SUPPLEMENTARY INFORMATION – S1

Materials and Methods

Isolation and characterization of polysaccharide fractions

Isolation of AGPs and RG II fractions

RG II and AGPs fractions were isolated from wine, following the procedure described in the literature[1,2]. AGPs fraction was also isolated from wine sample fractions after some chromatographic steps (anion-exchange and/or size-exclusion). A final fraction was then purified by affinity chromatography as it is reported in the literature[3]. Both RG II and AGPs fractions were then analyzed in terms of their neutral sugar composition.

Neutral sugar composition

Neutral sugar composition was determined as alditol acetates after TFA hydrolysis (75 min at 120 °C), reduction and acetylation, as described in the literature[4,5]. The different alditol acetates were identified from their retention time by comparison with that of standard monosaccharides. Neutral sugar amounts were calculated relatively to the internal standards (allose and myo-inositol).

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[1] Pellerin, P. et al. (1997). Complexation du plomb dans les vins par les dimères de rhamnogalacturonane II, un polysaccharide pectique du raisin. Journal International Science Vigne Vin 31, 33-41.

[2] Brandão, E. et al. (2017). The role of wine polysaccharides on salivary protein-tannin interaction: A molecular approach. Carbohydrates Polymers 177, 77-85.

[3] Vidal, S. et al. (2003). The polysaccharides of red wine: total fractionation and characterization. Carbohydrate Polymers, 54(4), 439-447.

[4] Apolinar-Valiente, R. et al. (2013). Polysaccharide Composition of Monastrell Red Wines from Four Different Spanish Terroirs: Effect of Wine-Making Techniques. Journal of Agricultural and Food Chemistry 61 (10), 2538-2547.

[5] Ducasse, M.A. et al. (2010). Isolation of Carignan and Merlot red wine oligosaccharides and their characterization by ESI-MS. Carbohydrates Polymers 79(3), 747-754.

SUPPLEMENTARY INFORMATION – S2

Materials and Methods

Fluorescence quenching

Fluorescence quenching studies were done in Horiba Jobin Yvon Fluoromax-4 Spectrofluorometer. Since procyanidins are known to possess intrinsic fluorescence (λ_{ex} max=282 nm) the λ_{ex} was set to 260 nm, and the emission spectrum was recorded from 300 to 500 nm. This λ_{ex} was established since allowed to minimize the maximum absorption of procyanidin B2 (282 nm) and at the same time it was possible to obtain a saliva fluorescence spectrum. Even in these conditions, a residual procyanidin B2 fluorescence was observed. So, a blank of procyanidin B2 was performed and subtracted in all fluorescence experiments with this tannin. Considering polysaccharides at this wavelength no significant polysaccharide fluorescence was noted. The possibility of fluorescence resonance energy transfer (FRET) between SP from saliva and procyanidin B2 was discarded after analysis of both absorption and emission spectra[6].

Due to a higher sensitivity of this technique, PNG and procyanidin B2 concentrations were the lowest used for all techniques (4 μM and 40 μM, respectively). In these experiments, saliva samples (S and DS^{-}) were added to a mixture tannin-polysaccharide (0.0-1.2 $g.L^{-1}$). For each experiment, a relative fluorescence value (%) was calculated as the ratio between the fluorescence of the measured sample (F) and that of the unquenched saliva (F_0, 100%).

[6] Gonçalves, R. et al. (2011). Mechanisms of Tannin-Induced Trypsin Inhibition: A Molecular Approach. *Lamgmuir* 27(21), 13122-13129.

SUPPLEMENTARY INFORMATION – S3

Materials and Methods

Nephelometry measurements

Nephelometry experiments were performed a Horiba Jobin Yvon Fluoromax-4 Spectro Fluorometer which was used as a 90° light scattering photometer. The excitation and the emission wavelengths were set at 400 nm because at this wavelength proteins, tannins and polysaccharides do not absorb the incident light[1,2]. A blank was made for each polysaccharide concentration, using a fixed concentration of tannins or saliva. A relative aggregation value (%) was then calculated as the ratio between the scatter intensity of each sample and the control condition (saliva with tannin in the absence of polysaccharide, 100%).

[1] Carvalho, E. et al. (2006). Influence of wine pectic polysaccharides on the interactions between condensed tannins and salivary proteins. Journal of Agricultural and Food Chemistry 54 (23), 8936-8944.
[2] Soares, S. et al. (2011). Mechanistic Approach by Which Polysaccharides Inhibit alpha-Amylase/Procyanidin Aggregation. Journal of Agricultural and Food Chemistry 57 (10), 4352-4358.

SUPPLEMENTAY MATERIAL – S4

Materials and Methods

SDS-PAGE

For some interactions such as the interactions between S/DS^- samples and tannins (PNG and procyanidin B2) it was necessary carried out more experiments to give evidences about the mechanism by which RG II or AGPs fractions were acting.

This method is based on a separation of proteins according to their sizes using a 16% acrylamide resolving gel. The precipitates were resolubilized in 25 µL of electrophoresis buffer (125 mM Tris-HCl pH 6.8, 20 % v/v glycerol, 4 % SDS, 10 % v/v β-mercaptoethanol, and 0.004 % bromophenol blue) and heated at 90 °C for 20 min.

The running buffer was 0.2 M Tris-HCl pH 8.3, 1.9 M glycine and 0.1 % SDS. Molecular weight markers were broad ranged (Precision Plues ProteinTM Unstained Standards, Bio-Rad). The separation was performed on a Bio-Rad MiniProtean Cell electrophoresis apparatus (Bio-Rad) at constant amperage (0.3 A). After electrophoresis, the gels were stained with Imperial Protein Stain - a Coomassie R-250 dye-based reagent - for 30 min. The destaining step was done by washing the gels overnight with water:methanol:acetic acid (70:20:10 v/v/v).

SUPPLEMENTAY MATERIAL – S5

Results and Discussion

Salivary-proteins (SP) identification

The major families of SP were already identified in previous works by ESI-MS and some of them are presented in Fig. S1[1,2]. After identification of the major proteins, the average molecular weight of each fraction was estimated based on ESI-MS signals intensity: bPRPs (M_w = 5 388 Da), aPRPs (M_w = 14 643 Da), statherin (M_w = 5 232 Da), P-B peptide (M_w = 5 792 Da) and cystatins (M_w = 14 300 Da). gPRPs average molecular weight was determined to be 16 000 Da by static light scattering[2].

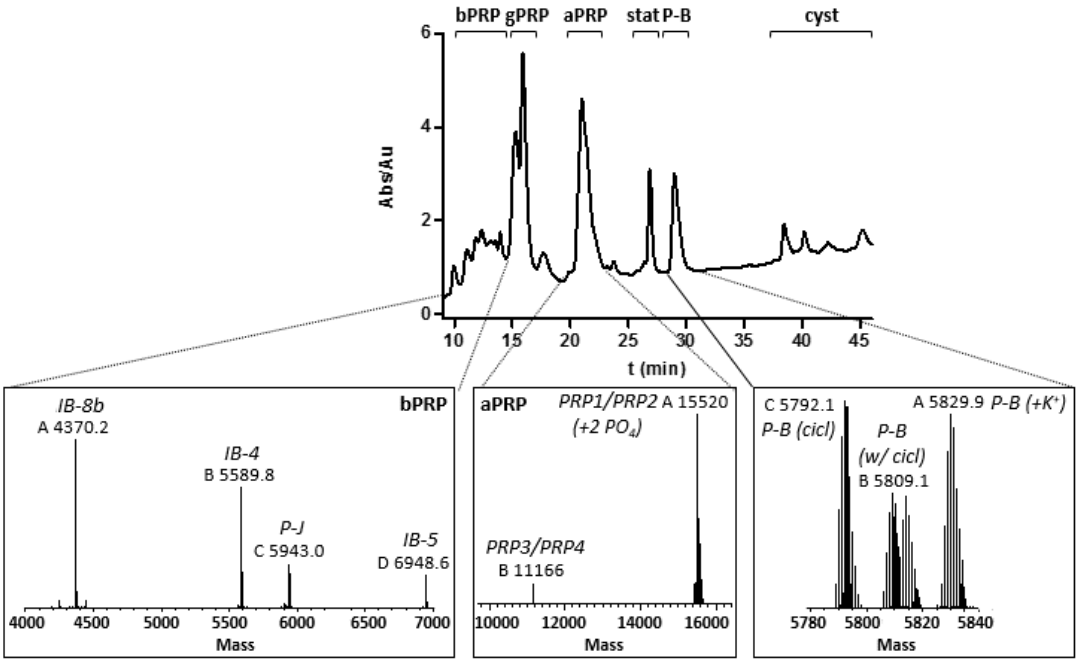

Figure S1. RP-HPLC profile (214 nm) of the acidified saliva used to isolate the different fractions corresponding to the families of PRPs and P-B peptide (upper figure). The identity of the SP families eluted in the different fractions are indicated in the top of the chromatogram. In the bottom, the deconvolution of the mass spectrum outlining the main proteins identified for each HPLC fraction is displayed.

[1] Soares, S. et al. (2011). Reactivity of human salivary proteins families toward food polyphenols. Journal of Agricultural and Food Chemistry 59(10), 5535-5547.

[2] Soares, S. et al. (2018). Study of human salivary proline-rich proteins interaction with food tannins. Food Chemistry 243, 175-185.

SUPPLEMENTAY MATERIAL – S6

Results and Discussion

Nephelometry measurements

Some control experiments with tannins or proteins and increasing polysaccharide concentrations were made to investigate if is there any aggregation between them. In general, for the control experiments between tannins and polysaccharides, the aggregation value increased with the increase of polysaccharide concentration. On the other hand, the aggregation value of the interaction between proteins and polysaccharides remained practically constant as polysaccharide concentration increased. For this reason, the blank of polysaccharides and tannins was subtracted to the aggregation value obtained for each experiment. Values lower than 100% indicate less scattered light, which may be due to smaller or less aggregates

FIGURES

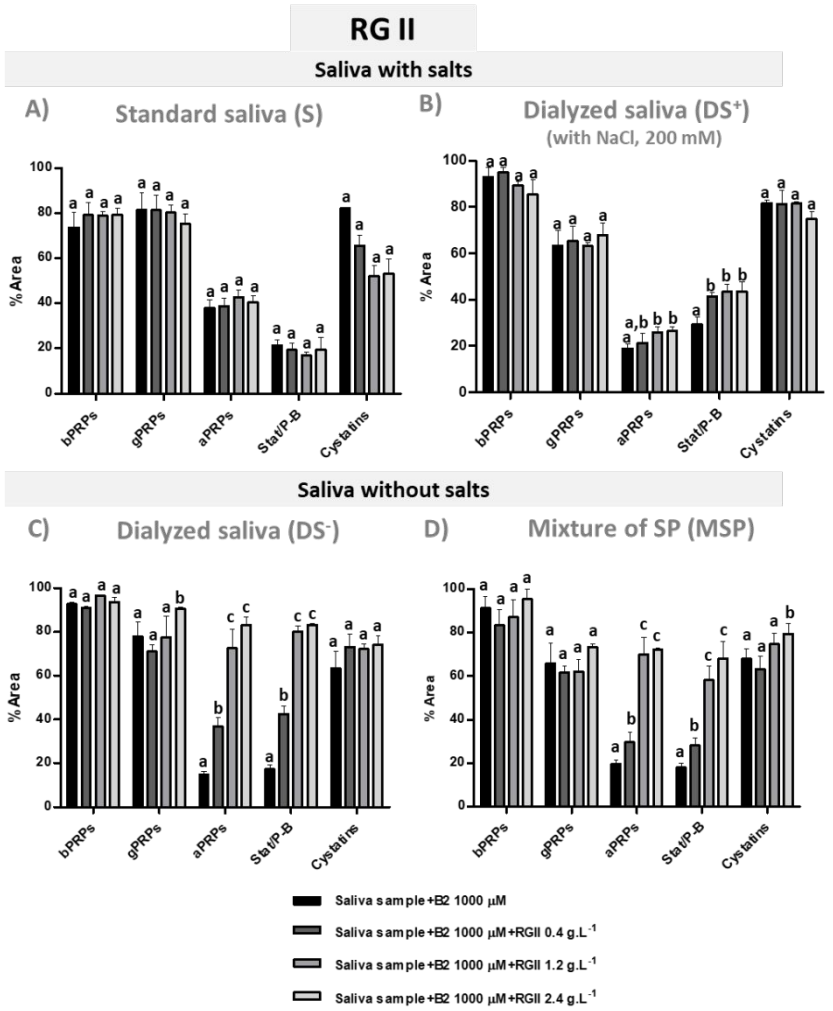

Fig. S2. Influence of concentration of RG II fraction on SP precipitation after interaction between saliva in the absence (DS^- and MSP) or presence of salts (S and DS^+) and procyanidin B2 (1000 µM). (A) S, (B) DS^+, (C) DS^-, and (D) MSP. These results represent the average of three independent experiments. Values with different letters within each column are significantly different ($P \leq 0.05$).

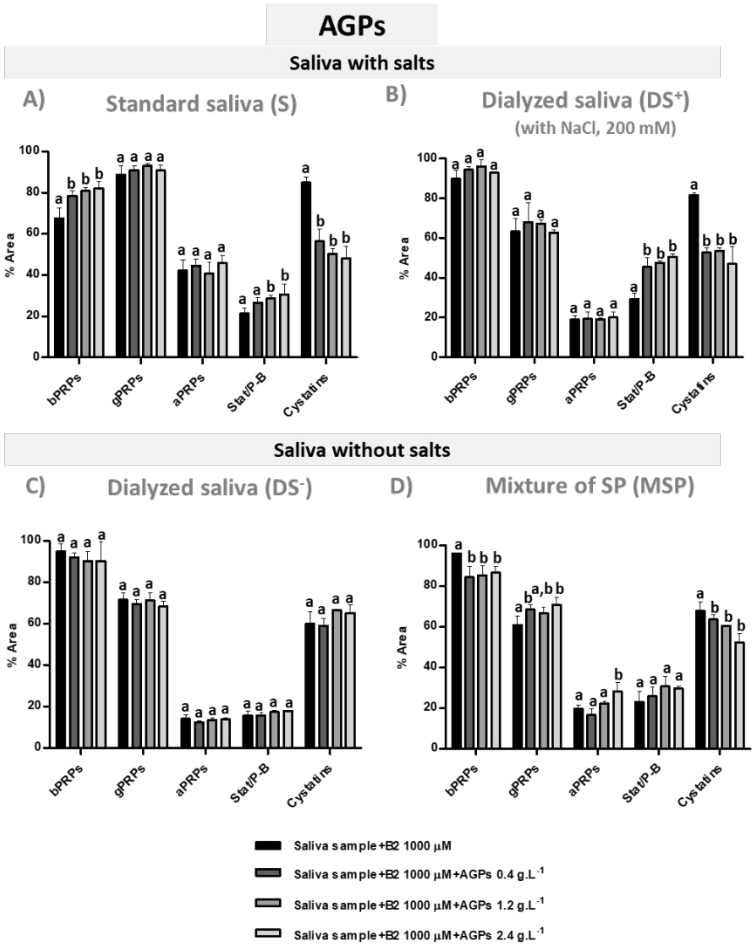

Fig. S3. Influence of concentration of AGPs fraction on SP precipitation after interaction between saliva in the absence (DS^- and MSP) or presence of salts (S and DS^+) and procyanidin B2 (1000 µM). (A) S, (B) DS^+, (C) DS^-, and (D) MSP. These results represent the average of three independent experiments. Values with different letters within each column are significantly different ($P \leq 0.05$).

TABLES

Table S1 – Neutral monossacharides composition (% molar) of the RG II and AGPs fractions.

	RG II	AGPs
Ara	25.3	38.5
Gal	15.8	50.8
Man	3.3	8.9
Rha	28.6	-
Xyl	-	-
Glc	2.5	1.9
Fuc	4.2	-
2-O-methyl-fucose	5.7	-
2-O-methyl-xylose	5.1	-
Apiose	9.3	-

Ara – arabinose; Gal – galactose; Man – mannose; Rha – rhamnose; Xyl – xylose; Glc – glucose; Fuc – fucose.