin and aPRPs have a high relative affinity toward condensed tannins complexation and precipitation comparatively to the other SP, in a competitive assay in a wine matrix. On the other hand, these results seem to indicate that bPRPs, when present in a wine matrix, have a low relative tannin affinity.

In order to study if the proteins present in WS and that are precipitated with TFA are important for the interaction with condensed tannin in wines, the TSA of WS and AS toward the several enriched red wines was also measured (Table 1).

From the results presented in Table 1, it is possible to observe that the aggregation is higher with AS comparatively to WS. However, these differences are more significant for the higher supplemented red wines. Proteins present in WS, such as α-amylase and mucins, that were removed with TFA, seem to affect negatively the formation of aggregates. These results suggest that the AS, which has been analyzed by HPLC, contains the most important proteins that react with condensed tannins and that may contribute to astringency sensation.

As expected, the TSA increased regularly with the concentration of OPC added to wine. This behavior concurs with the perceived astringency of those wines (Table 1). The analysis of the SP families of this AS showed that the different families could have different potentialities in developing astringency. From Figure 4, it is possible to conclude that for low wine volume and therefore lower concentration of tannins (10 µL of wine), the decrease of aPRPs is correlated with astringency intensity. However, for higher concentrations of tannins (20 µL of wine), the relative astringency between wines seems to be correlated to the gPRPs changes.

Conclusion

Astringency is a very complex sensation with different descriptives very hard to define by sensorial analysis. Those descriptives could be related with tannins' structure, concentration, wine medium, and class of SP precipitated.

The results presented show that human AS could be used for an in vitro evaluation of the astringency of a sample because both HPLC and TSA results using AS are in agreement with the results obtained by the sensory panel.

Moreover, this work shows for the first time that increasing the volume of red wine, as well as OPC concentration in red wine matrix, affects differently the several families of SP, with aPRPs and statherin being the most affected either for low volumes as for low concentrations of OPC. Nevertheless, for higher volumes of red wine and higher concentration of OPC, gPRPs are also significantly affected and precipitated. So, it seems that the several SP families have relative discriminatory functions in rating the wine astringency depending on the concentrations of condensed tannins and the volume of wines. In summary, the several families of

SP could be involved in different stages of the development of astringency.

The future work stands in studying the interaction of different human saliva proteins with different classes of tannins.

Funding

This work was supported by Fundação para a Ciência e Tecnologia by one phD grant [SFRH/BD/41946/2007] and one project grant [PTDC/AGR-ALI/67579/2006].

References

ASTM Materials. 1989. Standard terminology relating to sensory evaluation of materials and products. Annual book of ASTM standards. Philadelphia (PA): American Society of Testing and Materials.

Bate-Smith EC. 1954. Astringency in foods. Food. 23:124.

Bennick A. 1982. Salivary proline-rich proteins. Mol Cell Biochem. 45:83–99.

Darné G, Madero TJ. 1979. Mise au point d'une méthode d'extraction des lipides solubles totaux, des glucides solubles totaux et des composés phénoliques solubles totaux des organes de la vigne. Vitis. 18:221–228.

De Freitas V, Mateus N. 2001. Structural features of procyanidin interactions with salivary proteins. J Agric Food Chem. 49:940–945.

De Freitas V, Glories Y. 1999. Concentration and compositional changes of procyanidins in grape seeds and skin of white Vitis vinífera varieties. J Sci Food Agric. 79:1601–1606.

De Freitas V, Glories Y, Laguerre M. 1998. Incidence of molecular structure in oxidation of grape seed procyanidins. J Agric Food Chem. 46:376–382.

Green BG. 1993. Oral astringency: a tactile component of flavor. Acta Psychol. 84:119–125.

Hay DI, Bennick A, Schlesinger DH, Minaguchi K, Madapallimattam G, Schluckebier SK. 1988. The primary structures of six human salivary acidic proline-rich proteins (prp-1, prp-2, prp-3, prp-4, pif-s and pif-f). Biochem J. 255:15–21.

Helmerhorst EJ, Oppenheim FG. 2007. Saliva: a dynamic proteome. J Dent Res. 86:680–693.

Hofmann T, Glabasnia A, Schwarz B, Wisman KN, Gangwer KA, Hagerman AE. 2006. Protein binding and astringent taste of a polymeric procyanidin, 1,2,3,4,6-penta-o-galloyl-α-d-glucopyranose, castalagin, and grandinin. J Agric Food Chem. 54:9503–9509.

Kallithraka S, Bakker J, Clifford MN, Vallis L. 2001. Correlations between saliva protein composition and some t-i parameters of astringency. Food Qual Prefer. 12:145–152.

Lu Y, Bennick A. 1998. Interaction of tannin with human salivary proline-rich proteins. Arch Oral Biol. 43:717–728.

Mateus N, Pinto R, Ruão P, De Freitas V. 2004. Influence of the addition of grape seed procyanidins to port wines in the resulting reactivity with human salivary proteins. Food Chem. 84:195–200.

Mehansho H, Clements S, Sheares BT, Smith S, Carlson DM. 1985. Induction of proline-rich glycoprotein synthesis in mouse salivary glands by isoproterenol and by tannins. J Biol Chem. 260:4418–4423.

Mehansho H, Hagerman AE, Clements S, Butler LG, Rogler J, Carlson DM. 1983. Modulation of proline-rich protein biosynthesis in rat parotid glands by sorghums with high tannin levels. Proc Natl Acad Sci U S A. 80:3948–3952.

Messana I, Cabras T, Inzitari R, Lupi A, Zuppi C, Olmi C, Fadda MB, Cordaro M, Giardina B, Castagnola M. 2004. Characterization of the human salivary basic proline-rich protein complex by a proteomic approach. J Proteome Res. 3:792–800.

Oppenheim FG, Hay DI, Franzblau C. 1971. Proline-rich proteins from human parotid saliva. I. Isolation and partial characterization. Biochemistry. 10:4233–4238.

Oppenheim FG, Xu T, Mcmillian FM, Levitz SM, Diamond RD, Offner GD, Troxler RF. 1988. Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. J Biol Chem. 263:7472–7477.

Pascal C, Poncet-Legrand C, Cabane B, Vernhet A. 2008. Aggregation of a proline-rich protein induced by epigallocatechin gallate and condensed tannins: effect of protein glycosylation. J Agric Food Chem. 56:6724–6732.

Peleg H, Gacon K, Schlich P, Noble AC. 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. J Sci Food Agric. 79:1123–1128.

Preys S, Mazerolles G, Courcoux P, Samson A, Fischer U, Hanafi M, Bertrand D, Cheynier V. 2006. Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. Anal Chim Acta. 563:126–136.

Rossetti D, Yakubov GE, Stokes JR, Williamson AM, Fuller GG. 2008. Interaction of human whole saliva and astringent dietary compounds investigated by interfacial shear rheology. Food Hydrocolloids. 22:1068–1078.

Schlesinger DH, Hay DI, Levine MJ. 1989. Complete primary structure of statherin, a potent inhibitor of calcium phosphate precipitation, from the saliva of the monkey, Macaca arctoides. Int J Pept Protein Res. 34:374–380.

Schwarz B, Hofmann T. 2008. Is there a direct relationship between oral astringency and human salivary protein binding? Eur Food Res Technol. 227:1693–1698.

Shomers JP, Tabak LA, Levine MJ, Mandel ID, Ellison SA. 1982. Characterization of cysteine-containing phosphoproteins from human submandibular-sublingual saliva. J Dent Res. 61:764–767.

Singleton VL, Rossi JAJr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 16:144–158.

Soares S, Vitorino R, Osório H, Fernandes A, Venâncio A, Mateus N, De Freitas V. Forthcoming 2011. Reactivity of human salivary proteins families toward food polyphenols. J Agric Food Chem. 59(10):5535–5547.