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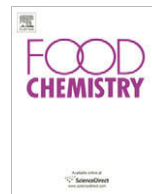
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## An assessment of the effects of wine volatiles on the perception of taste and astringency in wine

María-Pilar Sáenz-Navajas<sup>a,b</sup>, Eva Campo<sup>b</sup>, Purificación Fernández-Zurbano<sup>a</sup>, Dominique Valentin<sup>c</sup>, Vicente Ferreira<sup>b,\*</sup>

<sup>a</sup> Instituto de Ciencias de la Vid y el Vino (UR-CSIC-GR), Department of Chemistry, University of La Rioja, Madre de Dios 51, E-26006 Logroño, La Rioja, Spain

<sup>b</sup> Laboratory for Aroma Analysis and Enology, Department of Analytical Chemistry, Faculty of Sciences, University of Zaragoza, E-50009 Zaragoza, Spain

<sup>c</sup> UMR CSG 5170 CNRS INRA UB, CESC, 15 Rue Hugues Picardet, 21000 Dijon, France

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### ABSTRACT

The objective of this work is measuring the effect of different volatile extract compositions on the perception of taste, astringency, global intensity and persistence of wine. Six Spanish wines, two from Chardonnay and four from Tempranillo grapes, all of them showing different chemical and sensory characteristics, were selected. Wines were separated into volatile and non-volatile fractions by solid phase extraction and lyophilisation and further liquid extraction, respectively. Eighteen “reconstituted wines” were prepared, combining different volatile extracts and different non-volatile matrices and adjusting ethanol content to 12% (v/v), and were further described by a specifically trained sensory panel. Taste attributes (sweetness, acidity, bitterness), astringency, aroma intensity, global intensity and persistence were assessed in both, original and “reconstituted” wines by using a numerical category scale. The sensory properties of the original wines were retained by their corresponding “reconstituted samples”. The sensory assessment of the “reconstituted wines” showed that the addition of volatile fruity extracts from white wines brought about a decrease in astringency and bitterness and an increase in sweet perception in all cases. While global intensity and persistence of white wine matrices were also increased, they did not change in red wine matrices, which suggests that the volatile fraction plays only a secondary role in these attributes of red wines. Similarly, the effects of replacing the volatile fraction of a red wine by volatile extracts from other red wines were small and inconsistent, which confirms that taste and astringency are primarily driven by non-volatile molecules in these wines.

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### 1. Introduction

The overall flavour experience perceived during consumption of food is elicited by the simultaneous stimulation of several senses. It has been widely accepted that interactions can, and do, occur with stimuli (Noble, 1996) (aroma, taste, appearance, or mouth-feel). The presence of aroma–taste interactions has been largely studied and evidenced by the scientific literature. These interactions may result from physicochemical interactions (structure and binding effects) in the product itself, interactions at the receptor level or cognitive interactions (Small & Prescott, 2005). Since competition at the receptor site is highly unlikely because different receptors are involved among sensory modalities, perceptual interactions are more conceivable. It has been demonstrated that the orbitofrontal cortex is the structure most likely involved in these perceptual interactions. Stevenson, Boakes, and Prescott (1998) studied the associative learning between odour and taste in experiments

including conducting period. They were able to demonstrate the implicit nature of this learned synesthesia. In other words, the sweet taste was demonstrated to be processed along with the retro-nasal perception of the odour to produce a unitary sensation in the participant.

Many studies have shown that odours can suppress, enhance or have no effect on tastes (Caporale, Policastro, & Monteleone, 2004; Labbe, Damevin, Vaccher, Morgengegg, & Martin, 2006). These interactions have been demonstrated to occur in synthetic solutions (Welge-Lüssen, Drago, Wolfensberger, & Hummel, 2005) and in real samples, such as olive oil (Caporale et al., 2004), bitter cocoa and milk beverages (Labbe et al., 2006) or dairy desserts (Lethuaut et al., 2005). Moreover, interactions between aroma and other sensory modalities, such as touch, have recently been described. Kora, Latrille, Souchon, and Martin (2003) carried out a study on texture–flavour interactions in yogurts, revealing that olfactory perception enhanced product–perceived astringency. When the subjects perceived the flavour consisting of notes such as green apple, they may have associated this last perception with the astringency of unripe apple and given a higher score to the

\* Corresponding author.

E-mail address: [vferre@unizar.es](mailto:vferre@unizar.es) (V. Ferreira).

astringent intensity of the product in question. This observation was attributed to a cognitive association between the two perceptions (astringency and aroma).

Many studies have dealt with sensory taste–aroma interactions of molecules present in wines, e.g. sucrose interacting with vanillin (Welge-Lüssen et al., 2005) or the prune aroma (Prescott, Johnstone, & Francis, 2004), bitterness interactions with coconut aroma (rich in  $\gamma$ -lactones) (Labbe et al., 2006) or cut grass odorant (*cis*-3-hexen-1-ol) (Caporale et al., 2004). Furthermore, studies on the interactions between proteins, polysaccharides or polyphenols and selected aroma substances isolated from red wines have been carried out (Dufour & Bayonove, 1999a; Dufour & Bayonove, 1999b), revealing the existence of complexes driven mainly by hydrophobic forces.

Therefore, even if many studies on taste–aroma interactions have dealt with molecules present in wines, to our knowledge no one has focused on the aroma–taste and aroma–astringency interactions in real wine samples. In this context, the aim of this study is to obtain a preliminary measurement of the effect of the volatile composition of wine on some in-mouth sensory attributes, such as taste, astringency, intensity and persistence. In particular, the work will try to evaluate whether replacing the volatile composition of a given wine by other volatile extracts, e.g. taken from a different wine, has any measurable effect on those in-mouth sensory properties of the reconstituted wine and, in that case, to assess the type, magnitude and wine to wine consistency of such effects.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The chemical standards were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Sigma (St. Louis, Mo), Lancaster (Strasbourg, France), Polyscience (Niles, IL), Chem Science (West Chester, PA), International Express Service (Allauch, France) and Firmenich (Geneva, Switzerland), as indicated in Table 1. Dichloromethane, methanol, and ethanol, LiChrosolv quality, were from Merck (Darmstadt, Germany). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Polypropylene cartridges (6 ml), prepacked with LiChrolut EN resins, were also obtained from Merck (Darmstadt, Germany), whereas ammonium sulphate and  $\text{NaHCO}_3$  were supplied by Panreac (Barcelona, Spain).

### 2.2. Wines

A set of six commercial Spanish wines, with marked technological, sensory and aromatic compositional differences, was selected. The wines were a 1 year-old monovarietal Chardonnay wine fermented in stainless steel vats (W1), a 1 year-old monovarietal Chardonnay wine fermented in oak barrel (W2), a 1 year-old monovarietal Tempranillo red wine (W3), a high quality 4 year-old (18 months in oak barrel) 90% Tempranillo–10% Cabernet Sauvignon red wine (W4), a 3 year-old (18 months in oak barrel) monovarietal Tempranillo red wine with marked astringency (W5) and a 3 year-old (12 months in oak barrels) monovarietal Tempranillo red wine with marked woody aroma (W6). W1 was selected as the model for white wine, W2 as the model for a protein-rich white wine, W3 as the model for a neutral red, W4 as the model for a highly structured polyphenol-rich red wine, W5 as the model for a very astringent wine, and W6 was exclusively selected because of its typical woody aroma.

Conventional oenological parameters (ethanol concentration, pH, reducing sugars, titratable and volatile acidities) were determined in accordance with official OIV practices (O.I.V., 2005).

$\alpha$ -malic and lactic acids were determined by enzymatic methods in accordance with official AOAC analysis methods (AOAC, 2002, chap. 37). Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970).

### 2.3. “Reconstituted wine” preparation

#### 2.3.1. General

The volatile extracts of the six wines, named A1, A2, A3, A4, A5 and A6, respectively, and the non-volatile extracts of the five of them considered more relevant for the study selected as models of very different wine non-volatile matrices, named M1, M2, M3, M4 and M5, were separately obtained as detailed below (Sections 2.3.2 and 2.3.3).

#### 2.3.2. Volatile extracts preparation

SPE cartridges (in 6 ml reservoirs) filled with 2000 mg LiChrolut EN resins were put in the extraction unit (VAC ELUT 20 Station from Varian) and conditioned by passing (slowly) 20 ml of ethanol and 30 ml of a hydroalcoholic solution (12% ethanol (v/v), 5 g l<sup>-1</sup> of tartaric acid, pH adjusted to 3.0 with 0.1 M NaOH). After this, 600 ml of wine were loaded. The cartridge was then rinsed with 20 ml of the hydroalcoholic solution and volatile compounds were finally eluted with 20 ml of ethanol, using positive pressure to avoid air contact. The extract was spiked with BHA at 10 mg l<sup>-1</sup>, and was stored in vials with no headspace, sealed and stored at –25 °C prior to sample preparation.

#### 2.3.3. Non-volatile extracts preparation

Fifty millilitres of wine were lyophilised in 250 ml round flasks and, after this, samples were extracted with 3 × 10 ml of dichloromethane in order to eliminate remaining volatile compounds. Afterwards, dichloromethane was completely eliminated by forcing a stream of pure nitrogen (ca. 50 ml min<sup>-1</sup>) to pass through the sample for 20 min. The total absence of dichloromethane was assessed by headspace solid phase micro extraction (Carboxen/PDMS 75  $\mu\text{m}$  at 30 °C × 10 min) and GC with an electron capture detector (overall system detection limit 1 ng/sample). The extract was then dissolved in mineral water (Evian®, Evian-les Bains, France) and brought up to 10 ml (five times concentrated). After this, samples were placed in vials, with no headspace, in order to avoid sample-oxygen contact and stored at 5 °C prior to sample preparation.

#### 2.3.4. Sample reconstitution

“Reconstituted wines” were prepared by mixing 20 ml of ethanolic volatile extract (corresponds to the volatile extract of 600 ml of wine), 120 ml of non-volatile extract (corresponds to 600 ml of wine) and 52 ml of ethanol, and bringing the mixture to 600 ml with bottled mineral water (final ethanol content is 12% (v/v)). Eighteen samples were prepared by combining different volatile and non-volatile extracts from different wines, as shown in Table 2. Combinations (aroma × matrix) were selected, seeking for those exerting a most likely sensory impact on in-mouth attributes. Efforts were therefore concentrated on the red wine matrices, particularly in the most astringent (M5) in order to evaluate possible changes in astringency. Samples were stored at 5 °C in bottles with no headspace and hermetically closed in order to avoid contact with oxygen prior to sensory evaluation.

### 2.4. Wine characterisation

#### 2.4.1. General

The characterisation of the six wines used for the study was carried out by both sensory and chemical analyses.

**Table 1**

Volatile composition of the six studied wines (all data are expressed as microgrammes per litre). Bold numbers mean compounds above their odour threshold.

Compound	W1	W2	W3	W4	W5	W6	Odour description	Source	Odour threshold <sup>a</sup>
<i>Acids</i>									
3-Methylbutyric acid	<b>61.2</b>	<b>107</b>	<b>162</b>	<b>84.2</b>	<b>102</b>	<b>107</b>	Cheese	Aldrich	33 (Ferreira, Lopez, & Cacho, 2000)
2-Methylbutyric acid	<b>98.3</b>	<b>127</b>	<b>168</b>	<b>72.3</b>	<b>124</b>	<b>142</b>	Cheese	Aldrich	33 (Ferreira et al., 2000)
Isobutyric acid	670	700	1930	1760	2030	1880	Cheese	Aldrich	2300 (Ferreira et al., 2000)
Butyric acid	<b>1000</b>	<b>940</b>	<b>630</b>	<b>890</b>	<b>890</b>	<b>650</b>	Cheese	Polyscience	173 (Ferreira et al., 2000)
Hexanoic acid	<b>3320</b>	<b>3290</b>	<b>960</b>	<b>1330</b>	<b>1040</b>	<b>1310</b>	Cheese	Polyscience	420 (Ferreira et al., 2000)
Octanoic acid	<b>2240</b>	<b>2000</b>	<b>550</b>	<b>590</b>	<b>480</b>	<b>500</b>	Fatty/unpleasant	Fluka	500 (Ferreira et al., 2000)
Decanoic acid	720	720	190	190	160	180	Cheese	Polyscience	1000 (Ferreira et al., 2000)
<i>Alcohols</i>									
Furfuryl alcohol	47.8	197	nd <sup>b</sup>	64.7	13.7	29.0	Hay/mold	Fluka	2000 (Van Gemert & Nettenbreijer, 1977)
Methionol	177	188	549	177	212	558	Potato/coliflower	Aldrich	1000 (Ferreira et al., 2000)
Isobutanol	11,800	13,500	36,600	30,200	25,000	36,400	Fusel	Merck	40,000 (Ferreira et al., 2000)
1-Butanol	410	400	690	660	650	670	Fusel	Aldrich	150,000 (Etiévant, 1991)
Isoamyl alcohol	<b>85,800</b>	<b>115,000</b>	<b>199,000</b>	<b>131,000</b>	<b>119,000</b>	<b>165,000</b>	Fusel	Aldrich	30,000 (Guth, 1997)
1-Hexanol	780	590	1070	1110	970	1030	Grass	Sigma	8000 (Guth, 1997)
c-3-Hexenol	40	50	80	160	200	100	Grass	Aldrich	400 (Guth, 1997)
Benzyl alcohol	40	60	290	220	190	1450	Sweet, floral	Aldrich	200,000 (Aznar, Lopez, Cacho, & Ferreira, 2003)
β-Phenylethyl alcohol	9720	12,110	<b>25,300</b>	<b>17,200</b>	<b>13,300</b>	<b>31,600</b>	Roses	Fluka	14,000 (Ferreira et al., 2000)
<i>Aldehydes</i>									
Phenylacetaldehyde	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.50	Floral/honey	Aldrich	1 (Aznar et al., 2003)
Furfural	12.3	353	16.5	13.0	20.2	14.8	Almond	Fluka	14,100 (Ferreira et al., 2000)
5-Hydroxy-methylfurfural	nd <sup>b</sup>	24.1	3.35	3.23	3.23	0.00	Almond	Aldrich	100,000 (Van Gemert & Nettenbreijer, 1977)
Acetaldehyde	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	Green apple	Aldrich	500 (Guth et al., 1997)
<i>Esters</i>									
Ethyl isobutyrate	10.2	<b>26.8</b>	<b>53.8</b>	<b>36.1</b>	<b>77.1</b>	<b>60.0</b>	Fruity/strawberry	Fluka	15 (Guth, 1997)
Isobutyl acetate	56.1	37.9	58.8	22.0	20.3	22.9	Solvent	ChemService	1600 (Aznar et al., 2003)
Ethyl 2-methylbutyrate	<b>3.41</b>	<b>3.53</b>	<b>9.72</b>	<b>4.16</b>	<b>6.31</b>	<b>9.64</b>	Fruity/green apple	Fluka	1 (Guth, 1997)
Ethyl isovalerate	<b>8.95</b>	<b>7.28</b>	<b>18.4</b>	<b>12.4</b>	<b>15.7</b>	<b>16.7</b>	Fruity/anise	Fluka	3 (Ferreira et al., 2000)
Ethyl decanoate	113	189	59.7	59.2	60.8	57.7	Fruity	Polyscience	200 (Ferreira et al., 2000)
Phenylethyl acetate	141	104	92.1	20.2	16.7	23.5	Roses	ChemService	250 (Guth, 1997)
Ethyl cinnamate	0.96	<b>2.03</b>	0.40	0.90	0.68	0.69	Cinnamate/sweet	Aldrich	1.1 (Ferreira et al., 2000)
Ethyl butyrate	<b>230</b>	<b>200</b>	<b>140</b>	<b>150</b>	<b>150</b>	<b>110</b>	Fruity	Aldrich	20 (Guth, 1997)
Isoamyl acetate	<b>1910</b>	<b>1200</b>	<b>860</b>	<b>150</b>	<b>130</b>	<b>150</b>	Banana	ChemService	30 (Guth, 1997)
Ethyl hexanoate	<b>340</b>	<b>360</b>	<b>130</b>	<b>180</b>	<b>140</b>	<b>120</b>	Fruity/anise	Polyscience	5 (Guth, 1997)
Ethyl lactate	6570	6500	108,000	139,000	125,000	103,000	Fruity	Aldrich	154,000 (Etiévant, 1991)
Ethyl octanoate	<b>280</b>	<b>340</b>	<b>70</b>	<b>100</b>	<b>80</b>	<b>80</b>	Fruity/fresh	Polyscience	2 (Guth, 1997)
Ethyl 3-hydroxybutyrate	120	50	190	190	210	210	Fruity	Aldrich	20,000 (Aznar et al., 2003)
Diethyl succinate	590	1090	6140	8420	12,100	6930	Fruity	Fluka	200,000 (Etiévant, 1991)
<i>Volatile phenols</i>									
Guaiaicol	0.27	2.67	2.47	7.51	7.12	4.28	Phenolic/chemical	Aldrich	9.5 (Ferreira et al., 2000)
o-Cresol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.90	0.93	0.87	Leather/spicy	Aldrich	31 (Lopez et al., 2002)
4-Ethylguaiaicol	nd <sup>b</sup>	2.82	nd <sup>b</sup>	3.45	2.88	16.9	Leather/phenolic	Aldrich	33 (Ferreira et al., 2000)
4-Propylguaiaicol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.29	0.29	0.66	Leather animal	Lancaster	10 (Aznar et al., 2003)
Eugenol	1.06	<b>11.3</b>	nd <sup>b</sup>	<b>21.5</b>	<b>32.9</b>	<b>16.8</b>	Clove	Aldrich	5 (Guth, 1997)
4-Ethylphenol	nd <sup>b</sup>	nd <sup>b</sup>	7.2	2.7	21.2	189	Bitumen/leather	Aldrich	440 (Lopez et al., 2002)
Isoeugenol	0.43	2.83	0.28	2.63	2.45	0.84	Spicy	Lancaster	6 (Aznar et al., 2003)
4-Vinylphenol	72.1	76.4	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	Almond shell	Lancaster	180 (Boidron, Chatonnet, & Pons, 1988)
4-Allyl-2,6-dimethoxyphenol	nd <sup>b</sup>	7.04	2.20	10.7	11.2	6.62	Spicy smoky	Aldrich	1200 (Van Gemert & Nettenbreijer, 1977)
<i>Lactones</i>									
t-Whiskylactone	1.61	<b>110</b>	nd <sup>b</sup>	<b>161</b>	<b>81.0</b>	29.6	Coconut/woody	Aldrich	67 (Etiévant, 1991)
c-Whiskylactone	30.6	204	4.52	267	419	270	Coconut/woody	Aldrich	790 (Ferreira et al., 2000)
δ-Octalactone	5.32	5.82	5.00	4.95	6.44	4.19	Peach	Lancaster	400 (Van Gemert & Nettenbreijer, 1977)
γ-Nonalactone	5.90	5.05	10.2	6.98	7.08	11.5	Peach	Aldrich	30 (Nakamura, Crowel, Ough, & Totsuka, 1988)
γ-Decalactone	0.54	nd <sup>b</sup>	1.09	1.00	0.79	1.18	Spicy/woody/phenolic	Fluka	386 (Ferreira et al., 2000)
<i>Norisoprenoids</i>									
β-Damascenone	<b>2.44</b>	<b>1.51</b>	<b>2.23</b>	<b>1.17</b>	<b>0.72</b>	<b>1.11</b>	Backed apple	Firmenich	0.05 (Guth, 1997)
α-Ionone	0.24	0.18	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	Tabac	Sigma	2.6 (Etiévant, 1991)
β-Ionone	<b>0.12</b>	<b>0.12</b>	<b>0.30</b>	<b>0.30</b>	<b>0.23</b>	<b>0.23</b>	Violet	Sigma	0.09 (Ferreira et al., 2000)
<i>Terpenols</i>									
Linalool	<b>16.6</b>	<b>16.1</b>	4.8	5.4	4.6	5.0	Floral/muscat	Aldrich	15 (Guth, 1997)
α-Terpineol	10.8	11.7	5.97	5.27	5.74	5.29	Fresh/rosemary	Fluka	250 (Ferreira et al., 2000)
β-Citronellol	3.20	3.09	2.39	1.94	2.87	2.57	Green/lemon	Aldrich	100 (Etiévant, 1991)

(continued on next page)

Table 1 (continued)

Compound	W1	W2	W3	W4	W5	W6	Odour description	Source	Odour threshold <sup>a</sup>
Geraniol	7.60	6.32	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	Floral	Fluka	30 (Guth, 1997)
<i>Vanillins</i>									
Vanillin	nd <sup>b</sup>	36.8	nd <sup>b</sup>	32.1	33.6	25.3	Vanilla	Aldrich	200 (Guth, 1997)
Methyl vanillate	12.9	12.0	2.82	3.50	2.82	3.27	Vanilla	Lancaster	990 (Lopez et al., 2002)
Ethyl vanillate	7.15	8.11	33.0	53.8	39.5	77.9	Vanilla/honey	Lancaster	3000 (Lopez et al., 2002)
Acetovanillone	21.2	23.1	41.5	39.3	40.7	51.6	Vanilla	Aldrich	1000 (Aznar et al., 2003)
Syringaldehyde	2.42	40.9	2.42	23.9	21.8	5.42	Vanilla	Aldrich	50,000 (Van Gemert and Nettenbreijer, 1977)
<i>Miscellaneous</i>									
Benzoic acid	41.4	124	6.91	12.6	17.2	24.3	Sweat	Aldrich	1000 (Aznar et al., 2003)
Phenylacetic acid	7.36	8.69	24.6	14.9	19.5	38.6	Roses	Aldrich	1000 (Maga, 1973)
Ethyl furoate	5.43	6.22	2.93	2.64	3.67	3.37	Fruity	Fluka	16,000 (Ferreira et al., 2000)
Diacetyl	<b>450</b>	<b>2380</b>	<b>1490</b>	<b>680</b>	<b>7800</b>	<b>920</b>	Butter	Aldrich	100 (Guth, 1997)
Acetoin	830	830	4010	18,000	13,000	16,200	Fatty/wet	Aldrich	150,000 (Etiévant, 1991)
γ-Butyrolactone	2220	2570	1550	6660	7430	8590	Cheese	Aldrich	35,000 (Aznar et al., 2003)

<sup>a</sup> Reference from which the value has been taken is given in parenthesis.

<sup>b</sup> nd: not detected.

Table 2

Eighteen “reconstituted wines”, non-volatile (MX) and volatile (AY) extracts. Samples are referred to in the text as MXAY (formed by addition of the non-volatile extracts of wine X and the volatile extract of wine Y).

	M1	M2	M3	M4	M5
A1	x	x	x	x	x
A2					x
A3	x	x	x	x	x
A4			x	x	x
A5			x	x	x
A6					x

## 2.4.2. Sensory analysis

**2.4.2.1. Panel training.** In total, 36 students or staff members from the University of Burgundy (France) were recruited on the basis of their interest and their availability during 12 weeks (one 60 min session per week). They were not paid for their participation. Among these 36 panellists, 30 were selected to carry out the measuring sessions (12 males and 18 females from 20 to 69 years old).

The selection of panellists was carried out by calculating the reproducibility index ( $R_i$ ) proposed by Campo, Do, Ferreira, and Valentin (2008) for aromatic attributes, where the minimum  $R_i$  required to keep a judge response was established at 0.20. According to this, 30 panellists were selected and, with them, a three-way ANOVA for the in-mouth attributes, involving samples (S), judge (J) and replicate (R) as fixed factors and all first order interactions, was calculated to confirm the panel performance.

Panellists attended eight descriptive sensory training sessions over a period of 2 months, during which panellists worked in sub-groups. They were provided with a list of 110 terms obtained from the literature (Campo et al., 2008). During training, different reference standards, representative of aroma, taste and astringency terms, were presented. Standards were either commercially available odorants taken from International Flavour and Fragrances (Dijon, France), Sentosphère (Paris, France), “Le Nez du Vin” (Jean Lenoir, Provence, France) and Firmenich (Geneva, Switzerland), or natural products (fruits, juices, spices, vegetables) prepared at the beginning of each session. For taste and astringency, solutions, containing different concentrations of table sugar (0–12 g l<sup>-1</sup>) for sweetness, tartaric acid (0–1.5 g l<sup>-1</sup>) for acidity, quinine sulphate (0–10 mg l<sup>-1</sup>) for bitterness and potassium, and aluminium sulphate (0–5 g l<sup>-1</sup>) for astringency stimuli, were presented to the panel to aid with recognition, and discrimination between the different oral sensations.

The training period included two phases: a general and a product-specific training phase. During the general training phase (four sessions), panellists became familiar with aroma attributes and with intensity rating of sweetness, acidity, bitterness, astringency, aromatic and global intensity, as well as persistence. During a typical session, panellists had to evaluate 2–4 different wines, describing their odour properties by choosing up to five descriptors in the aroma list and by rating sweetness, acidity, bitterness, and astringency on a 10-point scale (0 = “absence”, 1 = “very low” and 9 = “very high”), while in-mouth aroma intensity, in-mouth global intensity and global persistence were measured on a 9-point scale (1 = “very low” and 9 = “very high”) since, for these last concepts, the 0 has no meaning. The wines selected for this training phase presented intense and easily recognisable taste and astringency properties and included red, white and rosé wines of diverse grape varieties and origins. The session ended with a discussion, during which the panel leader compared the aroma descriptors and the taste intensity scores given by panellists to describe each wine.

The specific training phase consisted of four sessions, during which panellists became familiar with the type of samples of the study. During this phase, panellists described odour properties and rated the intensities of sweetness, acidity, bitterness, astringency, aromatic and global intensity, as well as global persistence of ten Spanish commercially available wines and two repetitions (one repetition for session) of five “reconstituted wines” different from those used for the study (formed by the non-volatile extract of W6 and the volatile fractions of W1, W2, W3, W4, and W5, respectively).

**2.4.2.2. Wine evaluation.** Trained panellists described wines in duplicate. Ten millilitres wine samples were presented in dark ISO (1977)-approved wineglasses labelled with three-digit random codes and covered by plastic Petri dishes according to a random arrangement. Panellists were asked to smell each wine and to describe their odour by choosing a maximum of five attributes from the list of 110 according to the citation frequency method (Campo, Ballester, Langlois, Dacremont, & Valentin, 2010; Campo et al., 2008). Then, they were asked to rate the sweetness, acidity, bitterness, astringency, aromatic and global intensities, as well as the global persistence of the samples using the above mentioned structured scales for each wine. Panellists paused for 7 min intervals between sample evaluations to limit adaptation effects. During that time they were asked to rinse their mouths with water, to have some plain crackers and finally to rinse their mouths again with water.

All wines were served at room temperature and were evaluated in individual booths. Samples were stored at 5 °C. Panellists were not informed about the nature of the samples being evaluated.

#### 2.4.3. Chemical quantitative analysis

**2.4.3.1. Major compounds (liquid–liquid microextraction and GC-FID analysis).** Quantitative analysis of major compounds was carried out, using the method proposed and validated by Ortega, Lopez, Cacho, and Ferreira (2001). In accordance with this method, 3 ml of wine and 7 ml of water were salted with 4.5 g of ammonium sulphate and extracted with 0.2 ml of dichloromethane. The extract was then analyzed by GC with FID detection using the conditions described elsewhere (Ortega et al., 2001). Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs constructed by the analysis of synthetic wines containing known amounts of the analytes. 2-Butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone and 2-octanol were used as internal standards.

**2.4.3.2. Minor compounds (SPE and GC–ion trap–MS analysis).** This analysis was carried out using the method proposed and validated by Lopez, Aznar, Cacho, and Ferreira (2002). In accordance with the method, 50 ml of wine, containing 25 µl of butyl-hydroxy-anisole (BHA) solution and 75 µl of a surrogate standards solution (3-octanone, β-damascone, heptanoic acid, and isopropyl propanoate), were passed through a LiChrolut EN cartridge at about 2 ml/min. The sorbent was dried by letting air pass through (–0.6 bar, 10 min). Analytes were recovered by elution with 1.3 ml of dichloromethane. An internal standard solution was added to the eluted sample. The extract was then analyzed by GC with ion trap MS detection under the conditions described by Lopez et al. (2002).

#### 2.5. Sensory characterisation of “reconstituted wines”

The trained panel described the 18 “reconstituted wines” by rating sweetness, acidity, bitterness, astringency, aromatic and global intensities, as well as global persistence. The panel training and sample evaluation were carried out in the same way as for wines (see Section 2.4.2.2).

#### 2.6. Data analysis

##### 2.6.1. Evaluation of wine sensory data

On the data derived from the in-mouth sensory analysis of wines, a one-way analysis of variance (ANOVA) (in which wine was the factor and judges (mean of both replicates) were considered as repetitions) was performed. On the data derived from the sensory evaluation of aroma, carried out by the frequency of citation method, a chi-square ( $\chi^2$ ) analysis on the average citation frequency (two repetitions) of each term was calculated.

##### 2.6.2. Evaluation of the “reconstituted wines” sensory data

**2.6.2.1. One-way ANOVAs.** Five one-way ANOVAs with repeated measurements were performed on each of the seven attributes averaged across replicates. The first ANOVA was performed on the “reconstituted wines” formed by the non-volatile extract of W1 (M1A1 and M1A3), and the second ANOVA was computed on the samples formed by the matrix of W2 (M2A1 and M2A3). The third and fourth ANOVAs were calculated for two red non-volatile extracts (M3 and M4, respectively) to which the volatile extract of one white wine (A1) and three red wines (A3, A4, and A5) were added. Finally the fifth ANOVA was performed on the six samples formed by the red non-volatile extract of W5 and the volatile extracts of the six studied wines (A1–A6).

Four one-way ANOVAs, with repeated measurements, were calculated on each of the seven attributes averaged across replicates

for the four pairs of wine/reconstituted samples. The first ANOVA was calculated on the reconstituted wine M1A1 and the commercial wine W1, the second on M3A3 and W3, whereas the third and fourth ANOVAs were performed on M4A4 and W4, and M5A5 and W5, respectively. Student–Newmans–Keuls *post hoc* pairwise comparisons (95%) were carried out for significance effects.

**2.6.2.2. Two-way ANOVAs.** A two-way ANOVA, with repeated measurements, was performed on six attributes (sweet, acid, bitter, astringent, global intensity and persistence) averaged across replicates. The ANOVA model was computed on the data derived from the sensory analysis of the “reconstituted wines” formed by the volatile extract of the white wine W1 (M1A1, M2A1, M3A1, M4A1 and M5A1) and of the red wine W3 (M1A3, M2A3, M3A3, M4A3 and M5A3). Matrix colour (white vs red) and volatile extract (A1 vs A3) were considered as fixed factors and judges as random factors according to the following model: matrix colour + volatile extract + matrix colour × volatile extract. Besides, a two-way ANOVA, with repeated measurements, was calculated with volatile extract (red vs white) and white matrices (M1 vs M2) as fixed factors and judges as random factors in order to evaluate the effect of the volatile extract (from red or white wine) on the perceived attributes.

**2.6.2.3. Principal component analysis (PCA).** A normalised PCA was performed on the mean ratings over the panellists for the attributes for each “reconstituted wine”. PC dimensions with an eigenvalue higher than 1 (Kaiser criteria) were retained. All analyses were carried out with SPAD (version 5.5, CISIA-CESRESTA, Montreuil, France) and SAS (version 9.1, SAS Institute Inc. Cary, NY, USA) softwares.

### 3. Results and discussion

#### 3.1. Wine characterisation

Six quite different wines, in terms of both aroma and taste properties, were selected for the study. The aroma properties of those six original wines, measured by a recently described frequency of citation method (Campo et al., 2010), are given in Fig. 1. The most discriminant terms according to the  $\chi^2$  criterion are presented in Supplementary Material. These results reveal that the seven aroma families, together with the terms “alcohol” and “vegetable”, varied significantly among the six wines. As shown in Fig. 1, the white wine W1 was the fruitiest (FC = 62), followed by the second white, W2 (FC = 52), while all reds showed similar and rather low frequencies of citation for this term (from 37 to 42). On the other hand, reds scored higher than whites on the burnt-woody term (FC between 11 and 24 vs less than 7). Leaving aside W3, which represents a quite neutral and aromatically poor red wine, the other red samples had rather specific descriptors, such as vegetal and vegetables (W4), burnt (W5), black and dry fruits (W5 and W6) or woody (W6).

Some of these aroma properties can be explained in terms of the measured volatile composition, given in Table 1. As can be seen, the two white wines (W1 and W2) have the highest concentrations of butyric, hexanoic and octanoic acids, as well as of their corresponding ethyl esters, and also of isoamyl acetate. These highest levels in fruity esters, together also with relatively high levels in β-damasconone can explain the highest scores in the fruity attribute in comparison with reds. The white wines also have highest levels of terpenols and of ethyl cinnamate, which contribute to floral and sweet notes. By contrast, red wines presented highest levels of isobutyric acid, isoamyl alcohol, β-phenylethyl alcohol, ethyl isovalerate, acetoin and diacetyl, in accordance with previous

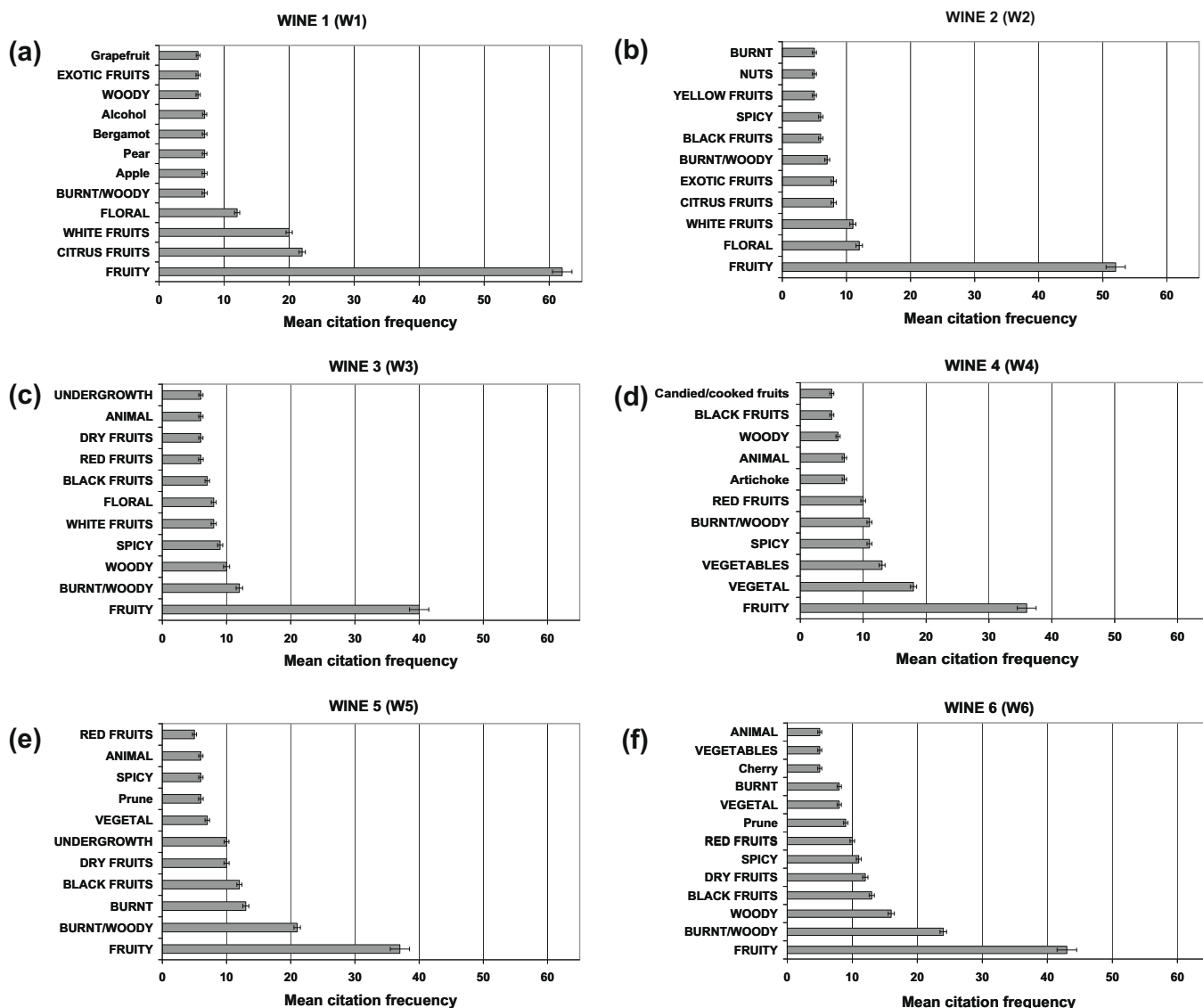


Fig. 1. Mean citation frequency of the 12 most cited odour attributes in the studied wines. Error bars are calculated as  $s/(n)^{1/2}$ ;  $s$ , standard deviation;  $n$ , number of panellists.

reports (Ferreira, Fernandez, & Cacho, 1996). Leaving aside W3, whose composition lies half-way between whites and reds, red wines are also richer in some volatile phenols, such as guaiacol, 4-ethylguaiacol, 4-ethylphenol, 4-propylguaiacol, and eugenol, which is consistent with their highest scores for the burnt-woody term.

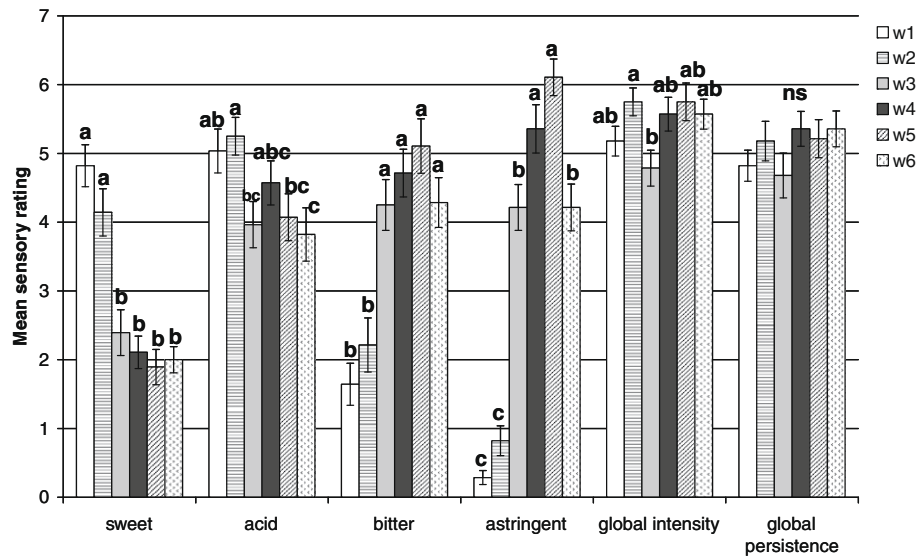
Leaving aside aromatic intensity, for which the panel was not able to provide a consistent assessment, the scores obtained for the measured in-mouth properties (taste, astringency, global intensity and persistence) are shown in Fig. 2. As can be seen, the two white wines, W1 and W2, are sweet, slightly acid and not astringent nor bitter, while the reds scored very low in sweetness and relatively high in astringency and bitterness. Wines W5 and W4 are the most astringent and bitter, although, in this case, differences were not significant. As for global intensity and persistence, the most remarkable fact is that W3 is the least intense, and that no significant difference was found for the attribute persistence among the studied wines.

Finally, the conventional oenological parameters of the studied wines are given in Table 3. The pH values were, as expected, higher in reds than in whites, and range from 3.36 to 3.74, while the highest value for the titratable acidity was observed for W5 ( $4.13 \text{ g l}^{-1}$ ).

It should be noted that pH is not correlated ( $r^2 = 0.4376$ ;  $P = 0.520$ ) with the acidity perceived on the studied wines, which is in accordance with the results published by Etaio et al. (2008), although it should be noted that white wines have the highest levels of malic acid whose taste threshold has been estimated as  $1.9 \text{ mg l}^{-1}$  (Hufnagel & Hofmann, 2008). Reducing sugar contents are low, as expected for dry table wines, and the maximum value for this parameter is found in W3. All the wines were relatively rich in ethanol, which ranged from 13.7% to 14.8%. Red wines had highest levels of TPI (total polyphenol index), and the maximum value of this parameter is observed for W4 (68.2).

### 3.2. "Reconstituted wines"

The major goal of the sample preparation procedure was to achieve completely odourless tastant fractions and tasteless odourant fractions. Those objectives were best achieved by using a combination of lyophilisation and liquid-liquid extraction for sample dearomatization, and direct solid phase extraction for the extraction of the volatile fraction, avoiding different strategies of distillation, which in most cases induced the formation of artifacts or involved a quite complex setup during which it is difficult to pre-



**Fig. 2.** Comparison of the mean sensory ratings of the studied wines. Error bars are calculated as  $s/(n)^{1/2}$ ;  $s$ , standard deviation;  $n$ , number of panellists. Different letters indicate the existence of a significant difference between samples ( $\alpha < 0.05$ ) (Student–Newman–Keuls test); ns, no significant differences; same letters indicate a tendency in perception ( $\alpha < 0.1$ ).

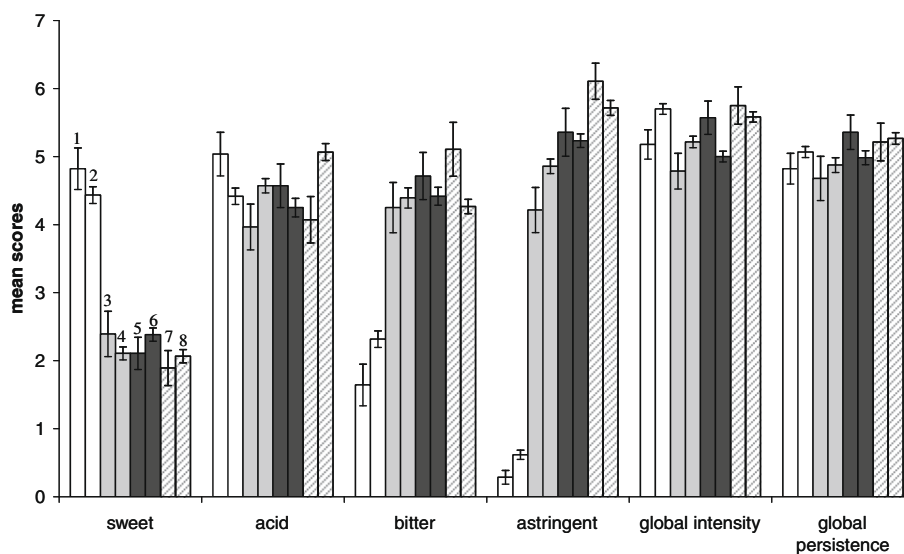
**Table 3**

Conventional oenological parameters of the studied wines.

	pH	Volatile acidity <sup>a</sup>	Titrateable acidity <sup>a</sup>	Reducing sugar <sup>b</sup>	Malic acid <sup>b</sup>	Lactic acid <sup>b</sup>	Ethanol (v/v)	TPI
W1	3.47	0.36	3.71	3.7	2.87	0.05	13.7	10.1
W2	3.36	0.29	3.78	2.7	1.91	0.54	14.6	13.6
W3	3.66	0.32	3.62	5.8	0.29	1.89	13.1	59.5
W4	3.74	0.46	3.53	2.2	0.10	2.08	14.7	68.2
W5	3.59	0.51	4.13	3.0	0.00	1.87	14.8	60.1
W6	3.57	0.38	3.51	2.1	0.02	1.56	13.9	64.0

<sup>a</sup> Expressed as g tartaric acid per litre.

<sup>b</sup> Expressed as g per litre.

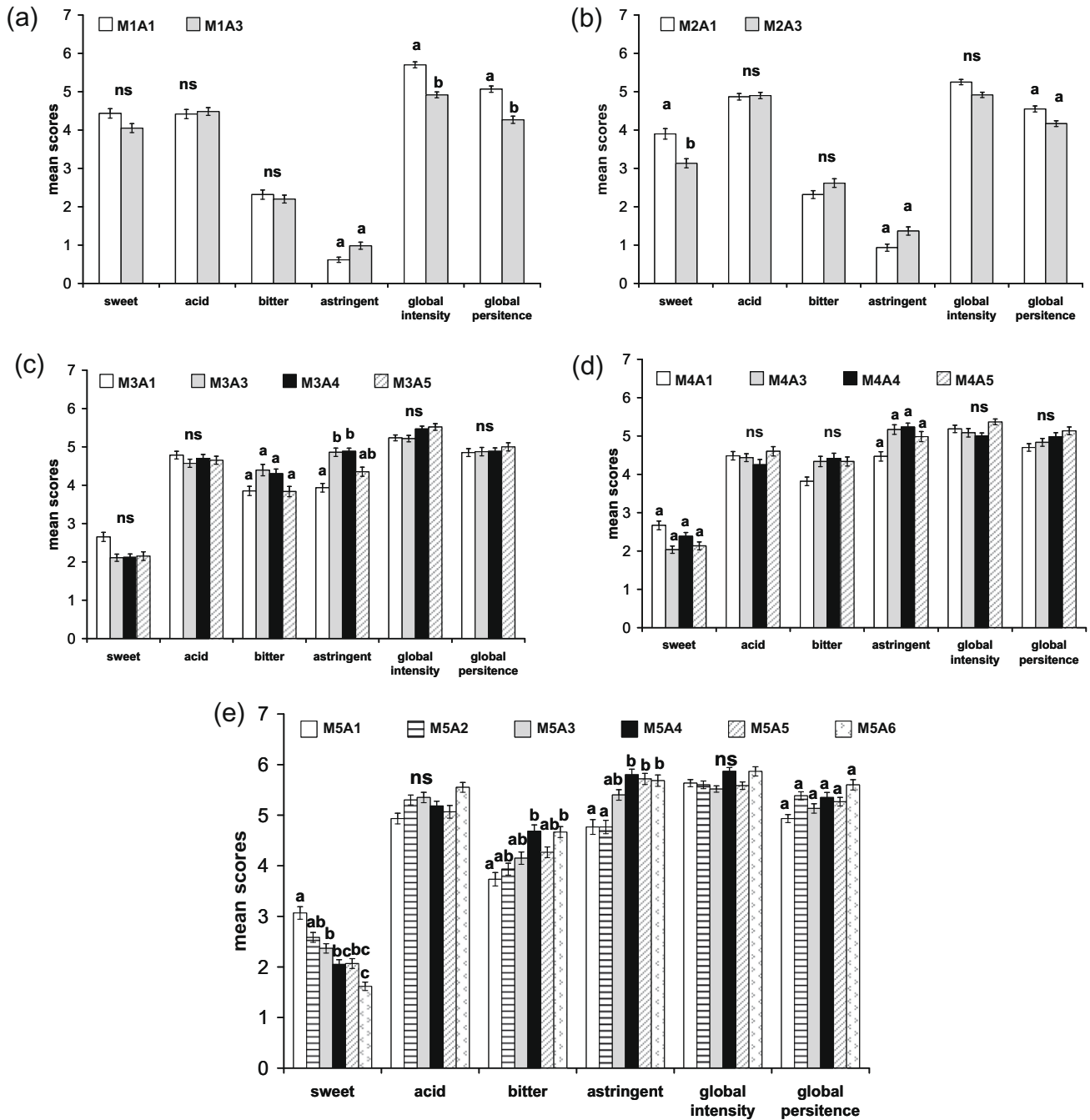


**Fig. 3.** Comparison of the mean sensory ratings of the four pairs of wine sample/reconstituted sample. Error bars are calculated as  $s/(n)^{1/2}$ ;  $s$ , standard deviation;  $n$ , number of panellists. Samples: W1 (1), M1A1 (2), W3 (3), M3A3 (4), W4 (5), M4A4 (6), W5 (7) and M5A5 (8).

serve sample integrity. The process worked reasonably well, since the odour of the tastant fraction was residual, as was the taste of the odorant fractions and, more importantly, “reconstituted wines” did not differ from normal commercial wine samples from the sen-

serial point of view and most of the sensory properties of the original wines were also present in the corresponding “reconstituted samples”. These observations are corroborated by the sensory scores given in Fig. 3 for four pairs of wine sample/reconstituted





**Fig. 4.** Graph of the mean sensory ratings of reconstituted wines formed by the non-volatile extract (a) M1, (b) M2, (c) M3, (d) M4 and (e) M5. Different letters indicate the existence of a significant difference between samples ( $\alpha \leq 0.05$ ) (Student–Newmans–Keuls test); ns, no significant differences; same letters indicate a tendency in perception ( $\alpha \leq 0.1$ ).

sample. As can be seen, reconstituted samples retain most of the sensory properties of the original wines and, in fact, only in a couple of comparisons (out of the 24 possible comparisons) were differences significant. Such differences were observed for the attribute “acid” for the comparison W5 vs M5A5, the reconstituted sample (M5A5) being evaluated as the most acid, and for the attribute global intensity for the pair M4A4–W4, in this case the reconstituted sample (M4W4) being evaluated as less intense.

### 3.3. In-mouth sensory properties of the “reconstituted samples”

The results of the sensory analysis of the “reconstituted wines” are summarised in Fig. 4, which shows the mean values and the

standard error of the attributes (except for aromatic intensity) assessed for the 18 “reconstituted wines”. The summaries of the different ANOVA statistics carried out on the data set are given in the [Supplementary Material](#), while Fig. 5 gives the projection of samples and variables on the PCA first two dimensions. The PCA plot, accounting for more than 89% of the original variance, can be used to gain a first idea of the hierarchy and intensity of the effects caused by different volatile extracts added to different non-volatile matrices.

As the PCA plot shows, samples are distributed primarily, as expected, according to the nature of the non-volatile matrix: the four samples with non-volatile matrices from white wines are on the left part of the plane, while the 14 samples made from red wine

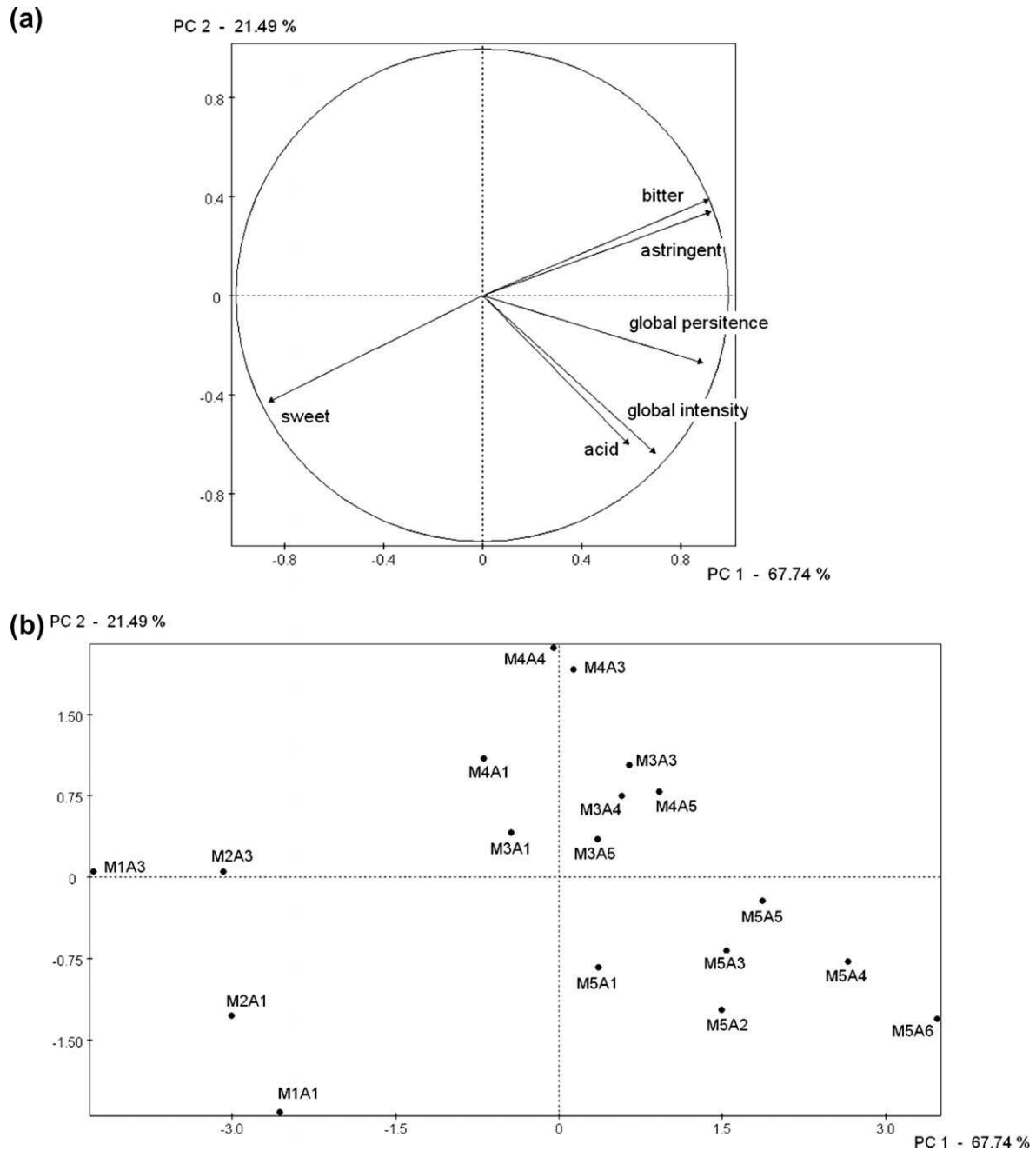


Fig. 5. PCA on the principal components 1 and 2. (a) Loadings of the *in-mouth* attributes, (b) projection of the 18 samples.

non-volatile matrices are on the right part. Next, the PCA shows that, while red wine matrix samples are grouped mainly according to the nature of the matrix (M4 samples centre up, M3 elements centre, and M5 elements down and right), white wine matrix samples are grouped according to the nature of the volatile extract (A3 with 0+ value in PC2, A1 with negative values of PC2); i.e. volatile composition exerts a major influence on the taste properties of white wines while, in reds, the influence seems to be much lower. Nevertheless, it can be observed that the three red wine matrix samples containing the volatile extract A1 from a fruity white wine are in a centred area, clearly displaced to the left of the other equivalent samples from the same matrix, which suggests that samples containing A1 are sweeter and less bitter and astringent than are the other samples from the same matrix.

These observations are corroborated by Fig. 4. In the case of the white wine matrix M1, Fig. 4a shows that global intensity and persistence were significantly perceived as different ( $P = 0.0089$  and  $P = 0.0021$ , respectively), which means that replacing A1 by A3 (from a neutral red wine), reduces the intensity of both attributes. In the case of M2, replacing A1 by A3 brings about a significant decrease of sweetness. It is important to note that there is a trend ( $P = 0.0898$  and  $P = 0.0749$ ) in the perception of astringency, which increases when A1 is replaced by A3 in both white wine matrices. Such increase could be related to the decrease in sweetness (significant only in the case of M2). In general, a repeated measures ANOVA, carried out on M1 and M2 (data not shown) confirms that in a white wine matrix, replacing A1 by A3 brings about significant decreases of the perceptions of sweetness ( $P = 0.002$ ), global intensity

and persistence ( $P = 0.007$ ) and an increase on the perception of astringency ( $P = 0.050$ ).

The second relevant observation from the PCA was the displacement of red wine matrix samples containing A1 to the left of the plane. As Fig. 4 confirms, such displacement can be explained by the increased sweetness of these samples (significant only in M5), and by decreased levels of bitterness (significant only in M5) and of astringency (significant in M3 and M5 and close to it in M4). The repeated measures ANOVA, carried out on these samples, confirms that the addition of A1 to any red wine matrix causes a significant increase of the perception of sweetness and significant decreases of bitterness and astringency. It is remarkable that, in red wine matrices, there is no significant effect linked to the presence of A1 on the intensity and persistence of the samples, as was previously observed with white wine matrices. This suggests that persistence and intensity of in-mouth perception in white wines is highly dependent on aromatic composition while, in reds, this does not happen; however, sweetness, bitterness and astringency are in all cases related to the wine volatile extract composition.

The effect of red wine volatile extracts on the taste and astringency attributes of red wine matrices is less clear, as both, PCA, Fig. 4 and different statistical treatments (not shown) indicate. There are some changes, but in most cases they are not significant, nor are they consistent in the different matrices. The single difference is due to the sweeter character of the sample composed of the most astringent matrix (M5) and the neutral wine (A3). Remarkably, that wine was the richest (among reds) in the fruity aroma components, isoamyl acetate, ethyl isovalerate and  $\beta$ -damascenone, as can be seen in Table 1.

It should be noted that we had expected, perhaps naively, that the volatile extract of complex and well-structured wines, such as W4, would have had some effect on the intensity and persistence of a neutral and rather poor wine, such as W3. Fig. 4 suggests, however, that, if there is an effect of the volatile extract on intensity and persistence, it is certainly very small; i.e. intensity and persistence in red wines are primarily related to the non-volatile composition. Similarly, it can be seen that the volatile extract of a very astringent and bitter wine, such as W5, does not elicit more bitterness when added to a different wine, and that the volatile fractions extracted from well-structured wines, such as W4 or W5, do not cause bitterness and astringency to decrease, which implies that, leaving aside the strong effect exerted by the volatile extracts of white wines, astringency and bitterness of a red wine are primarily and mainly related to its non-volatile matrix composition.

#### 4. Conclusions

This work has shown that sweetness of dry wine is closely related to fruity aroma, and that, as sweetness most likely affects the perceptions of astringency and bitterness, these two last percepts are also inversely related to fruity aroma. This suggests that not only are polyphenols responsible for astringency and bitterness perceptions in wines, but that such attributes are indirectly related to the astringency-sweetness interaction. It can also be concluded that global intensity and persistence seem to be closely related to the volatile composition in white wines but not in red wines. Nevertheless, the fact that replacing the volatile extract of a neutral or an astringent red wine by another, from a well structured wine, has small effects on astringency, bitterness, intensity and persistence, suggests that these percepts are primarily caused by non-volatile molecules.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2010.01.061.

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