Effect of β-lactoglobulin on perception of astringency in red wine as measured by sequential profiling

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Abstract

Astringency is a predominant sensory attribute that influences the overall quality of red wine. The application of whey proteins as functional and nutritional food additives is popular but their use is uncommon to enology. Here whey proteins as a suitable food component to improve the sensory quality of red wine were investigated. This work focused on the sensory perception of astringency in red wine treated with β-lactoglobulin and gelatin. Ovalbumin precipitation method was used to assess astringency pre- and post-treatment and compared to the perceived astringency. A sequential profiling sensory technique was used to evaluate astringency in relation to other attributes over repeated consumption of red wine. The intensity of astringency increased insignificantly over repeated sips at 60 sec intervals for the treated and untreated red wine. The difference in astringency perception ($p < 0.05$) between the wine samples was shown at 30 secs after swallowing. Wines treated with β-lactoglobulin and gelatin significantly reduced astringency and the total polyphenol content. The reduction in astringency indicates that these proteins actively bind and precipitate polyphenols which are known to contribute to perception of astringency. Furthermore, the good agreement between the chemical and sensory methods supports this mechanism for reduction of astringency.

Keywords: β-lactoglobulin, astringency, wine, gelatin, sequential profiling

1. Introduction

Red wines, beer, tea, fruits and vegetables are rich in polyphenols, which contribute to their sensory properties. Tannins are a major polyphenol group divided into hydrolysable and condensed tannins. Red wine, a fermented grape derived drink is rich mainly in the condensed tannins. The biological activities of tannins include their ability to interact with, and precipitate, proteins. Tannins contribute to the perception of astringency, which is described as a mouth feel of dryness, roughness and a puckering sensation on the oral cavity before and after ingestion of drinks such as red wine (Bacon and Rhodes, 2000; de Freitas and Mateus, 2001) and influences the overall quality and consumer acceptance of the wine. Wine makers treat red wine with protein-fining agents for the removal of protein- reactive tannins thus modulating astringency to a level that produces good organoleptic properties. The common fining proteins derived from animals include gelatin, egg ovalbumin, and caseinates which are positively charged and interact with the negatively charged tannins in red wine by a mechanism similar to that which occurs during wine tasting. Proteins derived from corn, soy, lentils, pea, rice potatoes (Simonato, Mainente, Selvatico,Violoni, & Pasini, 2013; Granato, Ferranti, Iametti , & Bonomi, 2018; Kang, Niimi, & Bastian, 2018; Gambuti, Rinaldi, Romano, Manzo, & Moio, 2016), grape seed extracts and pomace (Gazzola, Vincenzi, Marangon, Pasini, & Curioni, 2017; Jiménez-Martínez, Gil-Muñoz, Gómez-Plaza, & Bautista-Ortín, 2018) and fibre (Gil, Del Barrio-Galán, Úbeda, & Peña-Neira, 2018) were reported to reduce astringency by the removal of proanthocyanidins in wines. The mechanism for astringency perception has been reported to result from interactions of tannins with salivary proline-rich proteins in the mouth. Astringency is a tactile sensation that has been associated with alteration of mouth lubrication (Rossetti, Yakubov, Stokes, Williamson, & Fuller, 2008 and Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009) and increasing mouth friction (Dinnella, Recchia, Vincenzi, Tuorila, & Monteleone, 2009). Nongustatory mucosal surfaces and tissue movement are involved in the mouth friction, supporting astringency as a tactile sensation (Soares, Brandão, Mateus, & De Freitas, 2015). Astringency builds-up upon repeated tasting and involves a mechanical process as a sensation rather than a chemosensory process (Dinnella et al., 2009) such as taste. Astringency development and the intensity of its perception depend on the tannin and protein structure (Vidal et al., 2003; Sun et al., 2013; Soares, Sousa, Mateus, & De Freitas, 2012) and individual response, saliva characteristics (Dinnella et al., 2009 and Dinnella, Recchia, Vincenzi, Tuorila, & Monteleone, 2010), salivary flow rate (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006) and medium constituents including pH, ethanol, and polysaccharides (Rinaldi, Gambuti, & Moio, 2012a; Carvalho et al., 2006).

Attempts have been made to use instrumental methods such as chromatography (Kennedy, Ferrier, Harbertson, & Des Gachons, 2006), colourimetry (Cáceres-Mella et al., 2013; Aleixandre-Tudo, Buica, Nieuwoudt, Aleixandre, & du Toit, 2017), Nephelometric (Monteleone, Condelli, Dinnella, & Bertuccioli, 2004), methyl cellulose precipitation (Mercurio & Smith, 2008), physical measurements (Laguna, Álvarez, Simone, Moreno-Arribas, & Bartolomé, 2019) and protein precipitation using proteins such as ovalbumin, Saliva, BSA, and Gelatin (Llaudy et al., 2004 ; Rinaldi, Gambuti, & Moio 2012b; Harbertson & Kennedy, 2002 and Glories, 1984) to assess astringency development at a molecular level and to correlate the data with its sensory perception. The assessment of astringency in wine is best quantified through sensory evaluation. The heterogeneous nature of tannins limits the analytical methods used for their quantification and characterization. Various precipitants including proteins and polysaccharides have been employed for the quantification of tannins with varying values obtained (Mercurio & Smith, 2008; Llaudy et al., 2004). Ovalbumin precipitation method was shown to be simple, less time consuming and correlates with sensory evaluation (Llaudy et al., 2004)

Casein and gelatin has been used as a processing aid in the fining of white wine and red wine and this is well researched, unlike the use of whey proteins as fining agents. β-lactoglobulin, is a major whey protein which constitutes 50-58% of the bovine whey proteins. It is a globular protein consisting of beta-sheets and alpha helices, has an established secondary and tertiary structure. It has a molecular weight of 18,300 Da (18 kDa) and its isoelectric point is pH 5.2. One of the characteristics of this protein is that it binds hydrophobic molecules and it can interact with tea polyphenols (Kanakis et al., 2011) and complex with particular polyphenols (von Staszewski et al., 2012). However, there are no reports of its application to the reduction of red wine astringency by binding tannins.

In our previous work (Jauregi, Olatujoye, Cabezudo, Frazier, & Gordon, 2016) we employed an analytical method to assess the effect of β-lactoglobulin in reducing astringency and found that β-lactoglobulin was as effective as gelatin in reducing astringency and had a similar selectivity for the polyphenols which are markers for astringency. β-lactoglobulin was even better as it preserves catechin more than gelatin. Milk proteins are known for allergy. Although allergenic reactions to milk proteins are rare in adults than in children (Asero et al., 2009), in order to protect the sensitive consumers, the absence of β-lactoglobulin residue was ensured by applying a good manufacturing practice that includes the usage of low dose of fining agent and its removal by adequate filtration procedure in our present study. The absence of β-lactoglobulin residues after fining followed

by filtration and centrifugation was investigated and reported in our previous work (Jauregi et al., 2016). This implies potential for its application as a fining agent with no issue with allergenicity. Its greater solubility in wine compared to casein that requires a special dissolution preparation before mixing with wine was also an advantage.

In the present work we aimed at investigating the effect of β-lactoglobulin, in comparison with gelatin, on the perception of in-mouth attributes, particularly astringency, in red wine. The second goal of this study was to ascertain if there is agreement between the chemical method applied in the assessment of astringency and the sensory evaluation of astringency.

2. Materials and Methods

2.1. Materials

All reagents used for the analysis were of analytical grade. Bovine beta-lactoglobulin, bovine serum albumin (BSA), alpha-lactalbumin, bicinchoninic acid solution (BCA), copper sulfate solution, DEAE Sepharose®, ovalbumin, tannic acid, tartaric acid, FolinCiocalteu reagent, gelatin (Type B gelatin from bovine and 75 g bloom strength)were purchased from Sigma-Aldrich, (Dorset, UK). Flat sheet microfiltration membranes (0.45µm), and syringe driven PVDF Filters (0.45µm) were purchased from Millipore Corporation, (Bedford, UK). Potassium monophosphate, potassium diphosphate, sodium hydroxide, sodium carbonate, sodium chloride (NaCl), hydrochloric acid (HCl), trifluoroacetic acid (TFA), methanol, ethanol were purchased from Fisher Scientific (UK limited), Protease N 'Amano' Enzyme from Bacillus subtilitis was purchased from Amano Enzyme Inc., (Nagoya, Japan), Ultrospec 1100 pro UV/Visible Spectrophotometer was from Biochem Ltd., (Cambridge).Eppendorf CentrifugeMinispin plus G was from Fisher Scientific (UK Ltd).Amicon filtration cell was obtained from Amicon® a Grace company. Pasteurized skimmed milk and 100% Tempranillo Red wine, Valdubón (2012), from North Central of Spain (13% alcohol) were purchased from a local store.

2.2. Pilot Plant Production of the β -lactoglobulin rich whey fraction

4L of sweet whey was produced from pasteurized skimmed milk. Skimmed milk was heated to 35 ˚C in a water bath. Commercial rennet was added at a concentration of 0.3 ml per litre of milk with gentle stirring for 2 minutes. Incubation took place for one hour at that temperature and then the casein coagulum was cut in small squares to allow the remaining lactoserum to drain out of it. Incubation was extended for 20 additional minutes

and then the coagulum was scooped and filtered to drain most of the serum with the aid of vacuum. The whey was centrifuged at 3200 rpm to remove the last of the left over casein curds.

On a lab scale, the sweet whey was fractionated to obtain a β-lactoglobulin rich fraction following a method developed in our group based on a combination of adsorption and microfiltration (Welderufael, Gibson, & Jauregi 2012). However, for this work, the microfiltration step was replaced by a centrifugation process. To begin the purification process, 4L of whey (pH 6.4) and 400 ml of resin were placed in a jacketed bioreactor and stirred for 10 min. The mixture was transferred to the centrifuge unit where the non-adsorbed proteins in the supernatant were separated from the adsorbed proteins on the resin (DEAE Sepharose), an anion-exchanger. The resin was washed with 10 mM potassium phosphate buffer at pH 6.5 to further remove the non-adsorbed proteins. The adsorbed proteins include β-lactoglobulin and caseinomacropeptides (CMPs). For an enriched β-lactoglobulin fraction without CMP, a hydrolysis step was introduced while proteins were adsorbed onto the resin. Hydrolysis started after re-solubilising the adsorbed proteins with a pH 7, 10 mM potassium phosphate buffer, at 45°C in a jacketed bioreactor. Then, protease 'N'Amano enzyme was added to the mixture. After 2hrs, hydrolysed CMPs were centrifuged, removed as supernatant and finally, the non-hydrolysed protein remaining, β-lactoglobulin, was desorbed and eluted with a known volume of elution buffer,10 mM potassium phosphate buffer at pH 4.5 containing 0.5 M NaCl. Total protein content was analysed by the bicinchoninic acid assay (BCA) as described in section 2.3.

2.3. Chemical characterization of the β-lactoglobulin whey fraction

Total proteins were quantified according to the bicinchoninic acid assay (BCA). Briefly, 100 µl of standard or sample was mixed with 2 ml of the BCA working reagent (copper sulphate solution: BCA solution at a ratio of 1:50). The mixture was allowed to stand at 37 ^{O}C for 30 min, and then allowed to cool to room temperature for 5 min. Finally, absorbance was read for each sample/standard, at 562 nm within 8 minutes with water as a blank. Bovine serum albumin was used as a standard for protein quantification.

β-lactoglobulin was quantified using RP-HPLC. The samples were filtered with 0.45µm PVDF filter and analysed in a Dionex HPLC fitted with P680 HPLC pump, ASI-100 automated sample injector, thermostated column compartment TCC100, PDA-100 photodiode Array Detector with C18 column (250 x 4.6 mm). A gradient of solvent A which was prepared with 0.1% trifluoroacetic acid in HPLC grade water and solvent B prepared with 0.08% trifluoroacetic acid in HPLC grade acetonitrile was utilised. Solvent B was 0-45% over 60

minutes, 45-70% over 5 minutes, 70% over 10 minutes and solvent A was 100% over 15 minutes. Analysis was carried out using an injection volume of 50µl, flow rate of 0.8 ml/min, the peak areas were monitored at 214 nm and 280 nm while the temperature of the column was maintained at 25 ^{O}C. The standard calibration curve was obtained with β-lactoglobulin.

2.4. Concentrating and desalting whey protein

The β-lactoglobulin enriched fraction was concentrated and desalted by ultrafiltration. 10KDa MWCO Polyethersulfone (PES) membrane was placed into the 150 ml ultrafiltration magnetically stirred Amicon cell. To begin the process, 100 ml of β-lactoglobulin enriched fraction were added to the cell and stirred gently. The solution was filtered through the membrane, with the aid of positive pressure of air (2 bars). The solution volume was reduced to 10 ml. The filtrates and concentrates were analysed for protein content using the BCA method as described. By comparing the protein content of the feed (β-lactoglobulin enriched fractions), the filtrate and the resulting concentrate; the efficiency and low protein binding capacity of the membrane were determined. Concentrating the β-lactoglobulin enriched fraction was necessary in order to avoid diluting the red wine for the treatment with protein.

2.5. Protein-Wine treatment

The concentrated β-lactoglobulin solution and gelatin were added to wine at a final concentration of 0.1 mg/ml and water was added to the untreated sample (control). The protein concentration was chosen based on a previous work in our lab (Jauregi et al., 2016) and it is within the range of level usually for fining. The mixtures were rigorously mixed and allowed to stand for 10 min for adequate contact. Mixtures were centrifuged at 11700g for 10 min and supernatant was collected for astringency measurement and determination of polyphenolic content following the analytical methods described below and for sensory tests. All measurements were carried out in triplicate.

2.6. Analytical method for determination of astringency

Astringency of red wine was determined by the analytical method described by Llaudy et al. (2004) based on the precipitation of tannins by ovalbumin; they also established a correlation between the analytical method and the sensory perception of astringency. Tannic acid and ovalbumin solutions were prepared in a synthetic solution similar to wine. The synthetic solution was prepared with 4 mg/ml of tartaric acid, 95 mg/ml of ethanol and

adjusted to pH 3.5 with 5M sodium hydroxide. Solutions of tannic acid at concentration of 0.0-0.8 mg/ml were used as standards. Ovalbumin solutions at concentrations of 0.0, 0.4, 0.8, 1.6, 2.4, 3.2 and 4.0 mg/ml were used as protein to precipitate astringent tannins. Increasing concentrations of ovalbumin (0.5 ml) were added to tannic acid/red wine in the tubes. The tubes were thoroughly stirred for 10 secs, allowed to stand for 10 mins and then centrifuged at 11700g for 10 mins. Supernatants were diluted 50 times with distilled water and absorbance was read at 280 nm in a quartz cuvette with an optical path of 10 mm; experiments were carried out at room temperature and in triplicate.

2.7. Folin-Ciocalteu method for total polyphenol content

Folin-Ciocalteu's micro method as adapted for wine analysis by Waterhouse (2009) using gallic acid as the standard was used to determine the phenolic content. For the analysis, 20 µl of each calibration solution, treated red wine, red wine or blank were placed in a cuvette, and 1.58 ml water and 100 µL of Folin-Ciocalteu reagent were added, thoroughly mixed and allowed to stand between 30 seconds and 8 minutes. Then, 300 µL of the 20% w/v saturated sodium carbonate solution was added, mixed well and left at 20 ºC for 2 h, after which the absorbance of each solution was read at 765 nm using a spectrophotometer. Results were expressed as Gallic acid equivalents (mg GAE/L).

2.8. Sensory sequential profiling method

A sequential profiling technique was used by a trained expert sensory panel of 12 (n=12, 11 females; 1 male and age range 30-50)), each within a minimum trackable record of 6 months experience. A vocabulary session and three scorings were attended by the trained panel. Consideration was made for the recommended daily alcohol intake for each panelist, ensuring that no more than 0.52 units were consumed in any panel session. All scoring was carried out at room temperature (25 ± 2 ^{O}C) in isolated booths under artificial daylight.

The trained panelists developed seven (7) in-mouth attributes of the red wine in the consensus vocabulary session. These attributes were assessed with a sequential profiling technique, modified from that described by Methven et al. (2010). Sequential profiling was done by repeatedly scoring the attributes over four consecutive aliquots (5 ml) of red wine sample. In the scoring sessions, the trained panels scored the seven attributes as follows (1) during the consumption of each aliquot (SIP) (2) after- taste (AT1) at 30 seconds and (3) aftertaste (AT2) at 60 seconds post consumption. This method enables the dynamic nature of attribute perception to be

captured where the repeated sips at 1 minute intervals is used to simulate a natural wine drinking scenario. However, only four aliquots could be tested in order to control the alcohol intake of the panel.

The seven sensory attributes scored were sweetness, acidity, bitterness, astringency, dark fruity flavour, woody flavour and metallic taste (see Table 1). Although astringency is a quality attribute of wine that takes time to develop and build up upon repeated ingestion, other attributes may also change over repeated ingestion as reported by Meillon, Urbano, & Schlich (2009) with temporal dominance of sensation (TDS). Seven is the maximum number of attributes which can be scored within a sequential profiling method where repeated sipping is set at a minute intervals; if more attributes are used the time taken to score the attributes becomes too long. The attributes were agreed upon by the panel to represent the wine characteristics which appeared to be influenced by either the different samples (to which the panel were blinded) or by the repeated sipping. Sequential profiling which is also a multi-attribute method was chosen over TDS because of our interest in intensity over time rather than the dominance of the sensation. It was also better than time intensity (TI) that would have limited scoring to only one or two attributes hence consuming time. Three red wine samples; control, β-lactoglobulin and gelatin treated wine were sequentially profiled, two samples per day and duplicate scoring sessions were carried out on separate days. Samples were coded with three-digit numbers and all four aliquots of one sample were presented with the same code; panelists were not blinded to the sequential nature of the evaluation. Scoring for each sample set was performed without a resting or rinsing procedure between the 4 aliquots of the same sample. A 2-minutedelay was enforced after each sample during which time the trained panelists were required to cleanse their palates with low salt crackers followed by a water rinse (noting that this would have been a minimum of 3 minutes since tasting the previous sample aliquot). 5 ml of wine samples at 18^{o}C were presented to the trained panelists in ISO approved wine glasses. The intensity of each attribute was rated using an unstructured line scale with the appropriate anchors (0-100) from not to very. Data was acquired using Compusense Cloud sensory software (Ontario, Canada).

2.9. Data Analysis

All Statistical analysis were conducted using SPSS 21.0.Sequential profiling data was subjected to a mixed model analysis, treating the panelists as random factor and samples as fixed factors and the sequential time points (i.e. the 4 consecutive aliquots) as repeated effects. Multiple pairwise comparisons were carried out using Bonferroni. One-way analysis of variance (ANOVA) was used to determine the impact of the treatments on the polyphenolic content and astringency by absorbance measurements followed by a multiple pairwise comparison

using Tukey post hoc test. All data are expressed as the arithmetic mean ± standard deviation of three replicates unless stated otherwise.

3. Results and Discussion

3.1. Whey protein production

Sweet whey (4L) contained 38.24g total protein (9.56g/L) as determined by the total protein assay. The 4L of whey was processed as described in section 2.2 and the enriched β-lactoglobulin fraction analysed for total protein contained 5.68g/L. Protein content of whey and β-lactoglobulin (Table S1) is similar to that reported when prepared on a laboratory scale in our previous paper (Jauregi et al. 2016). The chromatographic profile of the pilot scale protein was also similar to that of the laboratory scale (Fig 1). Concentrated β-lactoglobulin (after the desalting step) contained 30 mg/ml total protein.

3.2. Total phenols

The results of the determination of total phenolic content in treated and untreated red wines analysed by the Folin-Ciocalteu micro method are presented in Fig. 2. There was a significant difference in the total phenolic content between the wine samples. Both β-lactoglobulin and gelatin were significantly different ($P < 0.05$) from control. This significant reduction of wine polyphenol indicates that β-lactoglobulin could be a good fining agent. Control had the highest average level of total polyphenols as expected. At the concentration (0.1 mg/ml) studied, β-lactoglobulin and gelatin had a similar impact on the total phenolic content after treatment and showed no significant difference in their effectiveness in reducing the total phenolic content. This similarity between gelatin and β- lactoglobulin treated wines is in agreement with our previous work (Jauregi et al. 2016). Phenol reduction by β-lactoglobulin and gelatin relies on a precipitation mechanism.

3.3. Whey protein and astringency

Tannic acid used as standard was precipitated upon the addition of ovalbumin and decreased the absorbance at 280nm. The slope of the logarithm curve obtained from the ovalbumin concentration against absorbance had a linear relationship (r^2=0.9989) to the initial tannic acid concentrations. This calibration curve was used in the determination of tannic acid in the wines as a measure of astringency. Tempranillo wine was used for this work based on its high astringency after the screening of three different varieties of commercial red wine (data not shown). The astringency of control (0.220 mg/ml- Fig 3) was within the range of values 0.112-0.566 mg/ml

reported by Llaudy et al.(2004) and significantly more astringent than the Merlot wine used in our previous study (Jauregi et al., 2016). β-lactoglobulin and gelatin reduced astringency to 0.17 mg/ml and 0.16 mg/ml respectively (Fig 3). The addition of the proteins led to a significant decrease in astringency of the commercial red wine and this is in agreement with our previous work (Jauregi et al., 2016). Although gelatin tended to reduce the astringency more than β–lactoglobulin, the difference was not significant ($p > 0.05$). β-lactoglobulin and gelatin reduced astringency by interacting with wine phenols which are major components contributing to astringency development. This form of interaction is mediated by hydrophobic and hydrogen bonding accompanied by aggregation and precipitation (Charlton et al.,2002). The primary structure of the protein influences polyphenol/protein interactions (Soares et al., 2015). Randomly coiled proteins have higher affinity for tannins than globular proteins (de Freitas & Mateus 2001). Other protein features such as molecular weight and number of proline residues and their sequence influence the interaction with tannins (Canon et al., 2013; Soares et al., 2015). The binding affinity of tannins to proteins increases with their molecular weight (Sarni-Manchado, Cheynier, & Moutounet, 1999). Factors such as temperature, salt concentration and pH affect the binding affinity of tannins to proteins (Shpigelman, Israeli, & Livney, 2010; Wang, Ho, & Huang, 2007)

3.4. Sequential profiling data

Data from sequential profiling was collected to observe the change in intensity of attributes over repeated consumption of 20 ml of red wine samples. Astringency, bitter taste, sweetness, acid taste, dark fruity flavour, woody flavour and metallic taste were selected as attributes for sequential profiling. Significant differences ($p < 0.05$) between red wine samples were found overall for astringency after swallowing and when scored as an aftertaste at 30 secs (AT1) (Table 2). Panelists perceived the astringency induced by β-lactoglobulin and gelatin treatments to be significantly lower at 30 sec post swallowing (AT1) compared to the control sample (Fig. 5). Overall mean astringency ratings for control were higher than for both β-lactoglobulin and gelatin treatments over repeated sips and aftertaste at 30 secs (AT1) and 60 secs (AT2) (Fig 4). This higher rating for control might be due to the presence of higher concentration of polyphenols available for interaction by the salivary proteins. The difference in astringency intensity between control and gelatin treatments was greater than the difference between control and β-lactoglobulin treatments. This shows that gelatin was more effective than β-lactoglobulin reducing astringency. However, there was no significant difference ($p>0.05$) between the gelatin and β-lactoglobulin.

In contrast, bitter taste, sweetness, acidity, dark fruity flavour, woody flavour and metallic taste showed no significant differences ($p > 0.05$) between the samples neither during sips nor during aftertaste ratings (Table 2). The lack of significant difference in these 4 taste and 2 key flavor attributes suggests that the addition of β-lactoglobulin caused no major modification to the red wine flavour; this is a desirable property of fining agents as they should not alter sensory properties of red wines except for astringency (Simonato et al., 2013). The lowest scoring attribute was metallic taste while the highest scoring attribute in the sequential profiling was astringency over repeated consumption (Table 2). Although the panelists were using an unstructured line scale and hence the values are relative rather than absolute; this does still imply that astringency was a predominant and important attribute in the perception and quality of red wine. The aftertaste values (30 and 60 sec) are high for astringency compared to the other attributes demonstrating that these sensations are just as prominent once the samples have been swallowed. The significant difference found for astringency between control, gelatin and β-lactoglobulin treatments could be caused by the decrease in tannin concentration as shown by the decrease in total polyphenol content (Fig. 2). Jiménez-Martínez et al (2018) reported a reduction in the phenolic content of red wine especially tannins by grape pomace, a by-product used as fining agent compared with casein. Fining red wines with potential fining agents are able to reduce astringency by decreasing the tannin content associated with astringency as seen in the treatment of wine with β -lactoglobulin (Jauregi et al., 2016).The intensity of astringency tended to build up over repeated exposure across the wine samples as expected, however the increase was not significant at the interval studied (60 secs) (Fig 4). At each sip astringency increased and reached a maximum and then the intensity decreased at 30 secs and further decreased at 60 secs until the next sip was taken where a slight increase was experienced. The non-significant increase may be due to the greater time interval between the sips. The time interval between sips affects intensification of astringency. Significant increases in maximum astringency intensity were reported when ingestions were taken with 20 and 25 secs intervals but not at 30 sec intervals on repeated ingestion of astringent stimuli (Guinard, Pangborn, & Lewis, 1986; Lesschaeve & Noble 2005; Noble, 2002). Astringency is a tactile long-lasting sensation with carry over effect upon repeated consumption of astringent samples and is not associated with the type of adaptation that is experienced with sweetness and bitterness (Methven et al.,2010; Lyman &Green 1990; Lee & lawless 1991). The binding of oral proteins and rupturing of the lubricating film induced by repeated exposure, consequently results in an increase in the perception of astringency (Dinnella et al., 2010; de Wijk & Prinz 2006). The perception of wine astringency reduced over time due to the flushing of phenols and restoration of saliva which acts as a lubricant.

Kennedy et al (2006) and Llaudy et al (2004) suggested protein precipitation as the best chemical method that correlated well with astringency perception. Monteleone et al (2004) showed a positive relationship between concentration of polyphenolic compounds and the sensory attribute of astringency. In this work we demonstrated that both total phenolic content and instrumentally measured astringency by the ovalbumin precipitation method were consistent with the perceived astringency of the wine samples. The trend during sips, and after swallowing at 30 and 60 secs (AT1 and AT2) interval showed that the control had a higher intensity of astringency than the β-lactoglobulin and gelatin treated wines. There was a similar trend and relationship between measured and perceived astringency between samples especially when assessed by the after taste at 30 sec intervals after the sips. Both sensory and chemical analysis showed that β-lactoglobulin had a similar ability as gelatin to react with tannins resulting in same effectiveness in reducing astringency in wine.

3.5. Mechanism of astringency reduction by β-lactoglobulin

The interaction between phenolic compounds and proteins in saliva form the basis of the mechanism that explains the perception of astringency (de Freitas and Mateus, 2001; Richardo da silva et al., 1991; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2003). The interaction of salivary proline rich proteins (PRPs) with tannins results in a loss of lubrication and increased friction in the mouth. Tannin-induced precipitation of salivary PRPs in the oral cavity has been established as a mechanism for perception of astringency by numerous research studies (Kallithraka, Bakker, & Clifford, 1998; Baxter, Lilley, Haslam, & Williamson, 1997; Luck et al., 1994; Bennick, 2002). The perception of astringency and its mechanism are affected by factors that include, tannin and protein structure, individual variability and are dependent on salivary protein composition, viscosity and flow rate (Vidal et al., 2003; Sun et al., 2013; Soares et al.,2012; Dinnella et al., 2009; Condelli et al., 2006).

Addition of β-lactoglobulin to the wine affected the concentration of polyphenols which is an important factor in the mechanism of astringency development. β-lactoglobulin bound the polyphenols in the red wine, thereby reducing the concentration available for salivary protein interactions and/or precipitation. This formed the basis of the chemical measurement of astringency which showed that β-lactoglobulin was as effective as gelatin in reducing astringency. Interestingly the same was concluded from the sensory study. The good agreement between the chemical and the sensory methods suggest that β-lactoglobulin reduces astringency in wine following the above mechanism.

4. Conclusions

This is the first sensory study of the impact of whey protein treatment on the perception of astringency in red wine using a sequential profiling technique. With this technique, seven attributes were evaluated over time for the wine samples. Astringency was the predominant attribute of the red wine during sips and in evaluation of aftertaste; samples prepared by different treatments were clearly differentiated by the panel. In this work we have demonstrated that both total phenolic content and instrumentally measured astringency by the ovalbumin precipitation method were consistent with the perceived astringency of the wine samples. The trend during sips, and after swallowing at 30 and 60 secs interval showed that the control had a higher intensity of astringency than the β-lactoglobulin and gelatin treated wines. There was a similar trend and relationship between measured and perceived astringency between samples especially when assessed by the after taste at 30 sec intervals after the sips. Moreover addition of β-lactoglobulin to wine did not alter other sensory attributes. Both sensory and chemical analysis showed that β-lactoglobulin had a similar ability as gelatin to react with tannins resulting in same effectiveness in reducing astringency in wine. The good agreement between the chemical and the sensory methods suggest that reduction of astringency by β-lactoglobulin wine is based on the same principle of protein precipitation: β-lactoglobulin binds the polyphenols in the red wine, thereby reducing the concentration available for salivary protein interactions and/or precipitation with the subsequent reduction in astringency perception. Moreover, this study has brought about a new potential application of β-lactoglobulin and/or processed whey as a fining agent and therefore, could contribute to add commercial value to sweet whey.

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Declaration of interest: None

Figure captions

Figure 1 HPLC Chromatogram of β-lactoglobulin fraction from integrative process; A) Lab scale production and B) Pilot plant production.

Figure 2 Total phenolic content of red wine treated with β-lactoglobulin (beta-lg) and gelatin as mg GAE/ ml. Values are means ± 2SE of duplicate analyses.

Figure 3 Astringency in red wine determined by analytical method as tannic acid equivalent (mg/ml). Values are Means ± 3SE of triplicate analyses. beta-lg (β-lactoglobulin)

Figure 4 Sequential profile of red wines; control, beta-lg and gelatin treatments for astringency over repeated consumption. Values are means ± 2SE of duplicate analyses. (1.) S1-S4, consecutive aliquots consumed (2.) AT1 and AT2, after-effects at 30 secs and 60 secs post consumption of aliquots S1-S4. beta-lg- Beta-lactoglobulin, S-Sips and AT- Aftertaste.

Figure 5 Mean astringency intensities of each aliquot's after-effects at 30 secs post consumption (S1AT1, S2AT1, S3AT1 and S4AT1) from sequential profiling of red wines; control, beta-lactoglobulin (beta-lg) and gelatin treatments. Values are means ± 2SE of duplicate analyses. Letters denote significant difference ($p < 0.05$) between samples.

Table captions

Table 1: Descriptions of attributes for sensory profiling

Table 2: Mixed ANOVA model. Effect of β-lactoglobulin and gelatin treatments on the in-mouth attributes of red wine. The p-value in each column represents the significance of the sample effect in each row.

Supplementary material.

Table S1: Protein content of sweet whey and β-lactoglobulin fractions from the integrative process (n=2±SE).

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