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Sensory assessment of grape polyphenolic fractions: an insight into the effects of anthocyanins on in-mouth perceptions

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ABSTRACT

Anthocyanins are extracted from grape skins during maceration and are responsible for the red colour of wine. Their contribution to in-mouth sensations is mainly related to their interactions with condensed tannins, which are largely responsible for wine astringency and mouthfeel-related features. Recently, the influence of several groups of polyphenols, together with other relevant non-phenolic wine constituents, was investigated in terms of their ability to modify the sensory perception of condensed tannins. The aim of this study was to investigate the influence of three acylation groups of anthocyanins (glucoside, acetylglucoside, and *p*-coumaroylglucoside) extracted from grape skins on in-mouth related features. An extract of total anthocyanins and their individual fractions were tasted using different sensory approaches (triangle test, check-all-that apply and descriptive analysis) and compared to polyphenols extracted from grape skins and seeds. The investigated sensations were overall astringency and astringency sub-qualities, which were divided into two groups: sensation during tasting (in-mouth, *particulates*) and sensation after expectoration (*surface smoothness*). Bitterness was also studied. Anthocyanin fractions were added to skin and seed extracts and tasted as mixtures to find out if anthocyanins modify in-mouth perception. Although the anthocyanin fractions showed a low sensory impact, total anthocyanins and the glucoside fraction were perceived at the concentration ranges found in wines (400 mg/L), and they were found to influence astringency intensity and soft astringency sub-qualities, such as "velvety" and "chalky". The addition of glucoside anthocyanin (400 mg/L) to skin and seed extract (1000 mg/L) modified in-mouth perception; in particular, seed extract was perceived as being more astringent and was characterised by harsher astringency sub-qualities (*surface smoothness* and *particulates)*. In contrast, the addition of glucoside anthocyanin to the skin extract led to lower *surface smoothness*, although the intensity of overall astringency was unchanged. These results confirm that the presence of anthocyanins can modify the perception of in-mouth sensations and interact to different extents with other polyphenols, thus leading to the modification of the intensity of astringency and its sub-qualities.

KEYWORDS

anthocyanins, winegrapes, check-all-that-apply (CATA), descriptive analysis (DA), sensory analysis, polyphenols

INTRODUCTION

In red wines, colour and "in-mouth" features strongly influence the sensory perception of quality (Peynaud, 1987; Parpinello *et al.*, 2009, Piombino *et al.*, 2020). These characteristics are mainly connected to the polyphenols extracted from black grapes. Among them, two groups are particularly relevant in terms of content: anthocyanins and condensed tannins (also generally referred to as proanthocyanidins), and their constituting monomers, the flavan-3-ols. Anthocyanins are responsible for the red wine colour, whereas condensed tannins are involved in both colour stabilisation and the "in-mouth" characteristics of wine, such as astringency and bitterness (Ma *et al.*, 2014). Recently, wine research has focused on the improvement of colour parameters and the modification of "in mouth" features with the development of technologies and practices aiming to improve the initial grape phenolic content and composition, their extraction into grape juice during maceration, and wine ageing management (Harrison, 2018). Nevertheless, wine tannin concentration and properties alone do not represent the full "in-mouth" complexity of wine. Wine technological parameters, such as ethanol content, total acidity and pH influence perception of astringency (Pickering and Demiglio, 2008; Fontoin *et al.*, 2008, Laguna *et al.*, 2017). Furthermore, in wine, other macromolecules (e.g., polysaccharides, proteins, and ellagitannins) derived from grape, yeasts, or external sources (e.g., wood used in ageing) can modulate the tannin effect or directly elicit astringency sensations (Glabasnia and Hofmann, 2006; Fukui *et al.*, 2002; Laguna *et al.*, 2017).

The role of anthocyanins in particular has been investigated to better understand how and to what extent they can modulate "in-mouth" properties (Table 1). Although they are known to contribute to depleting the astringency of tannins with the formation of complexes (Vidal *et al.*, 2004a) by the reduction of the available -OH group interacting with salivary proteins, the direct eliciting capacity of anthocyanins is far from clear. Pure anthocyanins have been reported to have a very "mild indistinctive taste" (Singleton and Noble, 1976), supporting the hypothesis that their role may only be relevant to the pigments formed with flavan-3-ols. In contrast, the addition of a mixture of isolated grape anthocyanins to seed and skin extracts has been found to increase astringency compared to the unspiked fractions (Broussard *et al.*, 2001). These results have been supported by winemaking experiments in which anthocyanins or grape pomace were added to white grape juices before fermentation, showing that anthocyanins increase astringency sub-qualities related to surface smoothness (*fine grain*), as well as other "in-mouth" attributes, such as "dry", "grippy" and perceived viscosity in final wines (Oberholster *et al.*, 2009). These descriptors agreed in part with the results of previous studies, in which anthocyanins were tasted in model wine solutions: they were found to contribute to "fullness", as well as to "chalkiness" and "coarseness" sensations (Vidal *et al.*, 2004b), and to increase "dryness" and "roughness" (Vidal *et al.*, 2004c). Anthocyanin purification of grape skins and wines in high quantities is still a challenge, since there are several compounds which interfer with sensory properties (mainly flavonol-belonging molecules) and which are difficult to avoid in extraction steps. In an improvement in purification, fractions of free glucoside and *p*-coumaroylated anthocyanins were shown not to differ from the unspiked model wine solution in both the wine range (13 % v/v) and at a reduced ethanol level (5 % v/v) (Vidal *et al.*, 2004a). Besides fraction composition and purity, such different results from sensory analyses may be due to the variability of the sensory technique used (e.g., different descriptors, reference standards and matrix solutions), as well as to differences in assessor training (Gawel, 1997; Gibbins and Carpenter, 2013; Sáenz-Navajas *et al.*, 2016)

Another possible option for evaluating the sensorial impact of polyphenols is the instrumental analysis of astringency and bitterness (Table 1). Astringency is a complex sensation involving several mechanisms, from sensory active molecules interacting with different salivary protein families to the formation of soluble and precipitable complexes. The onset of astringency involves hydrogen bonds, as well as hydrophobic and electrostatic interactions between sensory active molecules and proteins, and their complexity increases in mixed polyphenol solutions (Soares *et al.*, 2019). Moreover, additional phenomena can occur, such as the direct activation of mechanoreceptors, or direct interaction with the mouth epithelium (Gibbins and Carpenter, 2013; Soares *et al.*, 2016). Recently, the involvement of anthocyanins in the onset and formation of

TABLE 1. Studies which have carried out sensory and instrumental analyses on anthocyanin sensory properties.

Sensory analysis					
Year	Astringency	Bitterness	Mouthfeel	Notes	References
1976				"indistinct mild taste"	Singleton and Noble (1976)
2001	+	=	-	Anthocyanin extract added to seed and skin tannins in white wine	Brossaud *et al*. (2001)
2004	+		dryness, roughness, chalkiness	Anthocyanin extract (0.5 g/L, purity 78 %) added to model wine at 13 % ethanol	Vidal *et al.* (2004c)
2004	+	=	fullness, chalkiness, coarseness	Anthocyanin extract (0.5 g/L, purity 70 %) added to model wine at 11, 13, and 15 % ethanol	Vidal *et al.* (2004b)
2004			in-mouth dryness	Anthocyanin glucosides (0.5 g/L, purity 98 %) and coumaroylated (0.5 g/L, purity 87 %) in model wine at 13 % ethanol	Vidal *et al.* (2004a)
	=		-	Anthocyanin glucosides and coumaroylated (0.5 g/L) in un-buffered, 5 % ethanol solution	
2009	+	=	fine grain, dryness, grippiness, viscosity	Anthocyanin extracts (purity 95 %) added at 1.44 g/L in white juice during winemaking	Oberholster *et al.* (2009)
2014	+	+		Two *p*-coumaroylated anthocyanin contributors of bitterness, *p*-coumaroylated petunidin and malvidin-3-*O*-gucoside contributors to astringency	Gonzalo-Diago *et al.* (2014)
2015	+			Glucoside anthocyanin extract (purity 95 %), moderate velvety and astringency intensity	Ferrer-Gallego *et al.* (2015)
2017	+	+	dryness, bitterness, persistency	Oligomeric anthocyanins from wine fractions redissolved in 7 % ethanol buffer	Sáenz-Navajas *et al.* (2017)
2018	+	+		Anthocyanin extracts, glucoside, acetylated, and coumaroylated fractions (purity 95, 98, 87 and 91 % respectively) in model wine at 12 % ethanol; BET[a] 255, 297, 68, and 58 mg/L, respectively	Paissoni *et al.* (2018)
Chemical analysis					
Year	Astringency	Bitterness	Interaction	Notes	
2013		+	TAS2Rs[b] activation	Malvidin-3-*O*-glucoside activates TAS2R7 bitterness receptors (threshold of activation 6.0 µM), whereas cyanidin-3-*O*-glucoside did not activate TAS2Rs studied	Soares *et al.* (2013)
2015	+		Precipitable/soluble complexes with saliva PRPs[c]	Individual glucoside formed complexes with acidic PRPs. Evidence of soluble complexes between malvidin-3-O-glucoside with Histatin and PRPs.	Ferrer-Gallego *et al.* (2015)
2018	+		Precipitable complexes with saliva	Decrease in acetylated and coumaroylated fractions when saliva was added. Precipitation occurs depending on the anthocyanin acylation.	Paissoni *et al.* (2018)
2019	+		Electrostatic interactions	Malvidin-3-*O*-glucoside and (-) epicatechin synergic interaction with PRPs. The first driven by electrostatic interactions, the latter by hydrophobic and hydrophilic interactions. Interactions are increased in the presence of mixture. At high concentration non-specific reactions	Soares *et al.* (2019)
2020	+		Oral cell interactions	Anthocyanins (glucosides extract) are retained by different oral cells (HSC-3 from tongue and TR146 from buccal mucosa). Saliva and mucosal pellicle on the cell line decreased the anthocyanins retained.	Soares *et al.* (2020)

Symbols "+" and "=" indicate increased or unchanged perception respectively. [a] BET=Best estimated threshold, [b] TAS2Rs = human bitter taste receptors encoded by the TASTE 2 Receptor (TAS2R) gene family, [c] PRPs = Proline-rich proteins.

complexes has been demonstrated (Ferrer-Gallego *et al.*, 2015; Paissoni *et al.*, 2018; Soares *et al.*, 2019). Considering bitterness, anthocyanins have been found to activate the corresponding taste receptors (Soares *et al.*, 2013). Nevertheless, testing this stimulus in an alcoholic media is still impossible and it is difficult to study complex polyphenol matrices, as is the case for wine. Therefore, despite the possibility to instrumentally study the different mechanisms driving astringency sensations, the final perception is influenced by the complexity of wine polyphenols and other sensory active molecules mixture. Therefore, together with the instrumental evidence of anthocyanin involvement in astringency-driver mechanisms and bitterness stimuli, sensory analysis is still necessary for understanding the final in-mouth features of solutions.

The aim of this study was to investigate the sensory properties of grape extracts, with a particular focus on grape anthocyanins. First, grape extracts were tasted to establish their sensory active thresholds, and the appropriate terminology was selected in order to evaluate their contribution to in-mouth sensory properties by using a check-all-that-apply (CATA) methodology. After that, the assessors received training in the selected categories and descriptors, and then carried out a descriptive analysis (DA) of different individual grape extracts: polyphenol extracts from skins and seeds and anthocyanin extracts divided according to acylation group. Moreover, to find out if anthocyanins can influence the in-mouth perception of wine, the anthocyanin extract and its derived fractions were added to the polyphenol-based extracts and evaluated following the same descriptive sensory procedure.

MATERIALS AND METHODS

1. Extraction and purification of grape polyphenols

Grape samples from *Vitis vinifera* L. cv Nebbiolo and Barbera were collected at maturity in the Piedmont region (Northwestern Italy) during the 2015 vintage. Grape skins were peeled using a laboratory spatula and grape seeds were manually removed. The obtained grape material was lyophilised and then ground in a ball grinder; the resulting powder was used to carry out the extraction of grape polyphenol fractions.

1.1. Anthocyanin extracts

Total anthocyanin (TA) and its derived glucoside (GF), acetylglucoside (AF), and coumaroyl-glucoside (CF) fractions were extracted and purified by means of Centrifugal Partition Chromatography (CPC) and preparative HPLC as described in detail by Paissoni *et al.* (2018. The final extract purity was 95 %, 98 %, 87 %, and 91 % for TA, GF, AF, and CF respectively, calculated using the area ratios of 520 nm and 280 nm by HPLC-DAD. Total anthocyanin extracts (TA) from Barbera and Nebbiolo were individually tasted in a preliminary evaluation (triangle test; ISO 4120:1983), whereas subsequent evaluations were performed on Barbera TA only. The fractions differentiated by acylation were obtained from a mixture of the two different varieties.

1.2. Total polyphenol extract

Skin and seed polyphenol extraction was performed as described by González-Centeno *et al.* (2012) with an ASE 350 Accelerated Solvent Extraction System (Dionex Corporation. Sunnyvale, CA, USA). A mixture of Barbera and Nebbiolo ground grape seeds and skins was used to produce the polyphenol extracts. Grape skins and seeds were separately subjected to eight solid/liquid consecutive extractions with acetone/water (80:20, v/v) for the solvent system (40 mL of the corresponding solvent system). The ASE experimental settings were reported in Ma *et al.* (2016). The extract was then evaporated under reduced pressure and lyophilised to create crude skin and seed extracts. The crude polyphenol extract (equal to 5 g of dried powder of skins or seeds) was solubilised in 250 mL of water/ethanol (95:5, v/v) and extracted three times with chloroform to remove the lipophilic material (Lorrain *et al.*, 2011). This aqueous extract was concentrated and lyophilised to obtain a dry powder, indicated as SkTOT and SdTOT for skin and seed extracts respectively. The obtained extracts were analysed via phloroglucinolysis (Lorrain *et al.*, 2011). Mean degree of polymerisation (mDP) was 15.5 ± 0.45 and 3.7 0± 0.10 for SkTOT and SdTOT respectively. The percentage of galloylation (G %) was 15.20 ± 0.27 for SdTOT and 2.20 ± 0.10 for SkTOT. The percentage of prodelphinidins (Pd %) was 34.00 ± 0.23 for SkTOT, whereas no

prodelphinidins were detected in SdTOT. For the skin extract, anthocyanin content was determined by HPLC-DAD (Paissoni *et al.*, 2018), accounting for 130.2 ± 2.8 mg malvidin-3-*O*-glucoside /g of extract, (Sigma–Aldrich, Saint Quentin Fallavier, France). All the extracts were lyophilised twice before sensory analysis to ensure the absence of solvents.

2. Sensory analysis of grape extracts

2.1. General procedure

The sensory analyses were conducted in a tasting room at the University of Bordeaux, Oenology research unit (ISVV, France). The room fulfilled the ISO 8589:2007 standard (sound insulation, constantly regulated temperature).

A panel of 18 volunteer assessors from the Oenology department at the University of Bordeaux (ISVV, France) took part in the experiment. All assessors are considered wine experts according to the definition of Parr *et al.* (2002). Nevertheless, a preliminary panel selection process was carried out, in which the assessors tasted standard solutions: aluminium sulphate (AlSu; 2000 mg/L) for astringency, quinine sulphate (Qsu; 15 mg/L) for bitterness, tartaric acid (5 g/L) for acidity, and catechin (1 g/L) for both astringency and bitterness (Chira *et al.*, 2012). In order to do this, two tests were performed: a discrimination test (triangle test; ISO 4120:1983) and an identification test. In the triangle test, the assessors were asked to recognise different samples in the series composed of spiked water solution and water without the standard molecules. For the identification test, the assessors were asked to taste four spiked water solutions, and to identify and describe the in-mouth sensations perceived. Only those who recognised the sensations elicited by the reference standards were accepted as assessors for the experiment (16 assessors remained in the experiment).

The assessors were informed of the purification methodology for obtaining the fractions conducted in the laboratory. If they chose to continue in the experiment, they signed a consent form. A total of nine sessions were conducted prior to the formal evaluation, comprising three preliminary assessments of the extracts and six training sessions on the attribute scales of the descriptive analysis (DA). Finally, the formal descriptive analysis (DA) of the investigated extracts was conducted in five sessions.

2.2. Preliminary extracts' evaluation

2.2.1. Triangle test

Two sessions were dedicated to determining a suitable extract concentration for the experiment. Triangle tests (ISO 4120:1983) were conducted taking into account the usual wine concentration ranges and detection thresholds. Two total anthocyanin extracts (from different grape cultivars, Nebbiolo and Barbera TA; 250 mg/L), glucoside fractions (GF; 300 and 400 mg/L), acetylglucosides and coumaroylglucosides fractions (AF and CF; 100 mg/L each), skin polyphenol extract (SkTOT; 500 mg/L), and seed polyphenol extracts (SdTOT; 500 and 750 mg/L) were tasted. The selected extracts were dissolved in model wine solutions (12.5 % v/v ethanol, 4 g/L of tartaric acid, pH 3.5), and were randomly tasted along with the unspiked model wine solutions. Nebbiolo *vs* Barbera TA extracts were also tested to find any differences between the two. Barbera TA *vs* un-spiked model wine and Barbera TA *vs* Nebbiolo TA were evaluated in duplicate (once per session) to confirm the obtained results. Seed extract and skin extract at 500 mg/L, GF at 300 mg/L, CF and AF at 100 mg/L were evaluated in the first session; whereas seeds extract at 750 mg/L and GF at 400 mg/L were evaluated in the second session, with the aim of achieving higher discrimination from the unspiked model wine. In addition to the triangle tests, the assessors were asked to report the descriptors that helped them identify the correct sample.

2.2.2. Check-All-That-apply (CATA) for the selection of descriptors

Four extracts representative of the groups under evaluation were selected for a Check-All-That-Apply (CATA) analysis, in order to identify the most frequently reported attributes and the terms which were able to best discriminate the extracts (Varela and Ares, 2012). The selected grape extracts were skin extract (SkTOT; 1000 mg/L), seed extract (SdTOT; 1000 mg/L), total anthocyanin extract from Barbera (TA; 400 mg/L), and glucoside fraction of anthocyanins (GF; 400 mg/L) in model wine solution (12.5 % v/v ethanol, pH 3.5, 4 g/L of tartaric acid). The selection of attributes was based on the following: results previously obtained by Paissoni *et al.* (2018), the descriptors reported in the triangle tests (only the ones by assessors who correctly discriminated

among samples), and bibliographic research on the tasting of grape/wine extracts and fractions, in particular anthocyanins (Oberholster *et. al.*, 2009; Gawel *et al.*, 2000; Vidal *et al.*, 2004a; Vidal *et al.*, 2004b; Vidal *et al.*, 2004c; Sáenz-Navajas *et al.*, 2017). The selected attributes (23 in total) belonged to taste (sweet, bitter, salty, acid), and the selection of astringent sub-qualities was based on the mouth-feel wheel terminology proposed by Gawel *et al.* (2000). The introduced sub-qualities were: *weight* (watery, viscous, dense), *texture* (oily), *heat* (hot), *irritation* (prickle, tingle), *drying* (dry), and those related to astringency including *surface smoothness* (emery, silky) and *particulates* (dusty, grainy, chalky), *complex terms* (soft, round, mouthcoating, aggressive), a *dynamic attribute* (adhesive), and the term "rich".

2.3. Descriptive analysis (DA)

2.3.1 Sensory training for descriptive analysis (DA)

The assessor training on astringency and bitterness scales was a slightly modified version of that proposed by Chira *et al.* (2012). During the first part (two sessions), the assessors were familiarised with the different sensations, using a stimuli concentration range. In the first session, low (500 mg/L AlSu and 2.5 mg/L QSu) and high (4000 mg/L AlSu and 30 mg/L QSu) reference standards of astringency and bitterness dissolved in water were evaluated. To improve the assessors' ability to distinguish bitterness from astringency, they were provided with solutions of the mixed stimuli and asked to score low/high astringency or bitterness; one solution was representative of low bitterness and high astringency (2.5 mg/L Qs and 4000 mg/L AlSu), and the other of high bitterness and low astringency (30 mg/L QSu and 500 mg/L AlSu). The assessors' scores allowed us to slightly modify the scale used for training based on the tasted fractions (section 2.2.2). Part of the second session focused on the reference standard solutions for astringency and bitterness in model wine solutions (12.5 % (v/v) ethanol, pH 3.5, 4 g/L of tartaric acid), by carrying out an ordination test of selected scales; i.e., for astringency (model wine, and model wine plus 1000, 2000, 4000, and 6000 mg/L AlSu) and for bitterness (model wine, and model wine plus 2.5, 5, 10, and 20 mg/L QSu). The assessors were also asked to distinguish in low, moderate, and high astringency and bitterness in mixed solutions (2.5, 10, 20 mg/L of QSu for bitterness; 1000, 4000, and 6000 mg/L of AlSu for astringency).

In the second part of the training (three sessions), the assessors were familiarised with the scales to be used for *overall astringency* and *bitterness*, and with the definitions of astringency sub-qualities. The assessors discussed the definitions of *particulate* (in-mouth sensation), *surface smoothness* (after expectoration) and *irritation*. Tactile standards were used when possible, whereas terms that did not have standards were agreed upon by written definition. Attribute rating was done on an 8-point scale: 0 =

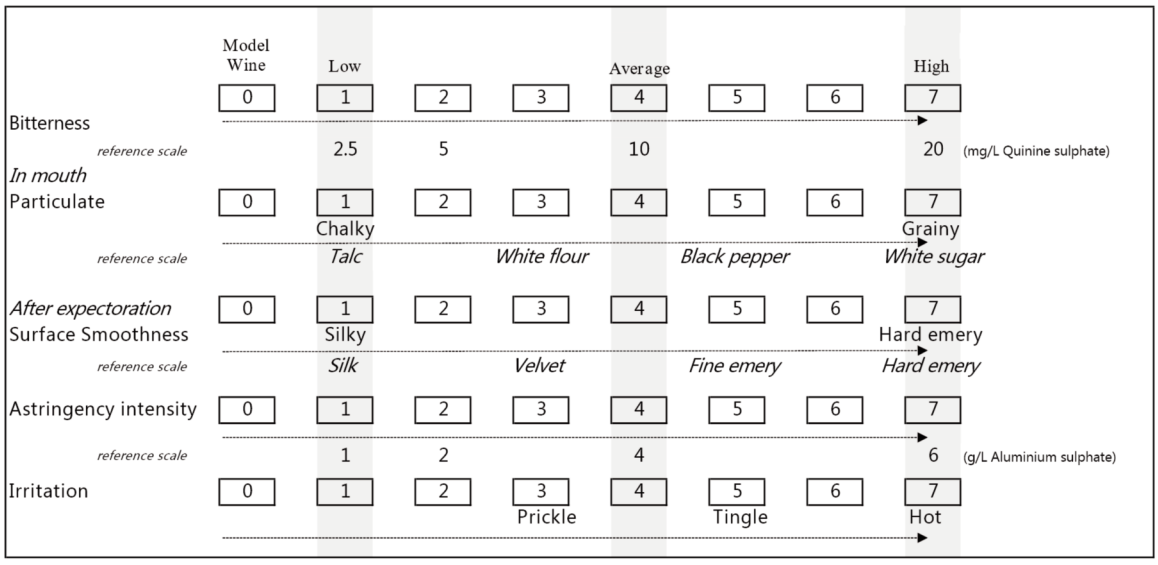

FIGURE 1. Tasting scorecard with anchored standard references.

TABLE 2. Descriptors, definitions, and reference standards used in descriptive analysis.

Descriptor	Standard or definition	Reference
Bitterness *(oral)*	Quinine sulphate (max 20 mg/L, average 10 mg/L)	
Overall astringency *(oral)*	Aluminium sulphate (max 6 g/L, average 4 g/L)	
Particulate powder *(manual)*	Feeling of particulate matter brushing against the surface of the mouth through the movement of the solution Talc: talc powder Dusty: white flour Sandy: grounded pepper Grainy: white sugar	Gawel *et al.* (2001)
Surface smoothness fabric *(manual)*	Texture felt on mouth surfaces when the different surfaces came in contact with each other (after expectoration) Silky: silk Velvety: velvet Fine grain: fine cotton Hard grain: corduroy	Gawel *et al.* (2001)
Irritation definition	Sense of irritation usually associated with carbonation Tingly (low) Prickly (moderate) Hot (high)	Pickering and Demiglio (2008)

"absence", 1 = "low" and 7 = "high"). "Low" and "high" levels were anchored with the use of reference standards for each attribute (Figure 1).

For example, for *overall astringency*, scores of 1,4 (corresponding to the average value) and 7 corresponded to 1000, 4000, and 6000 mg/L AlSu respectively, whereas for *bitterness*, these scores corresponded to 2.5, 10 and 20 mg/L QSu. Fabric samples were selected to anchor the *surface smoothness* line scale: "silky" (silk fabric) and "hard emery" (corduroy) were located along the scale at 1 and 7 points respectively. For the *particulate* attribute, "chalky" (talc powder) and "grainy" (white sugar) corresponded to 1 and 7 points. For *irritation*, definitions were given and placed on a low, moderate, and high scale (defined as "tingle", "prickle", and "hot" at 3, 5 and 7 respectively). The 0 score represented the model wine solution.

A summary of definitions, standard scales and references is provided in Table 2. During the last training session (the sixth), the assessors took part in a simulation of a descriptive analysis to become familiar with the tasting procedure and scorecard. They tasted solutions for stimuli enhanced by the samples under evaluation; in particular, a seed tannin extract from *cv* Nebbiolo (1250 mg/L), skin tannins (1000 mg/L skin commercial oenological tannin) and catechin and gallic acid solutions (both 1000 mg/L). At the end of each training session, the selected descriptors or intensity scores for each solution were compared in a discussion led by the panel leader.

2.3.2. Formal DA of grape extracts

The assessors evaluated a maximum of four extract solutions per session for a total of five sessions, in each session one sample was repeated to evaluate the assessor's performance (a maximum total of five samples per session). Each extract solution was coded with a three-digit random code, and black ISO 3591:1977 wine glasses were used and randomly placed in order not to bias perception.

In each evaluation session, the assessors were asked to assess the reference model wine solution (0 on the scale) and the medium point of the scale on which they were trained for *overall astringency* and *bitterness* (i.e., 4000 mg/L aluminium sulphate and 10 mg/L quinine sulphate for astringency and bitterness respectively) to help standardise the use of the scale before sample evaluation. Fabric samples for *surface smoothness* and powdery standards for *particulates* were provided during each session in each individual booth. The complete scales for *bitterness* and *overall astringency* were available in case assessors wanted to try them again before tasting. All fractions were served at room temperature and evaluated in individual booths. All extracts were evaluated in model wine (12.5 % v/v ethanol, 4 g/L tartaric acid, pH 3.5). The final concentrations were chosen according to the wine concentration range and triangular tests as explained above, which were 1000 mg/L for both SdTOT and SkTOT, 400 mg/L for TA and GF, and 100 mg/L for CF and AF.

The individual fractions of anthocyanins, TA, GF, and CF, were also added to the SkTOT and SdTOT solutions (1000 mg/L each) in the same concentration at which they were tasted individually (400 mg/L for TA and GF, and 100 mg/L for CF) to investigate if they could modify the sensory properties of other polyphenols in the wine concentration range.

To minimise fatigue and standardise the assessment process, a tasting and rinsing procedure was established: the assessors were asked to (1) sip and swirl the solution in their mouth for 5-10 s, (2) expectorate, and (3) fill out the scorecard. After each sample, the assessors were asked to rinse their mouth out with water, eat a piece of unsalted cracker and rinse again with water.

3. Data analysis

Data were analysed using R software (R Core team, Version 3.5.0; R Studio, Version 1.1.453). The panel performance for CATA questions was checked using the reproducibility index (*Ri*), as described by Campo *et al.* (2008), and calculated on the replicate sample to check assessor repeatability. For the CATA descriptors of selected data, the attributes on the list were ranked according to their citation frequency to identify the most relevant descriptors for tasting and to better distinguish each extract. A Correspondence Analysis (CA) was performed on the CATA frequencies table (descriptors in rows and evaluated extract in columns) using Factominer R package (Lê *et al.*, 2008). CATA significant attributes were assessed with Cochran's Q test (Varela and Ares, 2012). Since

CATA was performed as a preliminary approach to underlining attributes potentially relevant for further investigation, the criteria for significance was increased to $p < 0.1$ to avoid missing relevant terms.

The panel performance for descriptive analysis (DA) was evaluated using PanelCheck software (version 1.4.2). The panel performance was tested on the subset of replicate samples by three-way ANOVA with "replicate", "sample" (extract), and "assessors" as factors and their interaction. The evaluation was considered adequate when there were no significant differences ($p > 0.05$) in "assessors"*"replicates" and "assessors"*"sample" interactions. Principal component analysis (PCA) as Tucker-1 plots were performed for both attributes and assessors as described by Tomic *et al.*, 2010. The assessors' projections were checked in the loading plot for *bitterness*, *overall astringency*, *particulate*, *irritation* and *surface smoothness* in order to assess their agreement on each attribute.

Two-way ANOVA (assessors as random and extracts as fixed factors; R package nlme, Pinheiro *et al.*, 2020) was used to compare DA data from the formal sessions, and in case of significant differences, the Tukey HSD post-hoc test (R package multcomp, Hothorn *et al.*, 2008) was performed to establish differences among extracts.

RESULTS AND DISCUSSION

1. Triangle test results

The first experiment aimed to assess the concentrations at which the fractions could be perceived by the panel in a triangle test: the tested concentrations and results are reported in Table 3. The initial concentration to be tested was 500 mg/L total polyphenols from the skin (SkTOT) and seed (SdTOT) extracts. Although SdTOT had already been perceived as different by most of the panel ($p < 0.05$), SkTOT was not easily recognised as being different ($p > 0.05$). When the concentration of SdTOT increased to 750 mg/L, more differences were perceived ($p < 0.001$). Because the polyphenol range of a red wine can vary by up to several grams per liter, the concentration chosen for the tasting of these two fractions was 1000 mg/L, which fulfilled the red wine range and allowed the fraction to be clearly perceived by the assessors.

Concerning total anthocyanin extract (TA), two different extracts from *Vitis vinifera* L. cv Nebbiolo and Barbera (with a different ratio of individual anthocyanins and acylation; Paissoni *et al.,* 2018) were tasted at a concentration of 250 mg/L. Both the extracts were perceived as being different to the unspiked model wine solution ($p < 0.01$). Interestingly, when these two fractions were compared (400 mg/L), they were not perceived as being different. Therefore, just one (TA from *cv* Barbera) was used for the following experiments. To improve sensitivity, a concentration of 400 mg/L was chosen, which is in accordance with young red wine concentrations and is similar to other sensory assessments (Vidal *et al.*, 2004a; 2004b; Ferrer-Gallego *et al.*, 2015).

Regarding the individual fractions, glucoside anthocyanins (GF) were perceived as different at 300 mg/L ($p < 0.05$), but more so at 400 mg/L ($p < 0.01$); thus the latter concentration was chosen for further analysis. Acetylated (AF) and *p*-coumaroylated (CF) anthocyanins were found to differ at 100 mg/L ($p < 0.01$ and $p < 0.05$ for AF and CF respectively). Therefore, these concentrations were chosen after the triangle tests, as they could be easily perceived by the assessors, although they are very high compared to the concentrations found in commercial wines.

2. CATA results

Assessor performance in a wine CATA analysis is considered to be adequate if $Ri > 0.20$ (Campo *et al*., 2008). In our case, assessors who failed this repeatability test were excluded from further analysis (n = 2). The overall panel performance (n = 10) was $Ri = 0.43$, which fulfilled the repeatability requirement. Only descriptors cited by at least 20 % of the panel were considered for further analysis. A frequency table summarises each descriptor individually, which were grouped into arbitrarily chosen categories based on bibliography (Gawel *et al.*, 2000), namely *taste*, *mouthfeel*, *astringency sub-qualities* and *complexive astringency* (Table 4).

The frequency table shows the most cited terms and the most frequently used "group" to describe the selected grape extracts. The frequencies were also analysed in a Correspondence Analysis (CA, Figure 2) to find out if these terms differentiated the selected fractions. A combination of the most cited attributes and

TABLE 3. Triangle test results for the perception of phenolic fraction additions to the model wine solution.

Sample	Concentration (mg/L)	Reference solution	α value (correct answer) n = 16
Total anthocyanin extract (Nebbiolo, TA)	250	model wine	0.01 (11)[a]
Total anthocyanin extract (Barbera, TA)	250	model wine	0.01 (11)[a] 0.01 (11)[b]
Glucoside fraction (GF)	300	model wine	0.05 (9)[a]
Glucoside fraction (GF)	400	model wine	0.01 (11)[b]
Acetylated fraction (AF)	100	model wine	0.01 (11)[a]
Coumaroylated fraction (CF)	100	model wine	0.05 (10)[a]
Skin polyphenols (SkTOT)	500	model wine	ns (7)[a]
Seed polyphenols (SdTOT)	500	model wine	0.05 (10)[a]
Seed polyphenols (SdTOT)	750	model wine	0.001 (12)[b]
Total anthocyanin extract (Nebbiolo, TA)	400	total anthocyanin extract (Barbera, TA)	ns (8)[a] ns (4)[b]

[a] First session results and [b] and second session results.

TABLE 4. Frequencies of CATA analysis.

Descriptor	Grouping		Frequencies of citation			
	Group	Sub-qualities[a]	Rank	% in the group	% in total	p-value[b]
Bitter (amer)	**Taste**		**1**	**46.9**	**13.3**	0.464
Acid (acide)	**Taste**		**4**	**21.9**	**6.2**	0.476
Sweet (sucré)	Taste		13	7.8	2.2	0.738
Salty (salé)	**Taste**		**3**	**23.4**	**6.6**	**0.045**
Hot (brûlant)	**Mouthfeel**	**heat**	**4**	**22.2**	**6.2**	0.651
Dry (asséchant)	**Mouthfeel**	**drying**	12	9.5	2.7	**0.037**
Tingly (piquant)	**Mouthfeel**	**irritation**	**8**	**15.9**	**4.4**	0.641
Prickly (pointu)	Mouthfeel	irritation	11	11.1	3.1	0.478
Oily (onctueux)	**Mouthfeel**	**texture**	**7**	**17.5**	**4.9**	**0.069**
Watery (acqueux)	Mouthfeel	weight	9	14.3	4.0	0.762
Dense (dense)	Mouthfeel	weight	12	9.5	2.7	1.000
Emery (rugueux)	**Astringency sub-qualities**	**surface smoothness**	**6**	**24.0**	**5.3**	0.383
Dusty (poussiéreux)	**Astringency sub-qualities**	**particulate**	**5**	**26.0**	**5.8**	0.697
Grainy (granuleux)	Astringency sub-qualities	particulate	12	12.0	2.7	0.242
Chalky (talc)	**Astringency sub-qualities**	**particulate**	**8**	**20.0**	**4.4**	0.742
Silky (soyeux)	**Astringency sub-qualities**	**surface smoothness**	9	18.0	4.0	**0.040**
Rich (gras)	Complexive astringency		12	12.2	2.7	0.336
Adhesive (adhérant)	Complexive astringency	dynamic	9	18.4	4.0	0.801
Soft (doux)	**Complexive astringency**	**complex**	**8**	**20.4**	**4.4**	0.475
Mouthcoating (enrobant)	Complexive astringency	complex	10	16.3	3.5	0.928
Aggressive (agressif)	**Complexive astringency**	**complex**	**2**	**32.7**	**7.1**	**0.071**

[a] Sub-qualities grouping taken from Gawel *et al*. (2000). [b] *p*-value according to the Cochran's Q test for descriptors in discrimination of samples. Values in bold indicate relevant terms based on Cochran's Q test ($p < 0.1$) or rank of citation (1st to 8th most cited terms).

their ability to discriminate samples was taken into consideration for further sensory assessment of grape extracted fractions.

The three most frequently cited descriptors were "bitter" (13.3 %, 1st), "aggressive" (7.1 %, 2nd), and "salty" (6.6 %, 3rd) (Table 4). Interestingly, "bitter" was often reported, but did not allow the samples to be differentiated (p = 0.464). In contrast, "salty" and "aggressive" were relevantly used for the GF and SdTOT sample characterisation respectively, and showed better sample differentiation (p = 0.045 and p = 0.071, for "salty" and "aggressive" respectively). These descriptors, together with "acid" and "hot" (6.2 %, 4th) can be explained in part by the model wine solution, since tartaric acid and low pH are in accordance with acidic traits. Additionally, ethanol has been reported to be perceived as bitter and to induce a hot sensation (Vidal *et al*., 2004b). Likewise, phenolic compounds have been reported as bitter markers (Gonzalo-Diago *et al.*, 2014; Hufnagel and Hofmann, 2008), and they therefore are common descriptors for grape extracts.

Terms for astringent sub-qualities accounted for 22.1 % of the total citations, the terms for "dusty" and "emery" being the most cited with 5.8 % (5th) and 5.3 % (6th) of the total citation frequencies. Furthermore, "chalky" and "silky" were frequently cited (4.4 %, 8th and 4.0%, 9th respectively; Table 4**)**.

The CA biplot is shown in Figure 2**.** The two first dimensions account for 81.0 % of the explained variance among samples. Dimension 1 (Dim1) contributed 46.3 %, and it was mainly described by astringency sub-quality attributes: "silky" was negatively correlated (-0.805) and contributed to sample discrimination (p = 0.040), whereas "emery" mainly contributed to the positive loading (+ 0.394, p > 0.1). In general, samples on the right side of the plot can be considered to have harsher astringency sub-qualities ("emery"), whereas samples plotted on the left side evoked softer sensations ("silky"). This is in accordance with the "aggressive" attribute, which characterised the right side of the axis (p = 0.071), in contrast with "oily" (p = 0.069) and "silky" (p = 0.040), which characterised the left side.

On the other hand, Dimension 2 (Dim2) accounted for 34.7 % of the explained variance, and was positively correlated with "watery" and "mouthcoating" attributes (+ 0.396 and + 0.345 respectively), and negatively correlated with "dry" and "grainy" attributes (- 0.995, p = 0.037 and - 0.504 respectively). Nevertheless, these attributes were not able to significantly discriminate samples even with increased significance criteria (p < 0.1). Moreover, the "mouthcoating", "watery", "grainy", and "dry" attributes belong to different groups; i.e., *complex* (overall astringency), *weight* (mouthfeel), *particulate* (astringency sub-qualities), and *drying* (mouthfeel) respectively. Despite the variability of the group and the low discrimination ability (p > 0.1), the descriptors reported in the upper quadrant are linked by a general softer sensation with respect to those located in the lower quadrants of the plot. According to the plot description, Sk-TOT is related to harsher sensations, such as "grainy" and "dry", whereas SdTOT corresponds to "aggressive" and "emery" attributes.

Regarding anthocyanin-based fractions, TA and GF are in the upper part of the plot, and are therefore represented by "watery" and "mouthcoating" sensations. The glucoside fraction (GF), however, is differentiated by softer astringency sub-qualities, such as "silky", in contrast to total anthocyanins (TA), which are more associated with "emery". Interestingly, SkTOT and GF were well differentiated from SdTOT, whereas TA was slightly different from GF and SkTOT, and more like SdTOT in the tasted concentrations.

In terms of both frequency and discrimination ability, the most cited term, "bitter", was considered relevant enough to be investigated. Astringent sub-qualities such as surface-smoothness and particulate were also found to be both highly cited and able to discriminate the samples. Therefore, they were chosen to train assessors in using the scales. For example, particulate was described as "feelings of particulate matter brushing against the surface of the mouth through the movement of the solution", thus as "in-mouth" astringency; whereas surface smoothness was defined as "texture felt on mouth surfaces when the different surfaces came in contact with each other", therefore after expectoration. Reference standards and scales were defined, slightly modifying the scales proposed by Pickering and Demiglio (2008), for the evaluation of oral sensations elicited by white wine. *Overall astringency* was chosen for the scales to

summarise the intensity of both in-mouth and post expectoration astringency.

Moreover, given the high citation frequency of the term "acid" and "hot" (6.2 %, 4th) and also "tingly" and "prickly" (together 7.5 %), the panel discussed and attempted to scale these sensations and to verbally explain them. In this case, the Pickering and Demiglio (2008) definition of "tingly" was once again used, and the scale was divided into low, moderate and high irritation.

3. DA results

Panel performance was evaluated using a PCA approach (data not shown) to understand the consensus in the attributes investigated and ANOVA for the effects of assessors, extracts and replicates and their interactions in order to assess panel repeatability. Regarding consensus, assessor projections were grouped in the loading plot for *overall astringency*, *particulate*, and *surface smoothness*, showing that the panel had agreed on the interpretation of these terms. For *irritation*, however, the assessors were spread over the loading plot, which suggests that the assessors did not interpret this attribute in the same way. In addition, for the terms involved, the ANOVA test for assessors resulted in significant differences ($p < 0.05$), underlining the differences in the evaluation of these attributes on the scale. Hence, *irritation* was no longer considered in subsequent analyses, confirming that the use of standard references should be preferred to verbal description of terms. ANOVA also showed that there was a significant assessors* -sample interaction ($p < 0.05$) for the *attribute bitterness*. Examination of the PCA projection indicated that two assessors rated bitterness differently from the rest of the panel. Accordingly, none of the scores from these two assessors were taken into consideration for further analysis. Therefore, the final DA panel consisted of ten assessors.

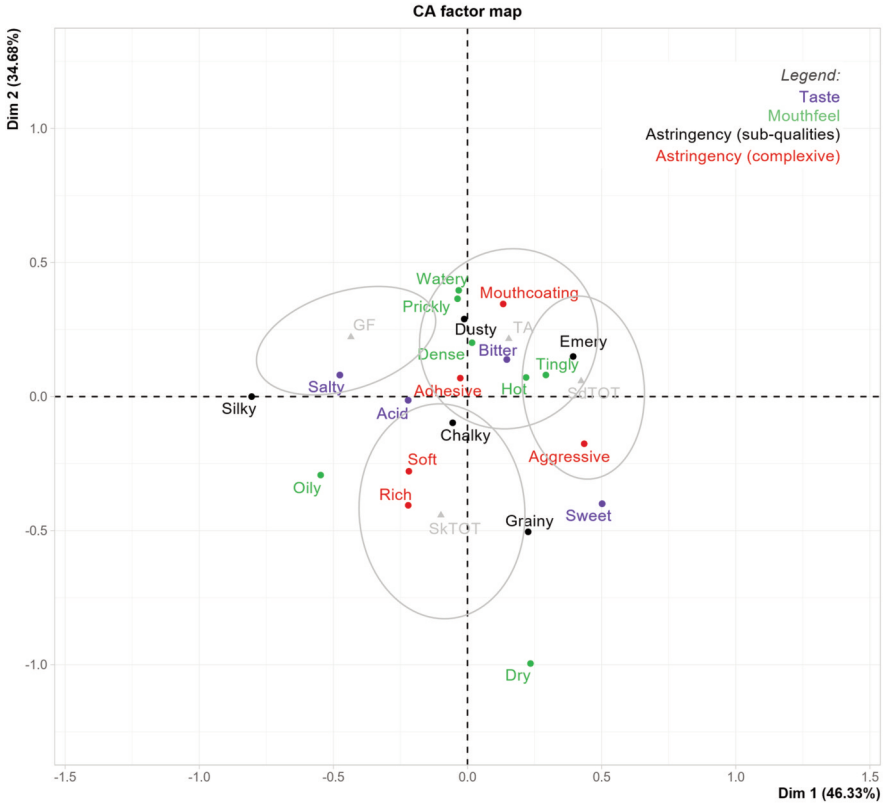

FIGURE 2. Correspondence Analysis (CA) of descriptors for the selected grape extracts performed on the citation frequencies.
GF = glucoside anthocyanin fraction; TA = total anthocyanin fraction; SdTOT = seed polyphenol extract; SkTOT = skin polyphenol extract. Ellipses represent confidence ellipses ($p = 0.05$).

3.1. Grape extract sensory characterisation

The result of the ANOVA test for the extract effect (Figure 3) shows that *overall astringency*, *particulate*, and *surface smoothness* were significantly different among extracts ($p = 0.003$, $p = 0.001$, and $p = 0.002$, respectively), but not *bitterness* ($p > 0.05$). Likewise, Brossaud et al. (2001) did not find significant differences between total anthocyanin extract and tannins from seeds and skins added to white wine, whereas differences were reported in citric acid solutions, in which seed extracts were significantly higher than skin and anthocyanin extracts. Our different results can be imputed to differences in polyphenol extracts. In fact, different content and composition of polyphenols in skin and seed extracts can vary depending on the grape features and the purification steps applied. Alcohol can also enhance bitterness (Mattes and DiMeglio, 2001), which may conceal individual differences between fractions.

In contrast, *overall astringency* differed among the fractions: SkTOT was the highest in astringency, but not significantly different from SdTOT and TA. Anthocyanin fractions (GF, AF, and CF) were rated lower in *overall astringency*, significantly less than SkTOT, but not different from SdTOT and TA. In previous research, anthocyanin astringency ratings have been found to be lower than seed or skin extracts, and differences have been found depending on the media in which they were tasted; i.e., model wine at different ethanol level, citric acid solution, and white wine (Brossaud *et al.*, 2001; Vidal *et al.*, 2004b). Regarding GF, anthocyanin glucoside has been reported to have moderate astringency (Ferrer-Gallego *et al.*, 2015).

Interestingly, the astringency sub-qualities, *particulate* and *surface smoothness*, differed among samples, and they agreed with the overall perceived astringency results. In fact, SkTOT was also perceived as the highest on the scale of sub-qualities, corresponding to anchored reference, "fine emery", for *surface smoothness*, and close to "grainy" for *particulate* attribute. This could agree with higher mean polymerisation (mDP) of skin tannins, which increases both the overall astringency and the puckery sensation in the mouth (Ma *et al.*, 2014). TA, GF, and CF fractions did not differ from polyphenol extracts in *particulate* attribute, although they were rated lower. Total anthocyanins added to white juice have previously been reported to result in a wine with an increased "fine grain" attribute (Oberholster *et al.*, 2009). Our results seem to agree with the CATA test, since TA rating corresponds to "dusty" and "sandy" on the scale, referring to the moderate particulate sensations in the mouth. The acetylated fraction (AF) was rated the lowest and was significantly different from SkTOT extract, evoking a "chalky" sensation previously reported in literature in an anthocyanin sensory analysis (Vidal *et al.*, 2004a; Vidal *et al.*, 2004b). *Surface smoothness* of AF and CF was significantly lower than that of skin polyphenol extracts ($p = 0.002$). According to the scale, AF and CF were closer to a "silky" sensation and TA and GF correspond to a "velvety" one, whereas SkTOT corresponded to "fine emery". Interestingly, a previous study (Ferrer-Gallego *et al.*, 2015) reported an increase in the descriptor "velvety" in wine with added anthocyanin glucoside

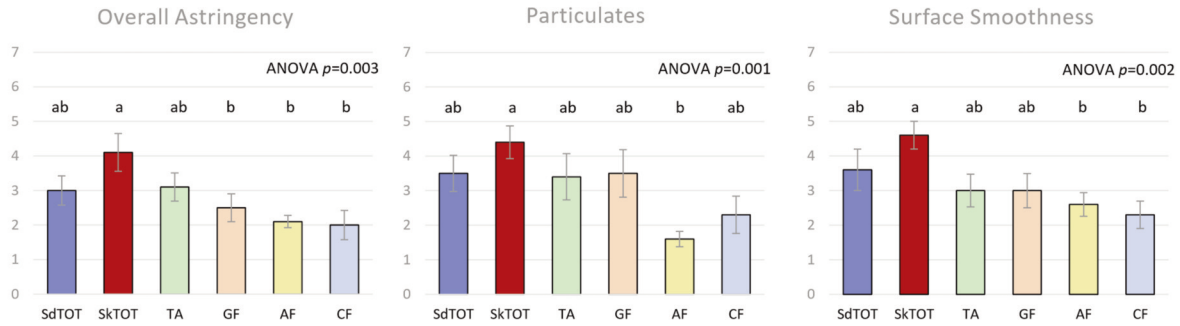

FIGURE 3. Descriptive analysis (DA) of grape extracts.
SdTOT = seed polyphenol extract; SkTOT = skin polyphenol extract; TA = total anthocyanin extract; GF = glucoside anthocyanin fraction; AF = acetylated anthocyanin fraction; CF = *p*-coumaroylated anthocyanin fraction. Data are expressed as means of assessor ratings and error bars are calculated as $s/(n)^{1/2}$, where s is standard deviation and n is the number of assessors. *p*-values are reported according to ANOVA, and different Latin letters indicate significant differences according to the HSD Tukey test ($p < 0.05$).

(400 mg/L), which is in line with the results of present study.

3.2. Mixed fractions sensory analysis

Anthocyanin fractions tasted alone were perceived as contributing to bitterness, overall astringency and its sub-qualities, *surface smoothness* and *particulate* to different extents, depending on their compositions and their concentrations. Although these contributions had lower ratings with respect to other phenol fractions, it is interesting to determine whether the sensory perceptions of polyphenol extracts are influenced by the addition of total anthocyanin extracts (TA) and their derived fractions GF and CF (Table 5). In previous research, total anthocyanin extracts were found to increase overall astringency when added to seed or skin extracts (Brossaud *et al.*, 2001; Vidal *et al.*, 2004b), although this increase depends on the concentration of proanthocyanidins (Vidal *et al.*, 2004b). Under our experimental conditions, when anthocyanin fractions were added to the SkTOT extract, no significant differences ($p > 0.05$) were found for any of the investigated attributes, except for *surface smoothness,* which was rated significantly lower ($p = 0.043$) in the sample with added GF. Conversely, a significant increase was found when GF was added to SdTOT for the *particulate* and *surface smoothness* sub-qualities ($p = 0.010$ and $p = 0.030$ respectively), as well as for *overall astringency* ($p = 0.009$). Depending on the polyphenol fraction, *surface smoothness* descriptors showed an inverse trend when anthocyanins were added, decreasing when glucoside anthocyanins were added to skin polyphenols ($p < 0.05$) and increasing when they were added to seed polyphenols ($p < 0.05$). Recently, Soares *et al.* (2019) showed that the mixture of epicatechin and malvidin-3-glucoside increased the interaction with salivary proline-rich proteins when compared to epicatechin alone, which may result in an increased perceived astringency, and thus justify the results obtained here for SdTOT. Nevertheless, this increase could be hidden in the skin polyphenol extract, because of the lower amount of monomeric flavanols usually reported in skin with respect to seeds. The condensed tannins of skins are conversely known to be highly polymerised, and increased polymerisation is positively correlated with perceived astringency (Ma *et al*., 2014). In fact, condensed tannins are the first drivers of in-mouth related sensations,

TABLE 5. Descriptive Analysis (DA) of mixed extracts.

Mixed extracts	Bitterness	Particulate	Surface smoothness	Overall astringency
SkTOT	3.6 ± 0.5	4.4 ± 0.5	4.6 ± 0.4 a	4.1 ± 0.5
SkTOT + TA	3.7 ± 0.5	4.3 ± 0.6	4.1 ± 0.5 ab	3.9 ± 0.5
SkTOT + GF	5.0 ± 0.5	5.3 ± 0.4	3.8 ± 0.3 b	5.4 ± 0.4
SkTOT + CF	4.3 ± 0.4	4.6 ± 0.5	4.0 ± 0.6 ab	4.0 ± 0.7
Sign	ns	ns	*	ns
p value	0.201	0.441	0.043	0.077
SdTOT	4.2 ± 0.5	3.5 ± 0.5 b	3.6 ± 0.6 b	3.0 ± 0.4 b
SdTOT + TA	5.2 ± 0.4	4.0 ± 0.5 ab	4.0 ± 0.4 ab	3.6 ± 0.3 ab
SdTOT + GF	3.8 ± 0.4	5.3 ± 0.5 a	5.1 ± 0.4 a	4.9 ± 0.5 a
SdTOT + CF	4.5 ± 0.6	3.3 ± 0.4 b	4.1 ± 0.4 ab	3.4 ± 0.4 b
Sign	ns	*	*	**
p value	0.177	0.010	0.030	0.009

SdTOT = seed polyphenol extract; SkTOT = skin polyphenol extract; TA = total anthocyanin fraction; GF = glucoside anthocyanin fraction; CF = *p*-coumaroylated anthocyanin fraction. Data are expressed as means of assessors rating and error bars are calculated as $s/(n)^{1/2}$, where s is standard deviation and n is the number of assessors. Sign: *, **, and ns indicate significance at $p < 0.05$, 0.01, and not significant respectively, for the differences among samples according to ANOVA. Different Latin letters indicate significant differences according to HSD Tukey test ($p < 0.05$).

particularly mouthfeel (Ferrero-del-Teso *et al.*, 2020). However, these results confirm the involvement - even if to a different extent - of other relevant groups of polyphenols (such as anthocyanins) in the final oro-sensory perception of wine, as has already been suggested by Ferrero-del-Teso *et al.* (2020).

CONCLUSIONS

The role of anthocyanins in the in-mouth sensory properties of wine has been evaluated in previous studies with contradictory results. The aim of the present study was to adapt common methodology for sensory analysis to the investigation of the contribution of anthocyanins alone and in more complex solutions, such as mixed polyphenol extracts. The results showed that anthocyanins extracted from grape skins are involved in the "in-mouth" perception of model wine solutions, although their contribution is less relevant than other polyphenolic groups, such as condensed tannins. At the wine level, glucoside anthocyanins were related to sensations of "velvety" sub-qualities, whereas when mixed with the other acylation groups (total anthocyanin extract) they showed higher overall astringency and harsher sub-qualities than when alone. In particular, glucoside anthocyanins led to a decrease in overall astringency in the presence of skin polyphenols, whereas they seemed to enhance the overall astringency and related sub-qualities of seed polyphenols. This behaviour could be relevant when explaining the final in-mouth perceptions of wine, and thus further studies would be required on the different interactions of fractions with a suppression or enhancing effects of sensory attributes.

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