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Accepted Version

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Olatujoye, J. B., Methven, L. and Jauregi, P. (2020) Effect of β -lactoglobulin on perception of astringency in red wine as measured by sequential profiling. LWT, 130. 109611. ISSN 0023-6438 doi: <https://doi.org/10.1016/j.lwt.2020.109611>
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Published version at: <http://dx.doi.org/10.1016/j.lwt.2020.109611>

To link to this article DOI: <http://dx.doi.org/10.1016/j.lwt.2020.109611>

Publisher: Elsevier

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

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7 **Abstract**

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

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

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23

24 1. Introduction

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

53 Attempts have been made to use instrumental methods such as chromatography (Kennedy, Ferrier, Harbertson,
54 & Des Gachons, 2006), colourimetry (Cáceres-Mella et al., 2013; Alexandre-Tudo, Buica, Nieuwoudt,
55 Alexandre, & du Toit, 2017), Nephelometric (Monteleone, Condelli, Dinnella, & Bertuccioli, 2004), methyl
56 cellulose precipitation (Mercurio & Smith, 2008), physical measurements (Laguna, Álvarez, Simone, Moreno-
57 Arribas, & Bartolomé, 2019) and protein precipitation using proteins such as ovalbumin, Saliva, BSA, and
58 Gelatin (Llaudy et al., 2004 ; Rinaldi, Gambuti, & Moio 2012b; Harbertson & Kennedy, 2002 and Glories,
59 1984) to assess astringency development at a molecular level and to correlate the data with its sensory
60 perception. The assessment of astringency in wine is best quantified through sensory evaluation. The
61 heterogeneous nature of tannins limits the analytical methods used for their quantification and characterization.
62 Various precipitants including proteins and polysaccharides have been employed for the quantification of
63 tannins with varying values obtained (Mercurio & Smith, 2008; Llaudy et al., 2004). Ovalbumin precipitation
64 method was shown to be simple, less time consuming and correlates with sensory evaluation (Llaudy et al.,
65 2004)

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

74 In our previous work (Jauregi, Olatujoye, Cabezudo, Frazier, & Gordon, 2016) we employed an analytical
75 method to assess the effect of β -lactoglobulin in reducing astringency and found that β -lactoglobulin was as
76 effective as gelatin in reducing astringency and had a similar selectivity for the polyphenols which are markers
77 for astringency. β -lactoglobulin was even better as it preserves catechin more than gelatin. Milk proteins are
78 known for allergy. Although allergenic reactions to milk proteins are rare in adults than in children (Asero et al.,
79 2009), in order to protect the sensitive consumers, the absence of β -lactoglobulin residue was ensured by
80 applying a good manufacturing practice that includes the usage of low dose of fining agent and its removal by
81 adequate filtration procedure in our present study. The absence of β -lactoglobulin residues after fining followed

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

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

90 **2. Materials and Methods**

91 **2.1. Materials**

92 All reagents used for the analysis were of analytical grade. Bovine beta-lactoglobulin, bovine serum albumin
93 (BSA), alpha-lactalbumin, bicinchoninic acid solution (BCA), copper sulfate solution, DEAE Sepharose®,
94 ovalbumin, tannic acid, tartaric acid, FolinCiocalteu reagent, gelatin (Type B gelatin from bovine and 75 g
95 bloom strength) were purchased from Sigma-Aldrich, (Dorset, UK). Flat sheet microfiltration membranes
96 (0.45 μ m), and syringe driven PVDF Filters (0.45 μ m) were purchased from Millipore Corporation, (Bedford,
97 UK). Potassium monophosphate, potassium diphosphate, sodium hydroxide, sodium carbonate, sodium
98 chloride (NaCl), hydrochloric acid (HCl), trifluoroacetic acid (TFA), methanol, ethanol were purchased from
99 Fisher Scientific (UK limited), Protease N 'Amano' Enzyme from Bacillus subtilis was purchased from
100 Amano Enzyme Inc., (Nagoya, Japan), Ultrospec 1100 pro UV/Visible Spectrophotometer was from Biochem
101 Ltd., (Cambridge). Eppendorf Centrifuge Minispin plus G was from Fisher Scientific (UK Ltd). Amicon filtration
102 cell was obtained from Amicon® a Grace company. Pasteurized skimmed milk and 100% Tempranillo Red
103 wine, Valdubón (2012), from North Central of Spain (13% alcohol) were purchased from a local store.

104 **2.2. Pilot Plant Production of the β -lactoglobulin rich whey fraction**

105 4L of sweet whey was produced from pasteurized skimmed milk. Skimmed milk was heated to 35 °C in a water
106 bath. Commercial rennet was added at a concentration of 0.3 ml per litre of milk with gentle stirring for 2
107 minutes. Incubation took place for one hour at that temperature and then the casein coagulum was cut in small
108 squares to allow the remaining lactoserum to drain out of it. Incubation was extended for 20 additional minutes

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

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

126 **2.3. Chemical characterization of the β -lactoglobulin whey fraction**

127 Total proteins were quantified according to the bicinchoninic acid assay (BCA). Briefly, 100 μ l of standard or
128 sample was mixed with 2 ml of the BCA working reagent (copper sulphate solution: BCA solution at a ratio of
129 1:50). The mixture was allowed to stand at 37 °C for 30 min, and then allowed to cool to room temperature for 5
130 min. Finally, absorbance was read for each sample/standard, at 562 nm within 8 minutes with water as a blank.
131 Bovine serum albumin was used as a standard for protein quantification.

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

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

141 **2.4. Concentrating and desalting whey protein**

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

151 **2.5. Protein-Wine treatment**

152 The concentrated β -lactoglobulin solution and gelatin were added to wine at a final concentration of 0.1 mg/ml
153 and water was added to the untreated sample (control). The protein concentration was chosen based on a
154 previous work in our lab (Jauregi et al., 2016) and it is within the range of level usually for fining. The mixtures
155 were rigorously mixed and allowed to stand for 10 min for adequate contact. Mixtures were centrifuged at
156 11700g for 10 min and supernatant was collected for astringency measurement and determination of
157 polyphenolic content following the analytical methods described below and for sensory tests. All measurements
158 were carried out in triplicate.

159 **2.6. Analytical method for determination of astringency**

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

164 adjusted to pH 3.5 with 5M sodium hydroxide. Solutions of tannic acid at concentration of 0.0-0.8 mg/ml were
165 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
166 as protein to precipitate astringent tannins. Increasing concentrations of ovalbumin (0.5 ml) were added to tannic
167 acid/red wine in the tubes. The tubes were thoroughly stirred for 10 secs, allowed to stand for 10 mins and then
168 centrifuged at 11700g for 10 mins. Supernatants were diluted 50 times with distilled water and absorbance was
169 read at 280 nm in a quartz cuvette with an optical path of 10 mm; experiments were carried out at room
170 temperature and in triplicate.

171 **2.7. Folin-Ciocalteu method for total polyphenol content**

172 Folin-Ciocalteu's micro method as adapted for wine analysis by Waterhouse (2009) using gallic acid as the
173 standard was used to determine the phenolic content. For the analysis, 20 μ l of each calibration solution, treated
174 red wine, red wine or blank were placed in a cuvette, and 1.58 ml water and 100 μ L of Folin-Ciocalteu reagent
175 were added, thoroughly mixed and allowed to stand between 30 seconds and 8 minutes. Then, 300 μ L of the
176 20% w/v saturated sodium carbonate solution was added, mixed well and left at 20 °C for 2 h, after which the
177 absorbance of each solution was read at 765 nm using a spectrophotometer. Results were expressed as Gallic
178 acid equivalents (mg GAE/L).

179 **2.8. Sensory sequential profiling method**

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

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

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

193 The seven sensory attributes scored were sweetness, acidity, bitterness, astringency, dark fruity flavour, woody
194 flavour and metallic taste (see Table 1). Although astringency is a quality attribute of wine that takes time to
195 develop and build up upon repeated ingestion, other attributes may also change over repeated ingestion as
196 reported by Meillon, Urbano, & Schlich (2009) with temporal dominance of sensation (TDS). Seven is the
197 maximum number of attributes which can be scored within a sequential profiling method where repeated sipping
198 is set at a minute intervals; if more attributes are used the time taken to score the attributes becomes too long.
199 The attributes were agreed upon by the panel to represent the wine characteristics which appeared to be
200 influenced by either the different samples (to which the panel were blinded) or by the repeated sipping.
201 Sequential profiling which is also a multi-attribute method was chosen over TDS because of our interest in
202 intensity over time rather than the dominance of the sensation. It was also better than time intensity (TI) that
203 would have limited scoring to only one or two attributes hence consuming time. Three red wine samples;
204 control, β -lactoglobulin and gelatin treated wine were sequentially profiled, two samples per day and duplicate
205 scoring sessions were carried out on separate days. Samples were coded with three-digit numbers and all four
206 aliquots of one sample were presented with the same code; panelists were not blinded to the sequential nature of
207 the evaluation. Scoring for each sample set was performed without a resting or rinsing procedure between the 4
208 aliquots of the same sample. A 2-minute delay was enforced after each sample during which time the trained
209 panelists were required to cleanse their palates with low salt crackers followed by a water rinse (noting that this
210 would have been a minimum of 3 minutes since tasting the previous sample aliquot). 5 ml of wine samples at
211 18°C were presented to the trained panelists in ISO approved wine glasses. The intensity of each attribute was
212 rated using an unstructured line scale with the appropriate anchors (0-100) from not to very. Data was acquired
213 using Compusense Cloud sensory software (Ontario, Canada).

214 2.9. Data Analysis

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

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

222 **3. Results and Discussion**

223 **3.1. Whey protein production**

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

230 **3.2. Total phenols**

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

240 **3.3. Whey protein and astringency**

241 Tannic acid used as standard was precipitated upon the addition of ovalbumin and decreased the absorbance at
242 280nm. The slope of the logarithm curve obtained from the ovalbumin concentration against absorbance had a
243 linear relationship ($r^2=0.9989$) to the initial tannic acid concentrations. This calibration curve was used in the
244 determination of tannic acid in the wines as a measure of astringency. Tempranillo wine was used for this work
245 based on its high astringency after the screening of three different varieties of commercial red wine (data not
246 shown). The astringency of control (0.220 mg/ml- Fig 3) was within the range of values 0.112-0.566 mg/ml

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

261 **3.4. Sequential profiling data**

262 Data from sequential profiling was collected to observe the change in intensity of attributes over repeated
263 consumption of 20 ml of red wine samples. Astringency, bitter taste, sweetness, acid taste, dark fruity flavour,
264 woody flavour and metallic taste were selected as attributes for sequential profiling. Significant differences ($p <$
265 0.05) between red wine samples were found overall for astringency after swallowing and when scored as an
266 aftertaste at 30 secs (AT1) (Table 2). Panelists perceived the astringency induced by β -lactoglobulin and gelatin
267 treatments to be significantly lower at 30 sec post swallowing (AT1) compared to the control sample (Fig. 5).
268 Overall mean astringency ratings for control were higher than for both β -lactoglobulin and gelatin treatments
269 over repeated sips and aftertaste at 30 secs (AT1) and 60 secs (AT2) (Fig 4). This higher rating for control might
270 be due to the presence of higher concentration of polyphenols available for interaction by the salivary proteins.
271 The difference in astringency intensity between control and gelatin treatments was greater than the difference
272 between control and β -lactoglobulin treatments. This shows that gelatin was more effective than β -lactoglobulin
273 reducing astringency. However, there was no significant difference ($p>0.05$) between the gelatin and β -
274 lactoglobulin.

275 In contrast, bitter taste, sweetness, acidity, dark fruity flavour, woody flavour and metallic taste showed no
276 significant differences ($p > 0.05$) between the samples neither during sips nor during aftertaste ratings (Table 2).
277 The lack of significant difference in these 4 taste and 2 key flavor attributes suggests that the addition of β -
278 lactoglobulin caused no major modification to the red wine flavour; this is a desirable property of fining agents
279 as they should not alter sensory properties of red wines except for astringency (Simonato et al., 2013). The
280 lowest scoring attribute was metallic taste while the highest scoring attribute in the sequential profiling was
281 astringency over repeated consumption (Table 2). Although the panelists were using an unstructured line scale
282 and hence the values are relative rather than absolute; this does still imply that astringency was a predominant
283 and important attribute in the perception and quality of red wine. The aftertaste values (30 and 60 sec) are high
284 for astringency compared to the other attributes demonstrating that these sensations are just as prominent once
285 the samples have been swallowed. The significant difference found for astringency between control, gelatin and
286 β -lactoglobulin treatments could be caused by the decrease in tannin concentration as shown by the decrease in
287 total polyphenol content (Fig. 2). Jiménez-Martínez et al (2018) reported a reduction in the phenolic content of
288 red wine especially tannins by grape pomace, a by-product used as fining agent compared with casein. Fining
289 red wines with potential fining agents are able to reduce astringency by decreasing the tannin content associated
290 with astringency as seen in the treatment of wine with β -lactoglobulin (Jauregi et al., 2016). The intensity of
291 astringency tended to build up over repeated exposure across the wine samples as expected, however the
292 increase was not significant at the interval studied (60 secs) (Fig 4). At each sip astringency increased and
293 reached a maximum and then the intensity decreased at 30 secs and further decreased at 60 secs until the next
294 sip was taken where a slight increase was experienced. The non-significant increase may be due to the greater
295 time interval between the sips. The time interval between sips affects intensification of astringency. Significant
296 increases in maximum astringency intensity were reported when ingestions were taken with 20 and 25 secs
297 intervals but not at 30 sec intervals on repeated ingestion of astringent stimuli (Guinard, Pangborn, & Lewis,
298 1986; Lesschaeve & Noble 2005; Noble, 2002). Astringency is a tactile long-lasting sensation with carry over
299 effect upon repeated consumption of astringent samples and is not associated with the type of adaptation that is
300 experienced with sweetness and bitterness (Methven et al., 2010; Lyman & Green 1990; Lee & Lawless 1991).
301 The binding of oral proteins and rupturing of the lubricating film induced by repeated exposure, consequently
302 results in an increase in the perception of astringency (Dinnella et al., 2010; de Wijk & Prinz 2006). The
303 perception of wine astringency reduced over time due to the flushing of phenols and restoration of saliva which
304 acts as a lubricant.

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

315 3.5. Mechanism of astringency reduction by β -lactoglobulin

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

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

333 4. Conclusions

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

351 Acknowledgements

352 The authors would like to acknowledge the Federal Government of Nigeria (Tertiary Education Trust Fund
353 (TEFT)) for their financial support.

354 Declaration of interest: None

355 Figure captions

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

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

360 **Figure 3** Astringency in red wine determined by analytical method as tannic acid equivalent (mg/ml). Values
361 are Means \pm 3SE of triplicate analyses. beta-Ig (β -lactoglobulin)

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

366 **Figure 5** Mean astringency intensities of each aliquot's after-effects at 30 secs post consumption (S1AT1,
367 S2AT1, S3AT1 and S4AT1) from sequential profiling of red wines; control, beta-lactoglobulin (beta-Ig) and
368 gelatin treatments. Values are means \pm 2SE of duplicate analyses. Letters denote significant difference ($p <$
369 0.05) between samples.

370

371 **Table captions**

372 **Table 1:** Descriptions of attributes for sensory profiling

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

375 **Supplementary material.**

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

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385 **References**

386 Aleixandre-Tudo, J.L., Buica, A., Nieuwoudt, H., Aleixandre, J.L., & du Toit, W. (2017). Spectrophotometric
387 Analysis of Phenolic Compounds in Grapes and Wines. *Journal of Agriculture and Food Chemistry*, 65, 4009–
388 4026. <https://doi.org/10.1021/acs.jafc.7b01724>

389 Asero, R., Antonicelli, L., Arena, A., Bommarito, L., Caruso, B., Crivellaro, M. *et al.*(2009). EpidemAAITO:
390 Feature of food allergy in Italian adults attending allergy clinics: A multi-centre study. *Clinical and*
391 *Experimental Allergy*, 39, 547-555. <https://doi.org/10.1111/j.1365-2222.2008.03167.x>

392 Bacon, J., & Rhodes, M. (2000). Binding affinity of hydrolyzable tannins to parotid saliva and to proline-rich
393 proteins derived from it. *Journal of Agricultural and Food Chemistry*, 48, 838–843.
394 <https://doi.org/10.1021/jf990820z>

395 Baxter, N. J., Lilley, T.H., Haslam, E., & Williamson, M. P. (1997). Multiple interactions between polyphenols
396 and a salivary proline-rich protein, repeat result in complexation and precipitation. *Biochemistry*, 36, 5566-5577.
397 <https://doi.org/10.1021/bi9700328>

398 Bennick, A. (2002). Interaction of plant polyphenol with salivary proteins. *Critical Review in oral biology and*
399 *Medicine*, 13, 184 -196. <https://doi.org/10.1177/154411130201300208>

400 Canon, F., Paté, F., Cheynier, V., Sarni-Manchado, P., Giuliani, A., Pérez, J., Durand, D., Li, J., & Cabane, B.
401 (2013). Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EGCG. *Langmuir*, 29,
402 1926–1937. <https://doi.org/10.1021/la3041715>

403 Cáceres-Mella, A., Pena-Neira, A., Narvaez-Bastias, J., Jara-Campos, C., Lopez-Solis, R., & Canals, J.M.
404 (2013). Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with
405 perceived astringency. *International Journal of Food Science and Technology*, 48, 2588-2594.
406 <https://doi.org/10.1111/ijfs.12253>

407 Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E., & De Freitas V. (2006). Influence of Wine Pectic
408 Polysaccharides on the Interactions between Condensed Tannins and Salivary Proteins. *Journal of Agricultural*
409 *and Food Chemistry*, 54, 8936-8944. <https://doi.org/10.1021/jf061835h>

410 Charlton, A.J., Baxter, N.J., Khan, M.L., Moir, A.J.G., Haslam, E., Davies, A.P., & Williamson, M.P. (2002).
411 Polyphenol/peptide binding and precipitation. *Journal of Agricultural and Food Chemistry*, 50, 1593-1601.
412 <https://doi.org/10.1021/jf010897z>

413 Condelli, N., Dinnella, C., Cerone, A., Monteleone, E., & Bertuccioli, M. (2006). Prediction of perceived
414 astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine
415 samples. *Food Quality and Preference*, 17, 96–107. <https://doi.org/10.1016/j.foodqual.2005.04.009>

416 De Freitas, V., & Mateus, N. (2001). Structural features of procyanidin interactions with salivary proteins.
417 *Journal of Agricultural and Food Chemistry*, 49, 940–945. <https://doi.org/10.1021/jf000981z>

418 deWijk, R.A., & Prinz, J.F. (2005). The role of friction in perceived oral texture. *Food Quality and*
419 *Preference*, 16, 121-129. <https://doi.org/10.1016/j.foodqual.2004.03.002>

420 Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M., & Monteleone, E. (2009). Saliva characteristics and
421 individual sensitivity to phenolic astringent stimuli. *Chemical Senses*, 34, 295–304.
422 <https://doi.org/10.1093/chemse/bjp003>

423 Dinnella, C., Recchia, A., Vincenzi, S., Tuorila, H., & Monteleone, E. (2010). Temporary modification of
424 salivary protein profile and individual responses to repeated phenolic astringent stimuli. *Chemical Senses*, 35,
425 75–85. <https://doi.org/10.1093/chemse/bjp084>

426 Gambuti, A., Rinaldi, A., Romano, R., Manzo, N., & Moio, L. (2016). Performance of a protein extracted from
427 potatoes for fining of white musts. *Food Chemistry*, 190, 237–243.
428 <https://doi.org/10.1016/j.foodchem.2015.05.067>

429 Gazzola, D., Vincenzi, S., Marangon, M., Pasini, G., & Curioni, A. (2017). Grape seed extract: the first protein-
430 based fining agent endogenous to grapes. *Australian Journal of Grape Wine Research*, 23(2), 215-225.
431 <https://doi.org/10.1111/ajgw.12268>

432 Gil, M., Del Barrio-Galán, R., Úbeda, C., & Peña-Neira, Á. (2018). Effectiveness of fibers from “Cabernet
433 Sauvignon” (*Vitis vinifera*) pomace as fining agents for red wines. *Journal of Food Quality*, 1–13.
434 <https://doi.org/10.1155/2018/6408734>

435 Glories, Y. (1984). La couleur des vins rouges, 2eme Partie, Mes, Orig. Interpret. Connaiss. Vigne Vin, 18, 253-
436 271. <https://doi.org/10.20870/oeno-one.1984.18.4.1744>

437 Granato, T.M., Ferranti, P., Iametti S., & Bonomi, F. (2018). Affinity and selectivity of plant proteins for red
438 wine components relevant to color and aroma traits. *Food Chemistry*, 256, 235-243.
439 <https://doi.org/10.1016/j.foodchem.2018.02.085>

440 Guinard, J.X., Pangborn, R.M., & Lewis, M.J. (1986). The time-course of astringency in wine upon repeated
441 ingestion. *American Journal of Enology and Viticulture*, 37, 184-189.

442 Harbertson, J. F., Kennedy, J. A., & Adams, D. O. (2002). Tannin in skins and seeds of Cabernet Sauvignon,
443 Syrah, and Pinot Noir berries during ripening. *American Journal of Enology and Viticulture*, 53, 54–59.

444 Jauregi, P., Olatujoye, J.B., Cabezudo, I., Frazier, R., & Gordon, M.H. (2016). Astringency Reduction in Red
445 Wine by Whey Proteins. *Food Chemistry*, 199, 547-555. <https://doi.org/10.1016/j.foodchem.2015.12.052>

446 Jiménez-Martínez, M.D., Gil-Muñoz, R., Gómez-Plaza, E., & Bautista-Ortín, A.B. (2018). Performance of
447 purified grape pomace as a fining agent to reduce the levels of some contaminants from wine. *Food Addition*
448 *Contaminant Part A Chemical Analytical Control Exposure Risk Assessment*, 35, 1061–1070.
449 <https://doi.org/10.1080/19440049.2018.1459050>

450 Kallithraka, S., Bakker, J., & Clifford, M.N. (1998). Evidence that salivary proteins are involved in astringency.
451 *Journal of Sensory studies*, 13, 29-44. <https://doi.org/10.1111/j.1745-459X.1998.tb00073.x>

452 Kanakis, C. D., Hasni, I., Bourassa, P., Tarantilis, P. A., Polissiou, M. G., & Tajmir-Riahi, H. A. (2011). Milk
453 beta-lactoglobulin complexes with tea polyphenols. *Food Chemistry*, 127(3), 1046-1055.
454 <https://doi.org/10.1016/j.foodchem.2011.01.079>

455 Kang, W., Niimi, J., & Bastian, S.E.P. (2018). Reduction of red wine astringency perception using vegetable
456 protein fining agents. *American Journal of Enology and Viticulture*, 69, 22–31.

457 Kennedy, J. A., Ferrier, J., Harbertson, J. F., & Des Gachons, C. P. (2006). Analysis of tannins in red wine using
458 multiple methods: Correlation with perceived astringency. *American Journal of Enology and Viticulture*, 57,
459 481–485.

460 Laguna, L., Álvarez, M.D., Simone, E., Moreno-Arribas, M.V., & Bartolomé, B. (2019). Oral Wine Texture
461 Perception and Its Correlation with Instrumental Texture Features of Wine-Saliva Mixtures. *Foods*, 8, 190-204.
462 DOI: [10.3390/foods8060190](https://doi.org/10.3390/foods8060190)

463 Lee, C. B., Lawless, H. T. (1991). Time-course of astringent sensations. *Chemical Senses*, **16**, 225–238.
464 <https://doi.org/10.1093/chemse/16.3.225>

465 Lesschaeve, I., & Noble, A.C. (2005). Polyphenols: Factors influencing their sensory properties and their effects
466 on food and beverages preferences. *The American Journal of Clinical Nutrition*, **81**, 330S-335S.
467 <https://doi.org/10.1093/ajcn/81.1.330S>

468 Luck, G., Liao, H., Murray, N.J., Grimmer, H.R., Warminski, E.E., Williamson, M.P., Lilley, T.H., & Haslam,
469 E. (1994). Polyphenols, astringency and proline-rich proteins. *Phytochemistry*, **37**, 357-371.
470 [https://doi.org/10.1016/0031-9422\(94\)85061-5](https://doi.org/10.1016/0031-9422(94)85061-5)

471 Llaudy, M. C., Canals, R., Canals, J. M., Rozes, N., Arola, L., & Zamora, F. (2004). New method for evaluating
472 astringency in red wine. *Journal of Agricultural and Food Chemistry*, **52**, 742–746.
473 <https://doi.org/10.1021/jf034795f>

474 Lyman, B.J., & Green, B.G. (1990). Oral astringency: effects of repeated exposure and interactions with
475 sweeteners. *Chemical Senses*, **15**(2), 151-164. <https://doi.org/10.1093/chemse/15.2.151>

476 Maury, C., Sarni-Manchado, P., Lefebvre, S., Cheynier, V., & Moutounet, M. (2003). Influence of fining with
477 plant proteins on proanthocyanidin composition of red wines. *American Journal of Enology and Viticulture*,
478 **54**(2),105.

479 Meillon, S., Urbano, C., & Schlich, P. (2009). Contribution of the temporal dominance of sensation (TDS)
480 method to the sensory description of the subtle differences in partially dealcoholized red wines. *Food Quality*
481 *and Preference*, **20**, 490-499. <https://doi.org/10.1016/j.foodqual.2009.04.006>

482 Mercurio, M.D., & Smith, P.A. (2008). Tannin quantification in red grapes and wine: comparison of
483 polysaccharide-and protein-based tannin precipitation techniques and their ability to model wine astringency.
484 *Journal of Agricultural and Food Chemistry*, **56**, 5528-5537. <https://doi.org/10.1021/jf8008266>

485 Methven, L., Rahelu, K., Economou, N., Kinneavy, L., Ladbroke-Davis, L., O.B. Kennedy, *et al.* (2010). The
486 effect of consumption volume on profile and liking of oral nutritional supplements of varied sweetness:
487 Sequential profiling and boredom tests. *Food Quality and Preference*, **21**, 948–955.
488 <https://doi.org/10.1016/j.foodqual.2010.04.009>

489 Monteleone, E., Condelli, N., Dinnella, C., & Bertuccioli, M. (2004). Prediction of perceived astringency
490 induced by phenolic compounds. *Food Quality and Preference*, 15 (7-8), 761-769.
491 <https://doi.org/10.1016/j.foodqual.2004.06.002>

492 Noble, A.C. (2002). Astringency and bitterness of flavonoids phenols. In: Given P, Paredes D, eds. Chemistry of
493 taste mechanisms, behaviors and mimics. Washington, DC: *American Chemical Society*, 192-199.
494 **DOI:** [10.1021/bk-2002-0825.ch015](https://doi.org/10.1021/bk-2002-0825.ch015)

495 Ricardo-Da-Silva, J. M., Cheynier, V., Souquet, J.M., Moutounet, M., Cabanis, J.C., & Bourzeix, M. (1991).
496 Interaction of grape seed procyanidins with various proteins in relation to wine fining. *Journal of the Science of*
497 *Food and Agriculture*, 57, 111–125. <https://doi.org/10.1002/jsfa.2740570113>

498 Rinaldi, A., Gambuti, A., & Moio, L. (2012a). Precipitation of salivary proteins after the interaction with wine:
499 the effect of ethanol, pH, fructose, and mannoproteins. *Journal of Food Science*, 77, 485-90.
500 <https://doi.org/10.1111/j.1750-3841.2012.02639.x>

501 Rinaldi, A., Gambuti, A., & Moio, L. (2012b). Application of the SPI (Saliva Precipitation Index) to the
502 evaluation of red wine astringency. *Food Chemistry*, 135, 2498-2504.
503 <https://doi.org/10.1016/j.foodchem.2012.07.031>

504 Rossetti, D., Bongaerts, J. H. H., Wantling, E., Stokes, J. R., & Williamson, A. M. (2009). Astringency of tea
505 catechins: More than an oral lubrication tactile percept. *Food Hydrocolloids*, 23, 1984–1992.
506 <https://doi.org/10.1016/j.foodhyd.2009.03.001>

507 Rossetti, D., Yakubov, G. E., Stokes, J. R., Williamson, A. M., & Fuller, G. G. (2008). Interaction of human
508 whole saliva and astringent dietary compounds investigated by interfacial shear rheology. *Food*
509 *Hydrocolloids*, 22, 1068–1078. <https://doi.org/10.1016/j.foodhyd.2007.05.014>

510 Sarni-Manchado, P., Cheynier, V., & Moutounet, M. (1999). Interactions of grape seed tannins with salivary
511 proteins. *Journal of Agricultural and Food Chemistry*, 47, 42–47. <https://doi.org/10.1021/jf9805146>

512 Shpigelman, A., Israeli, G., & Livney, Y. D. (2010). Thermally-induced protein polyphenol co-assemblies: Beta
513 lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG. *Food Hydrocolloids*, 24(8), 735–743.
514 <https://doi.org/10.1016/j.foodhyd.2010.03.015>

515 Siebert, K.J., Troukhanova, N.V., & Lynn, P.Y. (1996). Nature of polyphenol-protein interactions. *Journal of*
516 *Agricultural and Food Chemistry*, 44, 80-85. <https://doi.org/10.1021/jf9502459>

517 Simonato, B., Mainente, F., Selvatico, E., Violoni, M., & Pasini, G. (2013). Assessment of the fining efficiency
518 of zeins extracted from commercial corn gluten and sensory analysis of the treated wine. *LWT-Food Science and*
519 *Technology*, 54, 549–556. <https://doi.org/10.1016/j.lwt.2013.06.029>

520 Soares, S., Brandão, E., Mateus, N., & De Freitas., V. (2015). Sensorial Properties of Red Wine Polyphenols:
521 Astringency and Bitterness. *Critical reviews in food science and nutrition*. 57(5), 937-948.
522 <https://doi.org/10.1080/10408398.2014.946468>

523 Soares, S., Sousa, A., Mateus, N., & De Freitas, V. (2012). Effect of condensed tannins addition on the
524 astringency of red wines. *Chemical Senses*, 37 (2), 191-198. <https://doi.org/10.1093/chemse/bjr092>

525 Sun, B., Sá, M. D., Leandro, C., Caldeira, I., Duarte, F. L., & Spranger, I. (2013). Reactivity of polymeric
526 proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *Journal of*
527 *Agricultural and Food Chemistry*, 61, 939–946. <https://doi.org/10.1021/jf303704u>

528 Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003).
529 The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science*
530 *of Food and Agriculture*, 83(6), 564–573. <https://doi.org/10.1002/jsfa.1394>

531 von Staszewski, M., Jara, F. L., Ruiz, A. L. T. G., Jagus, R. J., Carvalho, J. E., & Pilosof, A. M. R. (2012).
532 Nanocomplex formation between beta-lactoglobulin or caseinomacropetide and green tea polyphenols: Impact
533 on protein gelation and polyphenols antiproliferative activity. *Journal of Functional Foods*, 4(4), 800-809.
534 <https://doi.org/10.1016/j.jff.2012.05.008>

535 Wang, X., Ho, C.-T., & Huang, Q. (2007). Investigation of adsorption behaviour of (-)-epigallocatechin gallate
536 on bovine serum albumin surface using quartz crystal microbalance with dissipation monitoring. *Journal of*
537 *Agricultural and Food Chemistry*, 55, 4987-4993. <https://doi.org/10.1021/jf070590l>

538 Welderufael, F.T., Gibson, T., & Jauregi, P. (2012). Production of angiotensin-I-converting enzyme inhibitory
539 peptides from β -lactoglobulin- and casein-derived peptides: an integrative approach. *Biotechnology Progress*,
540 28, 746–755. <https://doi.org/10.1002/btpr.1541>

541 Waterhouse, A. (2009). Folin-Ciocalteu micro method for total phenol in wine.

542 (<http://www.waterhouse.ucdavis.edu/phenol/fofinmicro.htm>).

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