



**ORIGINAL RESEARCH ARTICLE**

# The relevant and complex role of ethanol in the sensory properties of model wines

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## ABSTRACT

In a context of increasing interest in the production of wine-based beverages with lower ethanol content, the present work explored the role of ethanol in the sensory properties of model wines (MW). Four sets of MWs (red and white) with alcohol contents ranging from 0.5 to 15 % (1st study) and from 12 to 15 % (2nd study), the non-volatile fraction of real wines and fixed aroma compositions were all prepared and characterised by sensory descriptive analysis. The results indicate that sensory effects were stronger in red MWs than in white MWs, and that alcohol levels below 10 % in reds and 7.5 % in whites cause the models to become unbalanced with excessive sourness. In whites, low-alcohol MWs also showed reduced levels of perceived bitterness, and only at high ethanol levels were body and alcoholic aroma more intense. In reds, the low alcohol MWs showed less positive aroma persistence (perceived retronasally) and less body; at 0.5 % ethanol the positive odour intensity (perceived orthonasally) was minimal and at higher ethanol levels, the models became sweeter and more alcoholic. Overall, although samples with similar alcoholic contents maintained some sensory similarity, there were relevant discontinuities caused a slight increase in ethanol (0,5 %) to have a dramatic sensory impact, with some sensory descriptors showing significant maxima or minima at these alcohol levels. In reds, sourness, alcohol and astringency perceptions were maximum at 13.5 % and 15 % and body at 13 % and 15 %; meanwhile, red fruit was minimum at 13 % and 15 %, peaking at 14.5 %. In whites, alcohol perception was also maximum at 13.5 % and 15 %, while sweetness was maximum at 13.5 % and 14.5 %. These results demonstrate that ethanol exerts profound effects on sensory properties, probably due to strong perceptual interactions with odour, taste and tactile properties.

**KEYWORDS:** Alcohol, sensory characterization, wine aroma, perceptual interactions

## INTRODUCTION

The greenhouse effect is responsible for raising the temperature of the planet (Burney *et al.*, 2013) and also causes drought and alters the life cycles of plants. In the case of grapevines, harvesting times and ripening periods have changed (Petropoulos *et al.*, 2017; Resco *et al.*, 2016) and, as a result, grapes are accumulating higher sugar contents at maturity, thus increasing alcohol levels in wines after fermentation. In most Spanish regions, the alcohol content of wines has increased significantly over time, even though harvest is being carried out increasingly earlier. While the alcohol content of red wines remained at 13 % in the period 1984-1997, it rose to around 15 % in the period 1998-2008 (de Herralde *et al.*, 2012). These high levels of alcohol are causing many problems, ranging from much higher taxes imposed in most countries to technological difficulties in completing fermentation (Gehrsitz *et al.*, 2021). These observations coincide with a 2 % decrease in alcohol consumption, especially among younger groups. Moreover, reducing or suppressing alcohol consumption in general is recommended on, for example, religious or medical grounds (Gehrsitz *et al.*, 2021; Moeller *et al.*, 2012). All these reasons have led the wine industry to look for different strategies to produce wines with reduced alcohol content, while ideally maintaining flavour profiles as similar as possible to those observed in the original wines. To this end, a number of strategies have been proposed, including the use of genetically modified yeasts or their adaptive evolution, the use of non-Saccharomyces yeasts (Gobbi *et al.*, 2014; Quiros *et al.*, 2014), the use of wines made from early harvest grapes (Kontoudakis *et al.*, 2011; Longo *et al.*, 2018) or even the use of industrial dealcoholisation processes (reverse osmosis, nanofiltration, pervaporation, vacuum distillation, osmotic distillation, spinning cone column and multi-stage membrane systems) (Kumar *et al.*, 2024). However, these strategies can affect the composition of the wine, affecting its flavour, taste and mouthfeel (Pham *et al.*, 2020; Pham *et al.*, 2019). In this context, it is important to note that ethanol concentration has a marked effect on the flavour perception in alcoholic beverages (Ickes and Cadwallader, 2017), partly related to the complex role of ethanol as a solvent. Increasing the ethanol content increases the solubility of aroma compounds, reducing the concentration in the headspace at equilibrium, and hence the gas-liquid partition coefficients. However, ethanol also improves mass transfer properties in the liquid phase, accelerating the transfer of volatile compounds to the headspace in dynamic systems (Ickes and Cadwallader, 2017), partially neutralising the decrease in gas-liquid partition coefficients. As normal wine tasting and consumption takes place under conditions closer to those of dynamic headspace systems (Escudero *et al.*, 2014), the indirect effect of ethanol on the headspace compositions may have been overestimated.

In any case, ethanol has additional relevant sensory properties on its own, as it can induce different odour, taste and trigeminal properties. The latter two include sweetness, bitterness, dryness and irritation/burning perception or even

sourness (Green, 1988; Nolden *et al.*, 2016; Scinska *et al.*, 2000). The intensity of these sensations depends on the alcohol level and, at the usual alcohol levels found in wines (i.e., 12-15 %, v/v), bitterness appears to be the most prominent sensation in ethanol-aqueous solutions (Nolden and Hayes, 2015). Effects of ethanol concentration on viscosity, density and body perception have also been reported. Pickering *et al.* (1998) showed that the most viscous MWs are those with alcohol percentages in the high range of their study (7-14 %), with maxima levels observed at 10 and 12 %.

In addition, and given the high concentration levels at which this sensory-active organic molecule is found in wines, ethanol can most likely induce relevant sensory changes via perceptual interactions with other odour, taste and trigeminal or tactile stimuli present in the product, although these aspects have been poorly addressed in previous studies. The suppressive effect of ethanol on the fruity notes of wine was already described in 2007 (Escudero *et al.*, 2007). In this work, the authors showed that the fruit intensity of MWs containing a fixed amount of fruity esters decreased as the alcohol content was increased, becoming non-perceptible at 14.5 % (v/v). This effect was confirmed by other authors (Goldner *et al.*, 2009; Villamor *et al.*, 2013), who also found a decrease in caramel nuances and an increase in herbaceous, chemical and woody notes with increasing alcohol content. Ethanol has also been found to increase the 'metallic' character of samples lacking polysaccharides (Jones *et al.*, 2008).

Since all the technological processes used to reduce the alcohol content of wine cause significant changes in the remaining volatile fraction of the wine (loss of volatile molecules, such as SO<sub>2</sub>, H<sub>2</sub>S, DMS or acetaldehyde), and may also induce chemical rearrangements in the non-volatile fraction (H<sub>2</sub>S is strongly related to the redox properties of wine and the conformation of its proteins (Nelson *et al.*, 2023)), the study of the sensory effects related to the alcohol content must be carried out using 'wine-like' models with controlled compositions of wine odorants and also of wine non-volatile components, as in previous work (de-la-Fuente-Blanco *et al.*, 2019; de-la-Fuente-Blanco *et al.*, 2016; Ferreira *et al.*, 2016; Sáenz-Navajas, Campo, Culleré, *et al.*, 2010; Sáenz-Navajas, Campo, Fernández-Zurbano, *et al.*, 2010; San-Juan *et al.*, 2011).

In this context and given the importance of understanding the sensory effects actually induced by alcohol on the perception of wine flavour, the present work aims at comprehensively study those sensory effects by carrying out compositionally controlled experiments on reconstituted model wines (imitating red and white wines), varying only in their alcohol content. This compositional control allowed us to identify the effects linked solely to the variation in the alcoholic content, without affecting the rest of the various compositional changes (volatile and non-volatile) that occur during the dealcoholisation process. To this end, two independent studies were carried out. In the first one, models with an alcohol content varying between 0.5 % and 15 % v/v were studied in order to identify sensory differences between MWs

with a wide range of alcohol level: from wines with no or low alcohol content (0.5 %) to contents similar to commercial and conventional wines (15 %). The second study focused on a more limited alcohol range (12 to 15 % ethanol v/v) in order to evaluate the specific sensory differences induced exclusively by limited alcohol changes resulting from the different technological solutions used to reduce the alcohol content of wines.

## MATERIALS AND METHODS

### 1. Compounds and standards

**Solvents:** LiChrosolv quality ethanol was purchased from Merck (Darmstadt, Germany), and Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

**Standards:** Chemical standards: isoamyl alcohol,  $\beta$ -phenylethanol, acetic acid, ethyl acetate, hexanoic acid, 3-methylbutyric acid, ethyl hexanoate, isoamyl acetate, ethyl 2-methylbutyrate, vanillin,  $\gamma$ -nonalactone, guaiacol,  $\beta$ -damascenone,  $\beta$ -ionone, 4-Hydroxy-2,5-dimethyl-3(2H)-furanone, 3-mercaptopentyl acetate, ethyl cinnamate, linalool, eugenol, whiskylactone and geraniol were supplied by Sigma-Aldrich (Madrid, Spain); 3-mercaptopentanol, ethyl vanillate, 2,3-butanedione were supplied by Cymit Quimica (Barcelona, Spain) and were of the highest available purity and food grade.

Standards of ethyl esters were isolated and purified with three extractions of 2 mL of 5 % bicarbonate solution according to the procedure described in de-la-Fuente-Blanco *et al.* (2019), and the higher alcohols isoamyl alcohol,  $\beta$ -phenylethanol and isobutanol were isolated and purified with the necessary minimum quantity of Sulfonyl hydrazine polymer-bound (Sigma-Aldrich) 2 g/10 mL according to the procedure described in de-la-Fuente-Blanco *et al.* (2016).

### 2. Sensory analysis

Both studies were conducted in compliance with the Declaration of Helsinki. Accordingly, participants were informed at the beginning of the experiment that they would remain anonymous and that the provided data would only be reported in the aggregate. They had to acknowledge an informed consent statement in order to participate in the study. Participants had the right to clarify any doubts by requesting more detailed information and to withdraw from the experiment at any time. They were informed of their right to request access to their personal data from the data controller and to request any rectification, deletion, limitation in their processing and data portability, or any other rights that may correspond to them, and to withdraw their consent at any time by contacting the experimenters. To participate in this study, they had to be over 18 years of age and not present any pathology that could be incompatible with the sensory analysis, not be intolerant to alcohol, not be pregnant and not be directly linked to the research. They did not receive financial compensation for their participation.

In order to investigate the effect of ethanol on wine flavour, two studies were carried out, in which different alcohol level ranges were carried out evaluating different alcohol levels in white and red MWs. Study 1 was dedicated to the investigation of a wide range of alcohol levels (0.5-15 %, v/v), and Study 2 investigated alcohol levels ranging from 12 % to 15 % ethanol.

#### 2.1. Study 1

##### 2.1.1. Preparation of red and white MWs

A total of 14 model wine samples were employed in Study 1. Seven of the samples were white MWs, mimicking a Verdejo white wine. The other seven samples were red MWs, mimicking a Tempranillo red wine. MWs were generated by mixing a set of common components of wines, both volatile and non-volatile fractions. The non-volatile fraction of MW was obtained by lyophilisation of a red (pH = 3.66, Total Polyphenol Index measured as absorbance at 280 nm = 67 a.u., reducing sugars = 2.3 g/L, total acidity = 5.6 g/L expressed in tartaric acid; and malic acid < 0.1 g/L) or a white wine (pH = 3.19, reducing sugars = 1.4 g/L, total acidity = 5.1 g/L expressed in tartaric acid, and malic acid = 1.64 g/L). The concentration of volatile compounds (Study 1, Table 1) varied within the natural ranges of appearance in commercial wines, the average concentration found in commercial samples for the compounds that form the common base aroma was used in this study (San Juan *et al.*, 2012). Reconstituted model wines were prepared by mixing the non-volatile fraction, the volatiles indicated in Table 1 and ethanol, and bringing the mixture to 300 mL with bottled mineral water. For each type of model wine (red or white), the composition was identical except for the ethanol content, which varied from 0.5 to 15 % (v/v) (0.5, 2.5, 5, 7.5, 10, 12.5 and 15 %).

##### 2.1.2. Descriptive analysis

**Participants:** This task was carried out by twelve trained panellists (9 men and 3 women, aged between 35 and 65, with an average age of 46 years) comprising members of the tasting panel of D.O. Campo de Borja (Ainzón, Spain). This official panel, which is part of the accreditation of wine as a product: ISO 17065, carries out sensory descriptions of wines based on quantitative descriptive analyses following the procedures described in ISO 17025. The panel had 6 years' experience in the evaluation of wine flavour.

**Procedure:** This task was carried out in two steps: 1) Panel training, and 2) evaluation of the samples of the study.

**Step 1: Panel training:** Participants attended three specific three-hour training sessions over a period of three weeks. During the sessions, panellists were presented with 20 aroma references commercially available from "Wine aroma training set" (Laboratorio de Análisis del Aroma y Enología -LAAE, Universidad de Zaragoza, Spain) covering the entire range of aroma families: positive descriptors ("alcohol", "white/yellow fruit", "tropical fruit", "exotic fruit", "red/black fruit", "citric", "dried/jammy fruit", "floral", "spice", "roasted/smoked", "lactic") and negative descriptors ("vegetal/fresh grass", "oxidation", "reduction" and "cork"), as well

**TABLE 1.** Concentrations and aromatic composition of MWs ( $\mu\text{g/L}$ ).

Compound	Study 1		Study 2	
	White MW	Red MW	White MW	Red MW
Isoamyl alcohol	120000	180000	120000	180000
$\beta$ -phenylethanol	20000	30000	20000	30000
Acetic acid	100000	150000	100000	150000
Ethyl acetate	-	50000	-	62000
Hexanoic acid	5000	2000	5000	2000
3-methylbutyric acid	-	300	-	300
2,3-butanedione	-	400	-	17400
Ethyl hexanoate	5000	1000	5000	1000
Isoamyl acetate	1500	1000	1500	5500
Ethyl 2-methylbutyrate	-	120	-	120
Ethyl vanillate	-	250	-	250
Vanillin	-	70	-	70
$\gamma$ -nonalactone	-	20	-	20
Guaiacol	-	10	-	10
$\beta$ -damascenone	3	4.0	3	7.0
$\beta$ -ionone	-	0.30	-	0.30
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furanol)	-	100	-	30
3-mercaptohexanol	0.40	-	0.60	-
3-mercaptohexyl acetate	0.15	-	0.35	-
Ethyl cinnamate	0.50	-	0.50	115
Linalool	5.0	-	5.0	7.0
Eugenol	-	15	-	-
Whiskylactone	-	300	-	-
Geraniol	-	-	-	0.13

as references for taste and mouthfeel (“sweet”, “sourness”, “bitterness”, “astringency”, “body”). The attributes “positive odour intensity” (POP), and “positive aroma persistency” (PAP) were also defined. POP refers to the amount of positive odour perceived orthonasally, evaluated by comparing with a reference that has an average intensity (= 2 in a 0-4 scale). POP ranges from absence of to very intense positive odour: *absence (0)* corresponds to a sample where “aromatic defects are perceived or no aromatic defects are perceived but positive odours are imperceptible”; *average (2)* is defined as “there are no aromatic defects and positive odours are perceived at a similar intensity to the reference”; and *very high (4)*: “there are no aromatic defects and the positive odours are perceived at a much higher intensity than the reference”. PAP refers to the time the positive aromas persist after the wine has been expectorated: *absence (0)* corresponds to a sample where “aromatic defects are perceived or no aromatic

*defects are perceived but positive aromas are imperceptible after expectorating*”; *average (2)* is defined as “the duration of the aromas in mouth after expectorating is between 2-4 seconds”; and *very high (4)*: “the duration of the aromas in mouth after expectorating is between >6 seconds”.

The samples subject to study were checked for defaults, and as none of them had defective aromas, the POP and PAP descriptors referred to the quantity of odour perceived orthonasally and to the time the aroma is retained in the mouth after expectorating respectively.

Panelists were trained to identify the 20 descriptors and to rank them by intensity on a 5-point structured scale (0 = “absence”, 1 = “low”, 2 = “medium”, 3 = “high”, and 4 = “very high”). Panelists carried out a suitability verification test before panel evaluation in order to confirm panel performance: they were presented with 12 wine samples



similar to those of the study and including four repeated samples to evaluate panel repeatability and consistency. In order to confirm panel performance, two three-way ANOVA models (one for white and one for red wine) involving sample (S), judge (J) and repetition (R) as fixed factors and all first order interactions were first performed. Panel performance were carried out using Panelcheck (version 1.4.2, Matforsk).

*Step 2: Sample evaluation:* Fourteen MWs were sensory described in two sessions taking place the same day, one session for white and one for red MWs, with an hour-long break in the middle. All participants evaluated the MWs in a sequential monadic manner. They were instructed to score the intensity of 20 attributes: fourteen odour attributes evaluated orthonasally (“positive odour intensity”, “alcohol”, “white/yellow fruit”, “tropical fruit”, “exotic fruit”, “red/black fruit”, “citric”, “dried/jammy fruit”, “floral”, “spice”, “roasted/smoked”, “vegetal/fresh grass”, “lactic” and “oxidation”) and six attributes in the mouth: three for taste (“sweet”, “sourness”, “bitterness”) and two for mouthfeel attributes (“astringency”, “body”) and one time-related attribute evaluated retronasally (“positive aroma persistency”) on a 5-point structured scale (0 = “absence”; 1 = “low”; 2 = “medium”; 3 = “high”; and 4 = “very high”). Samples were prepared the day before the sensory session, stored at 10 °C and served 15 min before the evaluation session. Twenty millilitres of sample (20 ± 1 °C) were presented in a random and different order to each judge in dark ISO approved wineglasses labelled with a three-digit code and covered with a Petri dish. All model wines were presented to the panellists at room temperature and evaluated in individual booths. Panellists were not informed about the nature of the samples. All responses were collected in paper ballots.

In order to assess the effect of alcohol level on the sensory attributes, a two-way ANOVA (panellists as random and sample as fixed factors) was performed on each of the two matrices studied (white and red reconstituted model wines) with the intensity scores for each attribute, followed by a Fischer post-hoc pairwise comparison (95 %) test for significant effects. Simple correlations between ethanol level and mean intensity (calculated as the average of the scores provided by the 12 participants) for each attribute were calculated for significant descriptors. Subsequently, two principal component analyses (PCA), one for each type of matrix, were performed on the panellists’ average intensity scores for significant attributes. All statistical analyses were carried out using XLSTAT (Addinsoft, version 2019).

## 2.2. Study 2

### 2.2.1. Preparation of red and white MWs

A total of 13 reconstituted MWs were studied containing a range of 12 to 15 % (v/v) ethanol. Six samples were red MWs, mimicking a Tempranillo red wine produced by carbonic maceration. The other seven samples were white MWs, mimicking a Verdejo white wine. MWs were generated by mixing volatile (Study 2, Table 1) and non-volatile fractions as described in Study 1. MWs were prepared by mixing the non-volatile fraction, the volatiles indicated in Table 1, and

ethanol, and bringing the mixture to 800 mL with bottled mineral water. For each type of MW (red or white), the composition was identical except for the ethanol content. Six different levels of ethanol content for red (12, 13, 13.5, 14, 14.5 and 15 %) and seven for white model wines (12, 12.5, 13, 13.5, 14, 14.5 and 15 %) were prepared.

### 2.2.2. Descriptive analysis

*Participants:* Twelve trained panellists (6 men and 6 women, aged between 24 and 50, with an average age of 33 years), belonging to the LAEE laboratory staff and very experienced in wine aroma description carried out the sensory tasks.

*Procedure:* This task was carried out in five steps: 1) general training, 2) selection of attributes, 3) specific training and familiarisation, 4) evaluation of panel performance, and 5) evaluation of the samples of the study.

*Step 1: General training:* Twelve participants attended five 30-min training sessions over a period of one week. During the sessions, panellists were presented with 42 aroma references commercially available from “Aromabar” (Hamburg, Germany) or “Wine aroma training set” (Laboratorio de Análisis del Aroma y Enología -LAEE, Universidad de Zaragoza, Spain) covering the entire range of aroma families, as well as references for taste and mouthfeel including sourness (1.5 g/L of tartaric acid), sweetness (12 g/L of table sugar), bitterness (10 mg/L of quinine), body (2 g/L of carboxymethyl cellulose) and astringency (5 g/L of aluminium sulphate). They had to smell aroma references and taste solutions, and to identify the aroma, taste or mouthfeel sensation using an established list of terms. They repeated the task until they had correctly identified all the references.

*Step 2: Selection of discriminant attributes:* this step consisted in two 60-min evaluation sessions, one for the seven white MWs and the other for the six red MWs. First, panellists were presented with a list of 121 aroma terms that were hierarchically structured (families, subfamilies and specific terms), as employed in previous work of the research group (Campo *et al.*, 2008), and a list for in-mouth sensations, including sourness, sweetness, bitterness, astringency and body. In each session, panellists were each presented with all the model wines (7 white and 6 red, depending on the session) simultaneously in dark glasses covered with a petri dish in a random and different order. Samples were coded with a three-digit number. Panellists were asked to orthonasally smell all the samples and identify the aroma descriptors that differentiated the samples, and then to taste them and note down the taste and mouthfeel properties that differentiated them. They could select as many terms as they considered necessary with no time restrictions. The total number of citations (NC) of the selected terms that differed between each sample set was counted. The maximum NC possible for a term was equal to the total number of judges (i.e., a maximum of 12). For each type of MW, white or red, the terms cited by at least 3 out of 12 panellists (representing 25 % of the panel) were selected. These terms were used in the training and evaluation sessions.

**Step 3: Specific training and familiarisation:** participants attended five 60-min descriptive training sessions over a period of three weeks. During these sessions, panellists were trained in the aroma, taste and mouthfeel attributes that they had selected in the previous step. Five odour (“white fruit”, “tropical fruit”, “dried fruit”, “floral” and “alcohol”) and four in-mouth (“sourness”, “sweetness”, “bitterness” and “body”) attributes were considered for white MWs, and five odour (“red fruit”, “dried fruit”, “spicy”, “toasted”, “alcohol”) and five in-mouth attributes (“sourness”, “sweetness”, “bitterness”, “body” and “astringency”) for red MWs. In sessions 1 and 2, panellists were asked to identify different reference standards representative of aroma descriptors. The standards were commercially available odorants, from “Aromabar” (Hamburg, Germany) or “Wine aroma training set” (Laboratorio de Análisis del Aroma y Enología -LAAE, Universidad de Zaragoza, Spain). Panellists had to be able to identify them correctly. In session 3, different levels of reference-spiked solutions representative of taste vectors (quinine hemisulphate monohydrate for “bitterness” from 0 to 45 mg/L, tartaric acid for “sourness” from 0 to 5 g/L, aluminium and potassium sulphate for “astringency” from 0 to 10 g/L, carboxymethylcellulose for “body” from 0 to 6 g/L (Hopfer & Heymann, 2014), and table sugar for “sweetness” from 0 to 10 g/L) were used to help the panellists with identification and to train them in ranking by intensity. In sessions 4 and 5, they were taught and trained in the use of the structured 15 cm-intensity scale (the extremes being 0 = “absence” and 15 = “very intense”) and they familiarised themselves with the sensory space object of study. To this end, different commercial wines with characteristic aroma descriptors were used. At the end of each training session, wines similar to those of the final study (i.e., MWs) were used to get panellists familiarised with the studied wines.

**Step 4: Panellist performance assessment:** Two selection sessions were held to evaluate the ability of panellists to rate the different descriptors and to evaluate their repeatability, reproducibility and consistency. For that purpose, the same eight wines were used in two different sessions: four duplicated wines, two white Verdejo wines (Valtropin and Marques de Caceres) and two Tempranillo red wines (Los Molinos and Chulato de Albeica); the glasses were labelled with a three-digit random code and covered with a Petri dish. In the first part, the panellists were asked to score 9 attributes of white wines: five aroma attributes (“tropical fruit/banana”, “dried fruit”, “alcohol”, “white fruit”, “floral”) and four in-mouth attributes (“sweetness”, “sourness”, “bitterness”, “body”) on a structured 15 cm- intensity scale (the extremes being 0 = “absence” and 15 = “very intense”). In the second part, they were asked to score 10 attributes of red wines: five aroma attributes (“red fruit”, “dried fruit”, “alcohol”, “spicy”, “toasted/wood”) and five in-mouth attributes (“sweetness”, “sourness”, “bitterness”, “body” and “astringency”) on the same scale as that described for white wines. Two three-way ANOVA models (one for white and one for red wine) involving sample (S), judge (J) and repetition (R) as fixed factors and all first order interactions were first performed. Then, for attributes showing a

significant interaction effect sample-by-judge ( $S * J$ ) a PCA was calculated on a table encoded in a sample x judge matrix, in which each cell represented the intensity evaluated by one judge in a sample. This PCA was run in order to assess any disagreement in scoring between the judges (for detailed information, see Supplementary material, Table S1 and Table S2). Panel performance were carried out using Panelcheck (version 1.4.2, Matforsk).

**Step 5: Sample evaluation:** Only those panellists who had been consistent and reproducible in the previous sessions carried out the evaluation sessions ( $n = 10$ ). Seven white MWs in duplicate underwent a sensory description in a formal session. In a second session, held on a different day, six red MWs in duplicate underwent a sensory description. In each session, panellists were instructed to score the intensity of the nine attributes of white MWs and the ten of red MWs listed in Step 4. Samples were prepared the day before the sensory session, stored at 10 °C and served 15 min before the evaluation session. Each judge received 38 mL of sample ( $20 \pm 1$  ° C) in dark ISO approved wineglasses labelled with a three-digit code and covered with a Petri dish in a random and different order. All MWs were served and evaluated in individual booths. Panellists were not informed of the nature of the samples. All responses were collected in paper ballots.

In order to assess the effect of alcohol level on each sample set (red or white MWs), a two-way ANOVA (panellist as random and alcohol level as fixed factor) was performed on the scores obtained for each attribute and all samples. Subsequently, a principal component analysis (PCA) was performed with the panellists’ average intensity scores for significant attributes. All statistical analyses were carried out using XLSTAT (Addinsoft, version 2019).

## RESULTS

### 1. Study 1 (panel 1). Large range of ethanol levels (0.5-15 % v/v)

Fourteen MWs, seven imitating white and seven imitating red wines, differing only in their alcohol contents (0.5, 2.5, 5, 7.5, 10, 12.5 and 15 %) were prepared and sensorily evaluated.

The ANOVA results of the sensory analysis are summarised in Table 2 (mean results for all attributes and samples are included as Supplementary material, Table S3-S4). The sensory effects of white MWs were limited to the aroma descriptor “alcohol” and to three in-mouth attributes: “body”, “sourness” and “bitterness”. In the case of reds, three aroma attributes including “positive odour intensity”, “alcohol” and “toasted/smoked”, and four in-mouth attributes; “sweetness”, “sourness”, “body” and “positive aroma persistence” were affected by ethanol levels.

The effects on the significant sensory descriptors of white WMs can be seen in detail in Figures 1 and 2. Figure 1 shows the PCA illustrating the distribution of samples and the variable loadings, while Figure 2 shows the sensory

**TABLE 2.** Results of the two-way ANOVA (participants as random and model wine as fixed factor) for the evaluation of the effect of alcohol on the intensity of different attributes in Study 1.

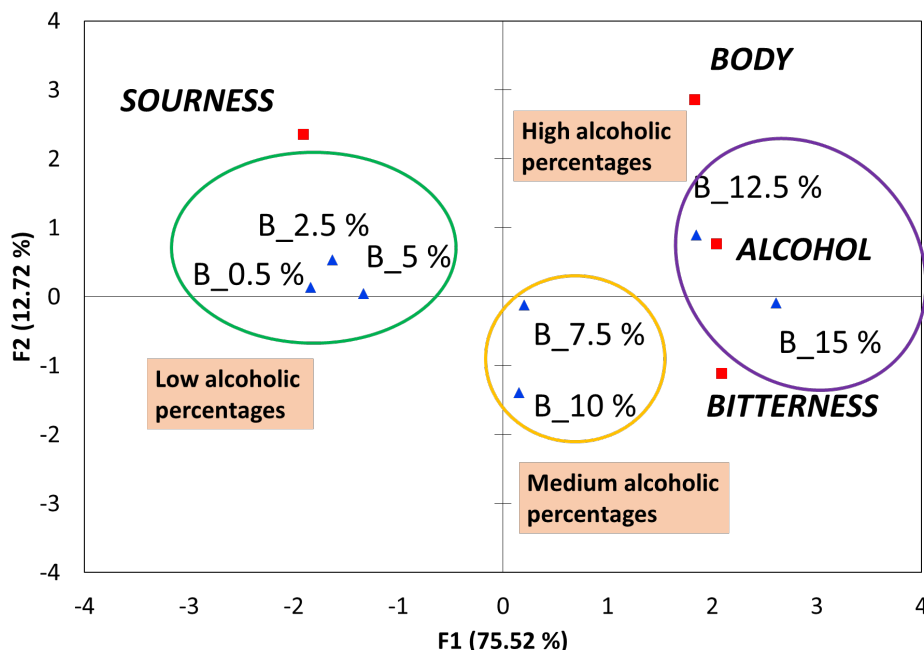
Descriptor	White MW		Red MW	
	F	P	F	P
Positive odour intensity	0.51	n.s.	2.42	<b>&lt; 0.05</b>
Alcohol	4.25	<b>&lt; 0.01</b>	2.45	<b>&lt; 0.05</b>
White/yellow fruit	0.49	n.s.	1.46	n.s.
Tropical fruit	0.64	n.s.	0.85	n.s.
Exotic fruit	0.87	n.s.	0.80	n.s.
Red/black fruit	1.63	n.s.	0.19	n.s.
Citric	0.77	n.s.	1.00	n.s.
Dried/jammy fruit	0.75	n.s.	0.45	n.s.
Floral	0.67	n.s.	1.46	n.s.
Spice	0.52	n.s.	2.08	n.s.
Toasted/smoked	0.82	n.s.	2.27	<b>&lt; 0.05</b>
Vegetal/fresh grass	0.52	n.s.	0.62	n.s.
Lactic	1.05	n.s.	1.33	n.s.
Oxidation	0.33	n.s.	0.92	n.s.
Sweetness	0.20	n.s.	3.68	<b>&lt; 0.01</b>
Sourness	3.17	<b>&lt; 0.01</b>	5.25	<b>&lt; 0.001</b>
Bitterness	3.29	<b>&lt; 0.01</b>	0.47	n.s.
Astringency	1.64	n.s.	1.16	n.s.
Body	2.23	<b>&lt; 0.05</b>	2.36	<b>&lt; 0.05</b>
Positive aroma persistence	0.75	n.s.	2.29	<b>&lt; 0.05</b>

<sup>a</sup>Significance of the effect; n.s.: not significant. Significant effects marked in bold ( $P < 0.05$ ). Positive odour intensity was perceived orthonasally, and positive aroma intensity was perceived retronasally.

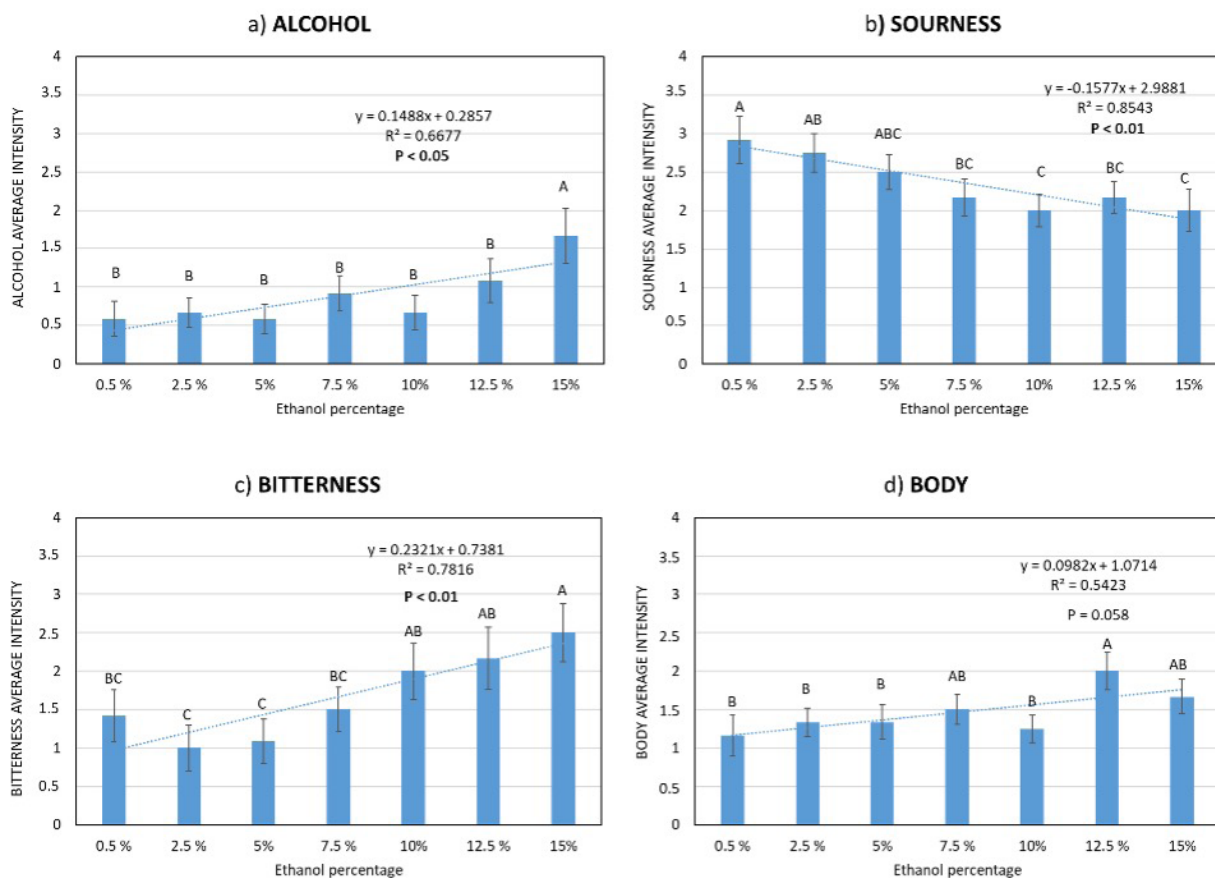
scores obtained at the different levels of ethanol. The PCA plot in Figure 1 clearly indicates that samples are grouped by alcoholic degree, and that, in general, lowering the alcohol content results in an increase in “sourness” and a decrease in “body”, “alcohol” and, particularly, in “bitterness”. This is further confirmed by the plots in Figure 2, in which it can be seen that “sourness” increases steadily and constantly when the alcoholic degree is lower than 10 %, while “bitterness” follows an opposite trend, increasing linearly with alcohol content higher than 5 %. In the cases of the sensory descriptors “body” and “alcohol”, the single significant difference is that they reach a maximum at 12.5 % or 15 % (v/v) respectively. It is worth noting that “alcohol” is a complex attribute perceived orthonasally, but which includes odour and tactile or trigeminal sensations, while “sourness” and “bitterness” are purely taste sensations and “body” is also a complex attribute comprising oral tactile and taste sensations.

The plots corresponding to red MWs are shown in Figures 3 and 4. The PCA plot in Figure 3 shows notable similarities to that corresponding to white MWs in Figure 1: the samples are grouped by alcohol content and, in general, a decrease

in alcohol level results in an increase in “sourness” and a decrease in a number of sensory descriptors, including “body” and “alcohol”. However, there are also other relevant differences, since the sensory effects of alcohol content seem to be much more intense in this case. While “bitterness” is not affected, “sweetness” and three aroma-related attributes are affected by alcohol content. As can be seen in Figure 4, “sourness” reaches a maximum at low alcohol levels (0.5-5 %) and a minimum at higher alcohol levels (10-15 %), with an approximately linear increase between 12.5 and 5 %. “Sweetness” scores high only at the highest alcohol level, while “body” scores high at ethanol levels above 7.5 %. In terms of orthonasally perceived sensations, “alcohol” scores low in the 0.5-10 % range and reaches a maximum at the highest alcohol levels (12.5 and 15 %). Similarly, for the red MWs “positive odour intensity” (perceived orthonasally) and “positive aroma persistence” (perceived retronasally) become minima at 0.5 % and are slightly higher at higher alcohol levels, with a more gradual and obvious effect of alcohol on “persistence”. Finally, the “toasted/smoked” aroma note follows a complex trend with two clear minima at 5 and 15 % and two maxima at 2.5 and 10 %.

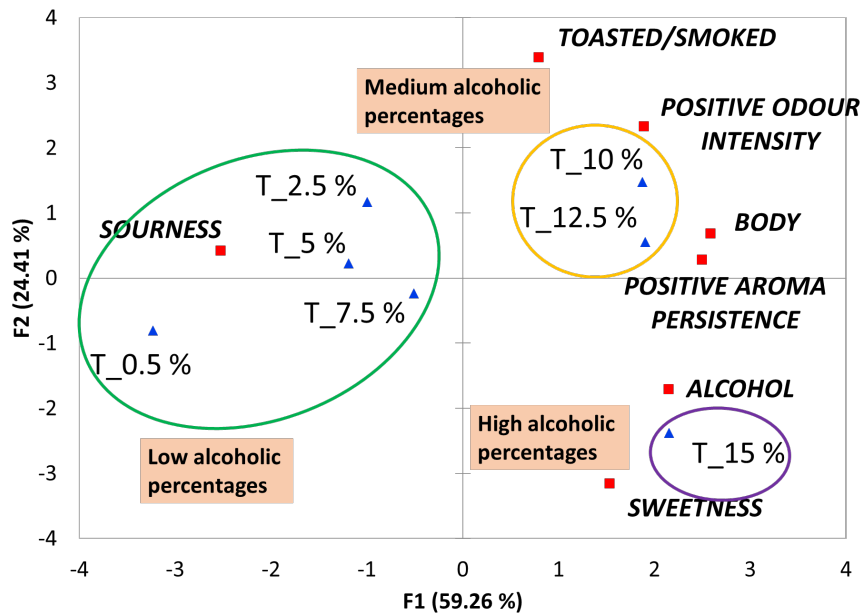


**FIGURE 1.** Biplot showing the projection of the significant descriptors and MWs on the first two principal components of the PCA space for white MWs in Study 1.

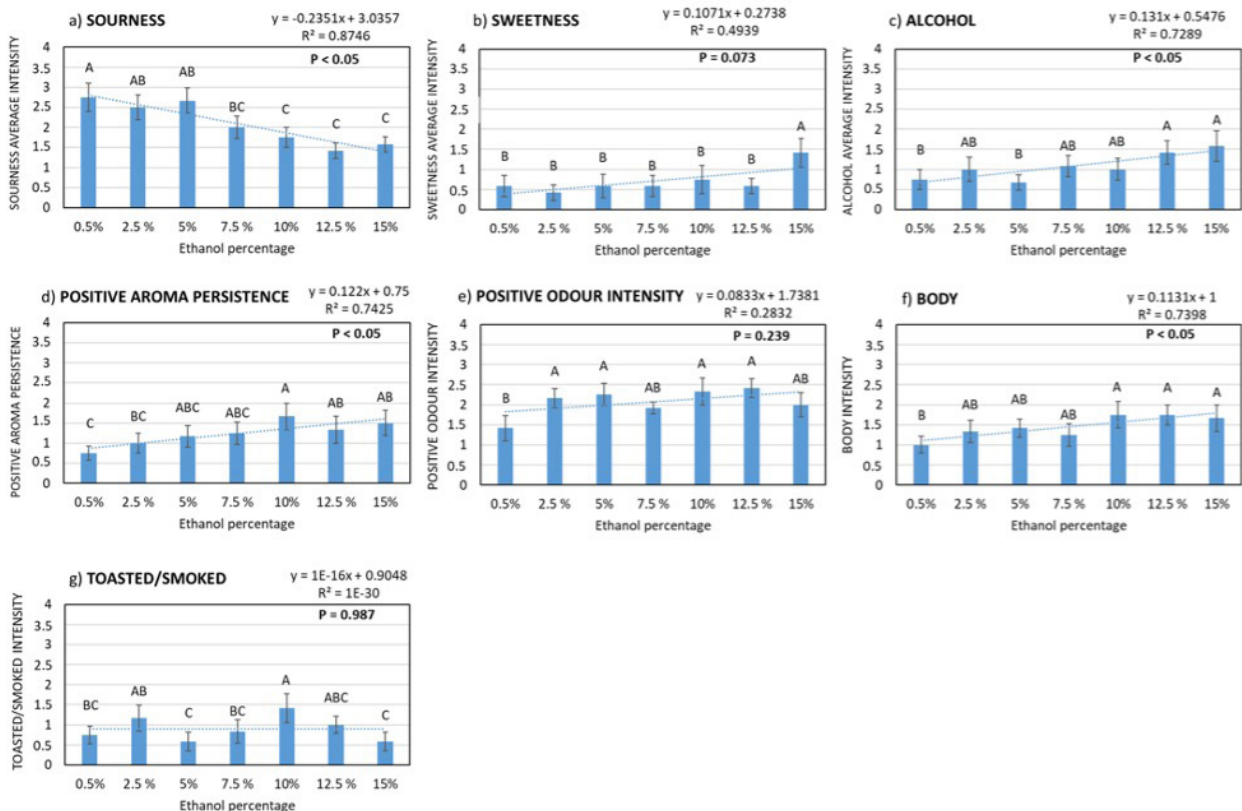


**FIGURE 2.** Bar plots showing the mean scores of the significant sensory descriptors at different ethanol levels in white MWs in Study 1. The lines and regression statistics are obtained by simple linear regression statistics: a) alcohol perception, b) bitterness, c) sourness, and d) body. Error bars are mean standard errors. For a given attribute, different letters indicate significant differences ( $P < 0.05$ ) among samples.





**FIGURE 3.** Biplot showing the projection of the significant descriptors and MWs on the first two principal components of the PCA space for red MWs in Study 1.



**FIGURE 4.** Bar plots showing the mean scores of the significant sensory descriptors at different ethanol levels in red MWs in Study 1. The lines and regression statistics are obtained by simple linear regression statistics for: a) sourness, b) sweetness, c) alcohol, d) positive aroma persistence (perceived retronasally), e) positive odour intensity (perceived orthonasally), f) body, and g) toasted/smoked. Error bars are mean standard errors. For a given attribute, different letters indicate significant differences ( $P < 0.05$ ) among samples.

## 2. Study 2 (panel 2). Narrow range of ethanol levels (12–15 % v/v)

In this experiment, the effects of six and seven different levels of ethanol content on red (12, 13, 13.5, 14, 14.5 and 15 % ethanol) and white (12, 12.5, 13, 13.5, 14, 14.5 and 15 % ethanol) MWs were studied.

Table 3 summarises the results of the ANOVA study carried out on the sensory descriptors (mean results for all attributes and samples are included as Supplementary material, Table S5-S6). In the case of the white MWs, only two out of the nine assessed attributes were significantly affected by alcohol content. These were the orthonasal perception of

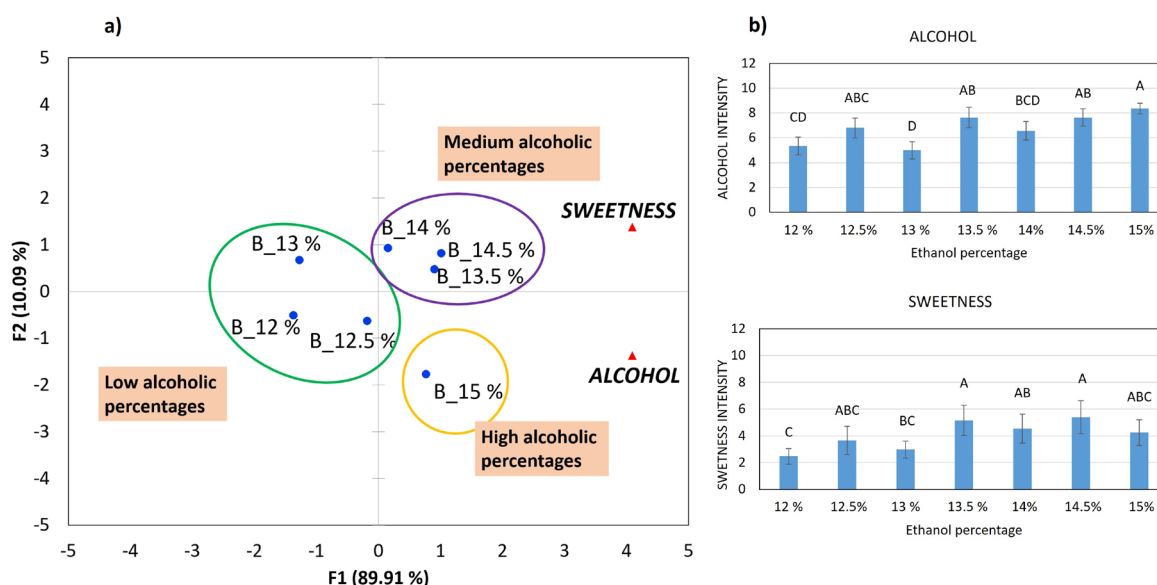
“alcohol” and the taste perception of “sweetness”. For reds, seven out of ten attributes were found to vary significantly with alcohol content, including all orally perceived attributes (except “bitterness”), “alcohol” and the two fruit-related odour attributes: “red fruit” and “dried fruit”. As observed in the previous study, the effects of ethanol content are much more pronounced on red MWs.

Similar conclusions can be drawn from the PCA plots shown in Figures 5a and 6. While in both cases it is evident that the samples are grouped according to degree of alcohol, the separation is much more marked in the case of the red MWs (Figure 6). Another rather noteworthy observation is that

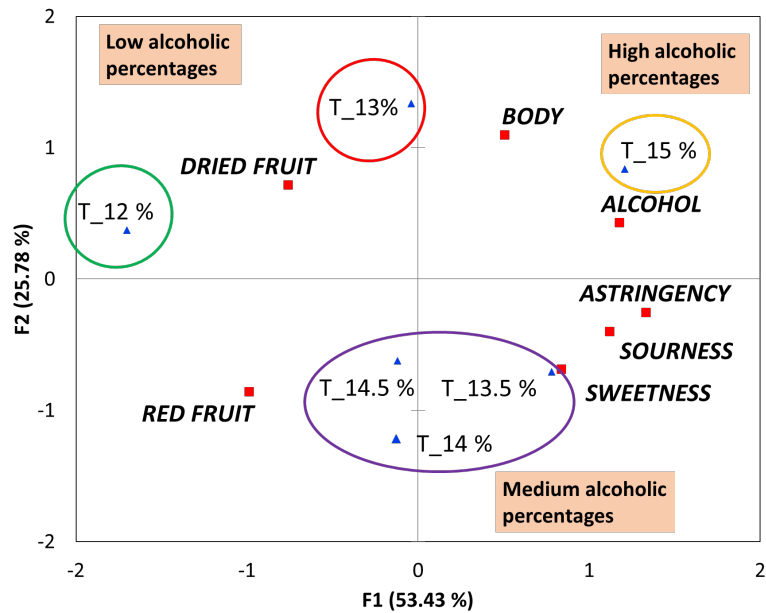
**TABLE 3.** Results of the two-way ANOVA (participants as random factor and samples as fixed factor) calculated to evaluate the effect of ethanol on the intensity of different attributes in white and red MWs in Study 2.

White MW			Red MW		
Descriptor	F	P <sup>a</sup>	Descriptor	F	P <sup>a</sup>
White fruit	0.58	n.s.	Red fruit	4.86	<b>&lt; 0.01</b>
Tropical fruit	0.82	n.s.	Dried fruit	4.63	<b>&lt; 0.001</b>
Dried fruit	1.10	n.s.	Spicy	1.94	n.s.
Floral	0.47	n.s.	Toasted	2.43	n.s.
Alcohol	3.89	<b>&lt; 0.01</b>	Alcohol	2.84	<b>&lt; 0.05</b>
Sourness	0.41	n.s.	Sourness	6.06	<b>&lt; 0.001</b>
Bitterness	0.59	n.s.	Bitterness	2.16	n.s.
Sweetness	2.57	<b>&lt; 0.05</b>	Sweetness	2.92	<b>&lt; 0.05</b>
Body	0.45	n.s.	Body	3.99	<b>&lt; 0.01</b>
			Astringency	4.30	<b>&lt; 0.01</b>

<sup>a</sup> Significance of the effect; n.s.: not significant. Significant effects marked in bold (P < 0.05).



**FIGURE 5.** Representations of white MWs in Study 2: a) biplot showing the projection of the significant descriptors and MWs on the first two principal components of the PCA space, b) bar plots showing the mean scores of the significant sensory descriptors in terms of alcohol and sweetness at different ethanol levels. Error bars are mean standard errors. For a given attribute, different letters indicate significant differences (P < 0.05) among samples.



**FIGURE 6.** Biplot showing the projection of the significant descriptors and MWs on the first two principal components of the PCA space for red MWs in Study 2.

samples containing 13.5, 14 and 14.5 % ethanol are clustered together in both cases, well separated from those with slightly lower (13.0 %) or slightly higher (15.0 %) alcoholic degrees. In the case of red MWs, this separation is radical, as can be seen in Figure 6, which shows the six models to have four clearly different sensory profiles depending on alcohol content; one of these profiles is shown for models with 13.5, 14 and 14.5 % alcohol.

In both cases, the reasons for these apparent jumps or discontinuities lie in the existence of relevant changes in the intensity of some sensory descriptors within very narrow and, apparently, quite specific ranges of alcohol content. For example, the attribute “alcohol” in the case of white MWs shows a clear minimum at 13 %, a local minimum at 12 %, local maxima at 12.5 % and 13.5 %, and an absolute maximum at 15 %, as can be seen in Figure 5b. Similarly, for “sweetness”, there is a clear local minimum at 13 %, an absolute minimum at 12 %, a local maximum at 13.5 % and an absolute maximum at 14.5 %. These jumps are responsible for the illustrated distribution of the samples in Figure 5a, and indicate that a mere 0.5 % increase in alcohol from 13.0 to 13.5 and from 14.5 to 15 % can produce strong sensory changes.

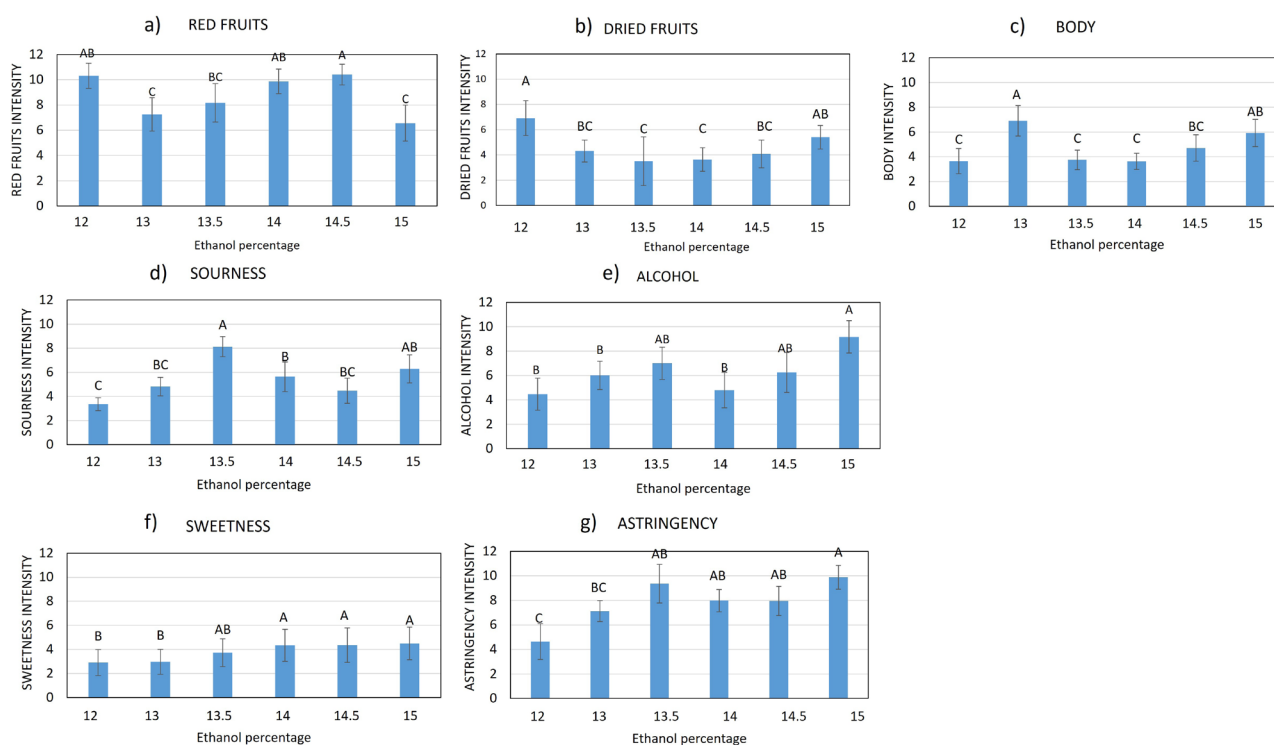
In the case of the red MWs, the PCA plot in Figure 6 reveals that the first component separates the samples into three groups. MWs with 12 % ethanol are projected on the far left, those with 15 % ethanol on the right, and the five with intermediate alcohol content in the middle. This shows that, in general, an increase in alcohol content is linked to a decrease in fruity character of the MW and an increase to “alcoholic” aroma, “astringency”, “sweetness” and “sourness”. Interestingly, the 2nd component basically separates the samples with alcohol levels in the range of

13.5-14.5 % from the others, especially from those with 13 % alcohol levels. This means that this group of samples has particularly high red/dried fruit and sweetness/body ratios (above 2.3 and 0.93 respectively), while the 13 and 15 % ethanol models have particularly low ratios (below 1.7 and 0.76 respectively).

A closer look at the scores shown in Figure 7, reveals that five descriptors change with alcohol content in a non-linear way, with clear maxima and minima. “Red fruit” has a relationship with alcohol content similar to a cubic curve, with a local minimum at 13 %, an absolute minimum at 15 % and two maxima at 12 and 14.5 %. “Dried fruit” follows a relationship similar to a quadratic trend, with a minimum at around 13.5-14 %, and “body” follows a cubic trend quite opposite to that observed for “red fruits”, with a maximum at 13 %, a second maximum at 15 % and minima at 12 % and 14 %. “Sourness” and “alcohol” show quite similar relationships, with two maxima at 13.5 % and 15 % - although the maxima at 13.5 % in the case of “alcohol” is not significantly different from the neighbouring MWs. “Astringency” also shows a similar dependence, but the minimum at 14 %-14.5 % are not significantly different from the maximal scores. Finally, “sweetness” shows a less complex relationship, with higher levels above 13.5 % and relatively stable plateaus in the low and high alcohol ranges.

## DISCUSSION

In the present work, two different experiments were carried out in order to investigate the effects of ethanol content on wine flavour by sensory description of four different sets of WMs.



**FIGURE 7.** Bar plots showing the mean scores of the significant sensory descriptors at different ethanol levels in Study 2 carried out on red MWs for: a) red fruits, b) dried fruits, c) body, d) sourness, e) alcohol, f) sweetness, and d) astringency. Error bars are mean standard errors. For a given attribute, different letters indicate significant differences ( $P < 0.05$ ) among samples.

The results show that alcohol content strongly modulated the sensory properties of the MWs, to such an extent that the sensory spaces in the four studies can be seen to be clearly influenced by ethanol content (Figures 1, 3, 5 and 6). Apart from the orthonasal (odour and trigeminal) perception of “alcohol”, which varied significantly across the four studies, the attributes the most influenced by ethanol content were three in-mouth attributes, including two taste-related properties: “sourness” and “sweetness”, and the trigeminal cue “body” (Cayeux, *et al.*, 2023). The effects of ethanol on these attributes were found to be independent of the MW matrix. “Bitterness” and “astringency” were also significantly influenced by ethanol content, but only in one out of the four studies on white and red MWs respectively. Finally, several aroma attributes, including “dried fruit”, “red fruit”, “toasted/smoked”, “positive odour intensity” (perceived orthonasally) and “positive aroma persistence” (perceived retronasally), were also found to change significantly, but only in one of the studies carried out on red MWs.

The results also reveal that while there is a more or less gradual change in the sensory descriptors over the range of 0.5–15% ethanol, complex highly non-linear trends emerge when monitoring narrower ranges of ethanol levels (Study 2). For instance, while the orthonasal descriptor “alcohol” increases at alcohol levels above 10% in both white and red MWs (Figures 2 and 4), significant local minima were observed

at 13% (Figure 5b) and 14% (Figure 7e) respectively. Similarly, the perceived “sourness” is particularly high at low ethanol levels (0.5–5%) in both sample sets (Figures 2 and 4), and is only significantly lower at ethanol levels above 7.5% (v/v). Interestingly, however, Study 2 shows that within the range of 12–15% of ethanol, the perception of “sourness” in red MWs follows a rather complex trend, with a maximum at 13.5% ethanol, followed by a minimum at 14.5% (Figure 7d). In addition, the term “body”, which is the third sensory characteristic the most generally influenced by ethanol level, reaches maxima at levels above 12.5% in white MWs (Figure 2d) and above 7.5% in red MWs (Figure 4f), where a local maximum was observed at 13% (Figure 7c). The results for “body” are consistent with those of Pickering and colleagues who observed that increasing ethanol levels of up to 7% resulted in an increase in the perception of “viscosity” and “density” in model wines (Demiglio and Pickering, 2008; Nurgel and Pickering, 2005; Pickering *et al.*, 1998). To the best of our knowledge, neither the prominence of “sourness” at low levels of ethanol nor the existence of complex relationships has been previously observed.

Increasing alcohol content leads to a perceptible increase in “sweetness” only at alcohol contents above at least 13% (Figures 4b, 5b and 7f); this contrasts with an old report (Hoopman *et al.*, 1993) of ethanol inducing a decrease



in “sweetness”, but is more in line with more recent work (Nurgel and Pickering, 2006; Zamora *et al.*, 2006) in which the sweetness of fructose-containing models increased with increasing ethanol content.

An interesting and positive effect of alcohol on the “bitter” perception of white MWs was also observed (Figure 2c), consistent with previous reports (Fontoin *et al.*, 2008; Jones *et al.*, 2008; Nolden and Hayes, 2015). Fontoin *et al.* (2008) observed that the contribution of ethanol to the perceived “bitterness” of tannin oligomers was particularly pronounced at typical wine ethanol levels (11-15 %), while Nolden and Hayes (2015) observed that the effect of ethanol on “bitterness” was especially notable in the 8-16 % ethanol range, in agreement with the results of the present study.

The observed increase in “astringency” of red MWs with alcohol content in the 12-15 % range (Figure 7g) is consistent with observations made on real wines. Sáenz-Navajas *et al.* (2019) reported a general positive correlation between “astringency” and ethanol content, in particular in wines with >14.4 % ethanol, which is further supported in different studies (Sáenz-Navajas *et al.*, 2010; Sáenz-Navajas *et al.*, 2012; Watrelot *et al.*, 2016). However, the effect of ethanol on “astringency” perception is not without discrepancies: older studies (Demiglio and Pickering, 2008; Fontoin *et al.*, 2008; Vidal *et al.*, 2004) reported an inverse effect of ethanol on perceived “astringency”. This effect was attributed to a decrease in hydrophobic interactions with increasing ethanol content, which is consistent with the assumption that “astringency” is the result of protein-tannin interactions (McRae *et al.*, 2015). However, the present results support the relevance of other mechanisms other than protein-tannin interactions for the perception of “astringency”, as suggested by Soares *et al.* (2017).

In terms of aroma attributes, “positive odour intensity” (perceived orthonasally) and “positive aroma persistence” (perceived retronasally) increased significantly in red MWs in the 0.5-10 % range of alcohol content, consistent with studies showing that ethanol has a significant and positive effect on “global aroma perception” in MWs (Jones *et al.*, 2008; Le Berre *et al.*, 2007). However, the effects of ethanol levels on specific aroma attributes seem to contradict the limited data available to date. Previous reports have generally indicated a reduction in “fruity” attributes (Escudero *et al.*, 2007; Fischer, 1996; Guth, 1997) with increasing ethanol levels. This may be due to both a reduction in the volatility of non-polar volatiles, such as ethyl esters, with increasing ethanol and perceptual suppression effects induced by ethanol. However, the results presented here suggest that the effects of ethanol content on fruity descriptors are extremely complex and product dependent, as demonstrated by the lack of effects in the case of the white MWs, in clear contrast to the strong effects observed in red MWs. In addition, in almost all cases, aroma attributes and alcohol content change according to rather complex non-linear functions, which means that reductions in the intensity of the aroma sensory attributes with alcohol content were only observed in narrow ethanol ranges; for instance, from 12 % to 13 % and from

14.5 to 15 % for “red fruits”, and from 12 % to 14 % for “dried fruits” (Figures 7a and 7b). This is also the case for the “toasted/smoked” descriptor, which decreases when the ethanol is increased from 2.5 to 5 % or from 10 to 15 % (Figure 4g).

All these observations suggest that the effects of ethanol on aroma are mainly the consequence of strong perceptual interactions induced by the olfactory and trigeminal properties of ethanol. These perceptual interactions would be particularly relevant in complex aroma models without dominant aroma vectors, which would explain why the effects are particularly noticeable in red MWs but not in white MWs, whose aroma is dominated by the varietal polyfunctional mercaptans 3-mercaptohexyl acetate (3MHA) and 3-mercaptohexanol (3MH).

It should be noted that in a previous report, the pairs of descriptors “dried fruits” and “tropical fruits” and “alcohol” by nose and “red fruit” in red MWs were found to be negatively correlated (Alegre *et al.*, 2020), which strongly supports the fact that these descriptors undergo perceptual interactions in these models. These perceptual interactions between odours are important in explaining the complex effects of ethanol content on the “alcohol” attribute perception of the mixture (Figures 5b and 7e).

Notably, the complex effects of the ethanol content on the taste or tactile attributes “sourness” and “body” seen in Figures 7c and 7d can also be clearly explained by the perceptual interactions between the oral perceptions of ethanol (sweet, bitter and pungent) and the rest of the tastants present in the models, which comprise acids and phenolics extracted from natural wines. Odour x taste interactions cannot be ruled out, but in previous work, it was observed that aroma compounds did not play any role in the “in mouth” properties of red wines (Sáenz-Navajas *et al.*, 2010). In all cases where complex relationships are observed, the perceptual interactions must be of a creative or configurational type (Ferreira *et al.*, 2021): maxima scores would correspond to the existence of a specific sensory profile associated with a familiar sensory object. For instance, the specific tastant composition of the red MW containing 13.5 % ethanol, would represent a sensory concept characterised by high “sourness” and “astringency” in the mouth (Figure 7), while that containing 13 % in ethanol would represent another sensory concept characterised by high “body” and reduced levels of “sourness” and “astringency”. A further reduction in 1 % ethanol results in a different sensory concept characterised by low “body”, “sourness” and “astringency”. This reveals that alcohol plays an extremely important role in determining the sensory properties of red MWs, including taste, tactile, trigeminal and odour perceptions, and that these perceptions can vary significantly and widely with very small changes in alcohol. Effects are also relevant, but to a lesser extent, in white MWs.

All these results show that there are major challenges associated with the production of wines with reduced alcohol content. One of the most relevant is the strong in-mouth

imbalance caused by the huge increase in “sourness” observed when ethanol is present at levels of 5 % or less (Figures 2b and 4). In white MWs, such an increase is compensated for by a strong reduction in “bitterness” (Figure 2c), but this means that the sensory profile is completely different. For red MWs, very low alcohol wines also have to face a strong decrease in “positive odour intensity” (perceived orthonasally), “persistence” (perceived retronasally) and “body” (Figures 4d-4f). For moderate reductions in alcohol levels, our results indicate that in red MWs both the odour and taste profiles change profoundly, and that small adjustments in the final alcohol content will have a strong influence on the sensory properties.

## CONCLUSION

Alcohol content strongly modulates the sensory perception of MWs, especially that of red MWs. The sensory descriptors most affected by the change in alcohol content are orthonasal “alcohol” perception, followed by the gustatory and trigeminal descriptors “sweetness”, “sourness” and “body”. “Astringency” for red MWs and “bitterness” for white MWs were also affected, while aroma attributes were only affected for red MWs.

The study showed that the reduction of ethanol at levels below 5 % in all cases caused a deep imbalance due to an excess of “sourness”. While in white MWs this is partly compensated for by a reduction in “bitterness”, in red MWs it is exacerbated by a considerable reduction in “body” and in “positive odour intensity” (perceived orthonasally) and “persistence” (perceived retronasally).

The study has also shown that most of the sensory descriptors change with ethanol content, following complex non-linear trends with different maxima and minima, which are evident when small changes in ethanol content are considered. The consequence, particularly evident in red MWs, is the existence of jumps or discontinuities in the sensory profile when the alcohol content changes from 13 to 13.5 % and from 14.5 to 15 %. The “red fruit” to “dry fruit” and “sweetness” to “body” ratios change at these transition points. This represents a challenge from both a technological and scientific point of view, and strongly suggests that ethanol exerts close perceptual interactions with odour, taste and tactile perceptions.

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## AUTHOR CONTRIBUTION

A.D.L.F.B.: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. M.P.S.N.: Conceptualization, Investigation, Writing – review & editing, Formal analysis. I.A.: Methodology, Formal analysis. A.E.:

Writing – review & editing, Project administration. V.F.: Conceptualization, Project administration, Writing – original draft, Writing – review & editing, Funding acquisition.

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