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The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium

Stéphane Vidal[a,b,*], Leigh Francis[a,b], Pascale Williams[c], Mariola Kwiatkowski[a,b], Richard Gawel[a], Véronique Cheynier[c], Elizabeth Waters[a,b]

[a]*The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia*
[b]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia*
[c]*Unité Mixte de Recherche Sciences pour l'Oenologie, INRA, 2, place Viala, 34060 Montpellier cédex, France*

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Abstract

Two fractions containing the major polysaccharides present in wine were isolated, one comprising a mixture of neutral polysaccharides, mannoproteins and arabinogalactan-proteins, and the other containing the acidic polysaccharide, rhamnogalacturonan II. A grape anthocyanin fraction was also prepared. A trained sensory panel, using formal sensory descriptive analysis methods to rate the intensity of mouthfeel attributes while the samples were held in the mouth and after expectoration, individually assessed the fractions, dissolved in a model wine at levels commonly encountered in red wines. Both polysaccharide fractions significantly increased the 'fullness' sensation above that of the base wine. The rhamnogalacturonan II fraction significantly decreased the attribute ratings associated with the astringency of the model wine whereas the neutral wine polysaccharide fraction had less affect on reducing the ratings for these attributes. The anthocyanin fraction tended to increase 'fullness' although the effect was not great enough to be statistically significant. Unlike the polysaccharides, this fraction also increased perceived astringency but this effect could be due to the presence of some derived tannins in the sample.

Keywords: Grape; Anthocyanins; Mannoproteins; Type II arabinogalactan-proteins; Rhamnogalacturonan II; Sensory descriptive analysis

1. Introduction

The macromolecular fraction of red wines is mainly composed of polysaccharides and polyphenolic compounds, such as proanthocyanidins (condensed tannins). The contribution of proanthocyanidins to the astringency of wine has been investigated by a number of groups (Brossaud, Cheynier, & Noble, 2001; Kallithraka, Bakker, Clifford, & Vallis, 2001; Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003) and these compounds are also believed to contribute to colour stability in red wines (Somers & Verette, 1988). The importance of anthocyanins and derived pigments to wine colour has already been established (Singleton, 1972; Somers, 1971); however, the extent to which these pigments contribute to the mouth-feel properties of wine is not clear. Anthocyanins have been reported, from an informal assessment, to have only a mild indistinctive flavour (Singleton & Noble, 1976). It has been suggested that anthocyanins could modulate the astringency perception in wine, either directly or through reactions with astringent proanthocyanidins that occur during ageing (Singleton & Noble, 1976). More recently, it has been observed that an anthocyanin fraction complemented grape proanthocyanidin astringency and did not contribute bitterness (Brossaud et al., 2001).

In comparison to wine polyphenolic compounds, wine polysaccharides, the other main macromolecular fraction in red wines, have been less studied. In the past decade, their structures have been characterised (Pellerin, Doco, Vidal, Williams, Brillouet, & O'Neill, 1996; Pellerin, Vidal, Williams, & Brillouet, 1995; Vidal,

Abbreviations: RG-II, rhamnogalacturonan II; MP, mannoproteins; AGP, arabinogalactan-proteins; GC, gas chromatography; ACN, anthocyanins

* Corresponding author. Fax: +61-8-8303-6601.
E-mail address: stephane.vidal@awri.com.au (Stéphane Vidal).

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Doco, Williams, et al., 2000) and some of their properties determined (Riou, Vernhet, Doco, & Moutounet, 2001; Vernhet, Dupre, Boulange-Peterman, Cheynier, Pellerin, & Moutounet, 1999; Vernhet, Pellerin, Prieur, Osmianski, & Moutounet, 1996). The main polysaccharides in wine are grape-derived type II arabinogalactan-proteins (AGP), and rhamnogalacturonans (RG-II), and yeast-derived mannoproteins (MP) (Pellerin & Cabanis, 1998).

Wine polysaccharides are regarded as probably being responsible for 'mellowness' (Semichon & Flanzy, 1927) but, to our knowledge, no rigorous sensory studies on purified wine polysaccharides have been carried out to determine their organoleptic properties in wine. Like that of anthocyanins, the role of polysaccharides in mouth-feel properties of wine may possibly be direct, by contributing mellowness, or indirect, through modulating astringency due to proanthocyanidins. There is currently no firm evidence for a direct effect of wine polysaccharides on mouth-feel properties of wine. One study demonstrated that removal of mannoproteins and other high molecular mass compounds from a Riesling wine by ultrafiltration had no impact on the sensory properties of that wine (Will, Pfeifer, & Dietrich, 1991). An indirect role for wine polysaccharides may be more likely. Smith and Noble (1998) showed that the extent and duration of the astringency sensation from a grape seed extract containing, inter alia, proanthocyanidins, was decreased in the presence of carboxymethyl cellulose. They speculated that this was because the viscosity of carboxymethyl cellulose compensated for the loss of lubrication caused by salivary protein precipitation by proanthocyanidins.

It was recently shown that wine RG-II favoured the self-aggregation of grape seed proanthocyanidins in wine-like solutions and indeed may have co-aggregated with the proanthocyanidins whereas wine MP and AGP tended to inhibit proanthocyanidin aggregation (Riou et al., 2001). Thus, in addition to wine polysaccharides providing increased viscosity, it is possible that the presence of polysaccharide–proanthocyanidin complexes in wine may also reduce astringency. This scenario appears to occur in persimmon fruit and juice, natural products relatively high in astringency, like red wine. Soluble persimmon pectins reduced the astringency of persimmon juice and isolated persimmon proanthocyanidins in solution (Taira & Ono, 1997) and this was considered to be due to the formation of soluble proanthocyanidin–pectin complexes that led to the precipitation of fewer salivary proteins than the persimmon proanthocyanidins alone. The existence of different classes of polysaccharides has been postulated, some being inhibitors of proanthocyanidin precipitation by proteins and others having no influence on that precipitation (Haslam, 1998).

In the present study, we isolated polysaccharide fractions and anthocyanins in sufficient amounts to carry out their sensory descriptive analysis. The aim of this work was to determine the intrinsic sensory properties of these classes of compounds in a model wine system before investigating whether these compounds modulated the astringency of grape proanthocyanidins in this medium. Two polysaccharide fractions were prepared, one containing the two main neutral polysaccharides in wine, AGP and MP, and the other containing the main acidic wine polysaccharide, RG-II.

2. Materials and methods

2.1. Purification of anthocyanins

Grapes (50 kg) of *Vitis vinifera* cv. Shiraz harvested at commercial maturity at the INRA experimental station of Pech-Rouge, France were manually peeled. Skins were immediately frozen in liquid nitrogen and ground using a Dangoumau blender (Prolabo, France). The resulting powder was sequentially extracted with ethanol/water (12:88, v/v), methanol and acetone/water (60:40, v/v), as described by Vidal et al. (2003). The acetone extract (2 l) was recovered by coarse filtration, acetone removed under vacuum at 30 °C and the extract freeze-dried.

This extract was loaded on Toyopearl TSK HW-50(F) gel from Tosoh Corp. (Tokyo, Japan), packed in a semi-preparative scale column (200×25 mm) and equilibrated in ethanol–water–TFA (55:45:0.05, v/v), using the chromatographic conditions previously described (Vidal et al., 2003). Anthocyanins were eluted with two bed volumes of ethanol–water–TFA (55:45:0.05, v/v). This eluate was concentrated under vacuum and loaded on a divinyl benzene–polystyrene (DVB–PS) resin column (25×50 cm) previously equilibrated in water for further purification. Elution was carried out under gravity. The column was sequentially washed with water (three bed volumes) and diethyl ether (two bed volumes) to eliminate flavan-3-ol monomers and simple phenolics, followed by ethyl acetate (two bed volumes) to remove flavonols and oligomer flavanols and, as a last step, methanol (one bed volume) was used to recover the anthocyanins adsorbed on the resin. The anthocyanin fraction (ACN) was lyophilised (1.9 g) and stored at room temperature under nitrogen.

2.2. Purification of polysaccharides

A fraction of neutral polysaccharides isolated from ethanol-precipitated wine colloids, using an anion-exchange method in a previous study (Pellerin et al., 1995), was used in this study. This fraction corresponds to the unbound fraction recovered from the anion-exchanger and referred to as AGP0 (Pellerin et al., 1995).

Acidic polysaccharides were purified from by-products of a commercial apple juice producer, "Les Vergers de Chateaubourg" (Chateaubourg, France). The retentate (3000 l) obtained after ultrafiltering (M8-Tech Sep membrane cut-off 50 kDa) apple juice, prepared by a total liquefaction process, was treated with pectolytic enzymes (Rapidase Liq, Gist-brocades, The Netherlands, 0.8 ml per l of retentate). Solid material remaining after this treatment was removed by filtration on a rotary filtration system with diatomaceous earth as the filtering agent. After concentration under vacuum to 40 l, the acidic polysaccharides were precipitated by the addition of 5 volumes of cold (4 °C) 96% (v/v) ethanol. Approximately 10% of the precipitate was resuspended in water (approximately 1 volume, 1 l) and dialysed (10–20 kDa cut-off membranes, Roth, Germany) against water for 24 h in a continuous system. The volume was adjusted to 10 l with 30 mM acetate buffer pH 5 and the sample loaded at 75 ml/min, using a Dynamax pump (Rainin, USA), on 6 l of Fractogel DEAE 650 M (Merck, Germany) equilibrated in 30 mM acetate buffer pH 5. After washing the column with the same acetate buffer (2 bed volumes), acidic polysaccharides were eluted by the same eluant containing 0.2 M NaCl (2 bed volumes). The fraction was desalted and concentrated by ultrafiltration on a 10 kDa cut-off membrane (Sartorius, Germany) and freeze-dried to give the acidic polysaccharide fraction.

2.3. Analytical methods

The intrinsic viscosity of the polysaccharide fractions was determined using an AVS-400 capillary viscosimeter (Schott Gerate, Germany) as described previously (Pellerin et al., 1995).

The molecular size of the RG-II fraction was determined by high resolution size-exclusion chromatography (HRSEC) using a Superdex-75 HR column (1×30 cm; Pharmacia) at 0.6 ml/min equilibrated in 30 mM ammonium formate pH 5.2, the elution being monitored with a Erma (ERC, Japan) refractometer. The column was calibrated using a Shodex pullulan standard P 82 kit (P-5, $M_W = 5800$; P-10, $M_W = 12\,200$; P-20, $M_W = 23\,700$; P-50; $M_W = 48\,000$; P-100; $M_W = 100\,000$; P-200, $M_W = 186\,000$; P-400, $M_W = 380\,000$; Showa Denko K.K.)

The neutral and acidic glycosyl-residue compositions of the wine polysaccharide fractions were determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C for 18 h), by gas chromatography (GC) of their per-*O*-trimethylsilylated methyl glycoside derivatives as previously described (Vidal, Doco, Moutounet, & Pellerin, 2000).

The glycosyl-linkage composition of the polysaccharides was determined by GC of the partially methylated alditol acetates. The procedure (Lerouge, O'Neill, Darvill, & Albersheim, 1993) was adapted for grape and wine polysaccharides as described previously (Vidal et al., 2000).

Direct HPLC analyses of the anthocyanin fraction were performed using a Waters Millenium HPLC-DAD system (Milford, MA) as described by Vidal et al. (2003). Anthocyanins were dissolved in 1% aqueous HCl. The elution was monitored on a Waters 996 photodiode array detector and Millenium 32 software. A calibration curve and response coefficient was based on peak areas at 520 nm and established using malvidin-3-glucoside prepared in the laboratory.

The anthocyanin fraction was submitted to thiolysis under the conditions described previously (Souquet, Cheynier, Brossaud, & Moutounet, 1996). Quantification of each terminal and extension unit was based on peak areas at 280 nm and calibration with external purified standards (Souquet et al., 1996).

2.4. Sensory analysis

2.4.1. General

A panel of 15 volunteer judges (13 males and 2 females, average age 35 years) from the Australian Wine Research Institute and from the University of Adelaide was convened. All panellists had participated on previous mouth-feel sensory descriptive panels. Furthermore, the panellists had regularly participated in aroma descriptive analysis and quality scoring wine sensory panels. The anthocyanin, neutral and acidic polysaccharide fractions were dissolved in model wine (ethanol/water 13:87 v/v saturated with potassium hydrogen tartrate, pH 3.6) at 0.5, 0.5 and 0.2 g/l, w/v, respectively, immediately before each sensory session as required.

2.4.2. Training

The panel attended daily training sessions for three weeks (15 sessions in total). The first part of the training was devoted to generating terms associated with the tactile sensations while the fractions were held in the mouth or after expectoration ('spitting'). The terms used were primarily derived by tasting the fractions from the study, but to aid term development, red wines and standards such as carboxymethylcellulose (2 g/l), glycerol (20 g/l), quinine sulfate (15 mg/l) or aluminium sulfate (2 g/l) were also presented. Any redundancy was discussed at the end of each session. The members of the tasting panel were very familiar with the tactile sensations of proanthocyanidins and there was good agreement among panel members on terms associated with these sensations because this work was performed immediately following a sensory descriptive analysis study of grape and apple proanthocyanidins (Vidal et al., 2003).

For the terms describing the surface smoothness sensation, touch standards (emery papers and silk cloth)

were used as recommended by Gawel, Oberholster, and Francis (2000). A list of definitions of the descriptors, as agreed by the tasters, is presented in Table 1.

The second part of the training dealt with rating the intensity of the different attributes using a labelled magnitude scale (Green, Dalton, Cowart, Shaffer, Rankin, & Higgins, 1996), with scores from 0 to a maximum of 10. After a series of discussion sessions, several rating sessions were carried out in isolated booths to familiarise the panellists with the concept of the computerised use of this scale and for practice. Data were collected using Fizz software (Biosystemes, Couternon, Version 1.3). The consistency of judges' ratings was evaluated before the formal sessions were started, and feedback provided to the judges.

2.4.3. Formal tasting

The samples (20 ml) were presented in coded black plastic disposable cups, with assessments carried out in isolated booths under virtual darkness, with lighting provided solely by the computer screen, at 22–24 °C in isolated booths. Each of the four samples under investigation (the three fractions added to the model wine, and the model wine with no addition) were presented at each of three sessions, with a randomised presentation order across panellists; thus each fraction was assessed in triplicate in a complete block design. The panellists were forced to have a rest of 30 s before being allowed to assess the next sample. Panellists were asked to score the intensity of the attributes while holding the sample in the mouth and also after expectoration.

2.4.4. Data analysis

All attribute variables were analysed with a mixed model of ANOVA, with judges treated as a random effect. Contrast tests were performed for each fraction versus model wine. Statistical analyses were performed both with Fizz (Version 1.3, Biosystemes, Couternon, France) and JMP™ (Version 3.1, SAS Institute, Cary, NC, USA) software.

Table 1
Sensory attributes rated by the panel and their definitions

Term	Definition
In mouth	
Fullness	Feeling of full, rounded sensation in the mouth, related to a 2 g/l carboxymethyl cellulose aqueous solution
In mouth and after expectoration	
Roughness	A roughening sensation felt on mouth surfaces when the different surfaces come into contact with each other
Dry	Feeling of desiccation or lack of lubrication in the mouth
Chalky	Feeling of fine particulate matter that mouth movements can displace
After expectoration	
Astringency	Overall level of astringent sensation, encompassing the above terms
Bitter	Bitterness, 15 mg/l aqueous quinine sulfate solution

3. Results and discussion

3.1. General

The aim of this study was to determine the influence that wine polysaccharides and anthocyanins could have on the mouth-feel properties of wine. Anthocyanins were purified from grape skin extract rather than wine to avoid, as far as possible, the presence of anthocyanin adducts that can form during fermentation and wine ageing (Mateus, Silva, Vercauteren, & Freitas, 2001; Somers, 1971). A neutral polysaccharide fraction, containing the two major neutral wine polysaccharides, mannoproteins (MP) and type II arabinogalactan-proteins (AGP), was purified from wine. An acidic polysaccharide fraction analogous to the major acidic polysaccharide in wine, rhamnogalacturonan II (RG-II), was isolated from apple. The structure and composition of RG-II is known to be well conserved within the plant kingdom and it has already been shown that RG-II in wine and RG-II isolated from apples, as described in this work, share similar structural properties (Doco, Williams, Vidal, & Pellerin, 1997), the minor discrepancies observed being restricted to the presence of different terminal sugars on some of the side-chains.

3.2. Characterisation of polysaccharide fractions

Glycosyl-residue analysis (Table 2) of the polysaccharide fractions revealed that the composition of the neutral fraction was dominated by mannose, galactose and arabinose, the main components of MP and AGP. The acidic fraction was mainly composed of RG-II, as shown by the simultaneous presence of its diagnostic sugars (apiose, 2-*O*-Me-fucose, 2-*O*-Me-xylose, aceric acid (3-*C*-carboxy-5-deoxy-L-xylose), DHA (3-deoxy-D-*lyxo*-heptulosaric acid) and KDO (3-deoxy-D-*manno*-octulosonic acid) in a molar ratio in accordance with the previously published composition for RG-II (Pellerin et al., 1996). Glycosyl-linkage composition analysis confirmed that the neutral fraction was composed of type II arabinogalactan-proteins (40%) and yeast mannoproteins (60%). The structural analysis of the acidic fraction revealed all the acetylated methyl ethers that are markers of RG-II molecules. HRSEC analysis showed that RG-II was exclusively present as a dimer in this fraction (data not shown), the predominant form in fruit-derived products (Doco et al., 1997).

Table 2
The glycosyl-residue composition (molar ratio) of polysaccharide fractions

Glycosyl-residue	MP + AGP	RG-II
Arabinose	11.8	6.9
Rhamnose	0.4	18.8
Galactose	27.8	7.2
Glucose	2.6	nd[a]
Mannose	57.4	3
Apiose	nd	2
Fucose	nd	3.5
2-*O*-Me-Fucose	nd	1.6
2-*O*-Me-Xylose	nd	1.9
Xylose	nd	5.9
Galacturonic acid	nd	43
Glucuronic acid	nd	1.9
Aceric acid	nd	1.6
DHA	nd	1.3
KDO	nd	1.5

[a] nd, non detected.

3.3. Characterisation of the anthocyanin fraction

The anthocyanin fraction obtained from grape skin extracts was composed of the typical series of grape anthocyanidin glucosides along with the corresponding acylated forms (acetylated and *p*-coumaroylated) but dominated by malvidin-3-glucoside. Additional material with absorbance at 280 nm, eluting as a broad unresolved 'hump' on the baseline between 20 and 40 min, accounted for 22% of the total peak area at 280 nm. The fraction was resistant to thiolysis (data not shown), demonstrating that the unresolved material probably did not contain grape proanthocyanidins but indicating that it may consist of derived-tannins formed in vivo or during the extraction steps.

3.4. Sensory analysis

3.4.1. Generated vocabulary

The polysaccharide and anthocyanin fractions were dissolved in model wine, an ethanolic solution saturated with potassium hydrogen tartrate pH 3.6, at concentrations close to those encountered in wines. The judges generated descriptive terms during the training sessions whilst tasting these fractions. Model wine itself was perceived as slightly astringent in line with previous observations that acids can elicit the sensation of astringency (Rubico & McDaniel, 1992). Rather than the acid nature, pH is considered to be the key parameter influencing astringency level (Lawless, Horne, & Giasi, 1996).

The specific descriptors (Table 1) generated by the panellists were derived predominantly from previous studies dealing with wine mouth-feel sensations (Gawel et al., 2000; Gawel, Iland, & Francis, 2001).

3.4.2. Analysis of variance

The data generated by the tasters during the formal sessions were examined by ANOVA and their reproducibility, discrimination and degree of agreement were examined. Four of the judges did not agree with the rest of the panel in their rating of several of the attributes, notably 'fullness', as assessed by large negative correlation coefficients between pairs of judges and between the judges' scores and the panel mean scores. Consequently, the results of 11 judges were retained for the final data analysis (ANOVA).

With the exception of bitterness, significant differences among fractions were observed for all the attributes.

3.4.3. Pair wise comparison of fractions

The mean scores for each sample, for each attribute, are shown in Table 3. Contrast comparison tests were used to determine the P values for significant differences between the scores for the samples and that of the model wine (Table 3).

3.4.4. Sensory properties of the polysaccharide fractions

The effects of both polysaccharide fractions on all the descriptors followed the same trends but with clear differences regarding the magnitude of the effects.

First of all, RG-II and neutral polysaccharides each increased the 'fullness' sensation above that of the base model wine, at a significance level of $P<0.05$ and $P<0.01$, respectively (Table 3). The fraction containing the neutral polysaccharides was found to have twice the intrinsic viscosity of that of RG-II (data not shown) and this probably explains why the panel gave a higher 'fullness' rating to this fraction. Amongst winemakers, mannoproteins are commonly believed to contribute to mellowness. The involvement of RG-II in such a sensation was, however, rather unexpected. As far as we know, our data represent the first evidence that isolated wine polysaccharides are likely to be involved in the fullness characteristics of wines.

Secondly, the two polysaccharide fractions were also perceived to be less intense in the astringent descriptors than the model wine alone. This result agrees with the previously reported observation that maximum intensity and total duration of astringency elicited by organic acids were decreased as the viscosity of the solution increased (Smith & Noble, 1998).

Our data showed that RG-II was responsible for a significant decrease of overall astringency of the model wine and also each of the other sub-qualifying astringent descriptors, both in the mouth and after expectoration, except for 'chalky' scored after expectoration (Table 3). 'Roughness' after expectoration and 'dry', scored in mouth or after spitting, were particularly affected by RG-II. Since these attributes describe astringent sensations localised on the mouth surfaces

Table 3
Mean attribute ratings[a] for the samples scored by the panel and estimates of significance[b] for the comparison between each fraction and the model wine

Attributes	Model wine	MP + AGP	RG-II	ACN
In mouth				
Roughness	0.57	0.45 ns	0.34*	0.94***
Chalky	0.3	0.21 ns	0.15*	0.42 ns
Dry	0.75	0.7 ns	0.55**	0.96**
Fullness	1.34	1.82***	1.67**	1.57 ns
After spitting				
Roughness	0.87	0.72 ns	0.55***	1.26***
Chalk	0.33	0.34 ns	0.26 ns	0.64***
Dry	1.29	1.08*	1.02**	1.41 ns
Astringency	1.16	1.07 ns	0.98*	1.44**
Bitter	0.32	0.37 ns	0.32 ns	0.42 ns

[a] Three repetitions × 11 panellists.
[b] Significance calculated with contrast tests of each fraction versus model wine: ns, not significant. *$P < 0.1$; **$P < 0.05$; ***$P < 0.01$.

and probably due to changes in mouth lubrication (Table 1), it is possible that the viscosity of RG-II compensates for the decreased mouth lubrication. This hypothesis is also in good agreement with results indicating that soluble pectins reduced persimmon tannin-mediated astringency (Taira & Ono, 1997) and that carboxy-methyl-cellulose, a viscous additive with similar properties to wine polysaccharides, decreased the perceived astringency of grape seed tannin (Smith & Noble, 1998).

The neutral polysaccharide mixture (MP + AGP) also tended to decrease the intensity of astringent attributes, but only the dry attribute scored after expectoration was significantly decreased ($P < 0.1$). This fraction was twice as viscous as the RG-II fraction, suggesting that viscosity is not the only factor responsible for the ability of these polysaccharide fractions to decrease perceived astringency. Indeed the recent observation that RG-II may have co-aggregated with proanthocyanidins (Riou et al., 2001) suggests that complexation with astringent compounds (Haslam, 1998) as well as an ability to provide mouth lubrication (Lyman & Green, 1990) may both be important factors.

3.4.5. Anthocyanin fraction

Unlike polysaccharides, anthocyanins contributed to an increased overall astringency when compared to model wine. Only some of the astringent attributes were significantly affected (Table 3). 'Dry', 'chalk' and 'overall astringency' descriptors were also used in a previous study dealing with the determination of the influence of the structural features of grape proanthocyanidins on astringency (Vidal et al., 2003). A comparison of the mean ratings for these particular attributes, in both studies, revealed that the anthocyanin fraction was scored lower than the least astringent proanthocyanidin fractions previously tasted. Thus, even though significant increase of intensities could be observed for the anthocyanin fraction, this increase was probably relatively unimportant. This tannin-like effect of the anthocyanin fraction on the astringent attributes, nevertheless needs to be further investigated as it is possible that the presence of derived-tannins in this fraction may be responsible for the observed enhancement of astringency. On the other hand, this anthocyanin fraction, dissolved in the model wine, increased the fullness perception of the solution slightly, although this difference was not large enough to be statistically significant.

4. Conclusions

This study has provided evidence that wine polysaccharides probably play an important role in the mouth-feel properties of wine. Both neutral and acidic polysaccharide fractions examined are likely to contribute a 'fullness' sensation to wine. The observation that the acidic polysaccharide, RG-II, significantly decreased the astringency of the model wine solution, whereas the neutral polysaccharide fraction had less effect, strongly suggests that the composition and structure of polysaccharide fractions, not just their overall viscosity, influence their capacity to decrease astringency.

The anthocyanin fraction studied only slightly increased astringency of the model wine solution to which it was added but this result remains to be confirmed due to slight contamination of the fraction by other unknown phenolic compounds.

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