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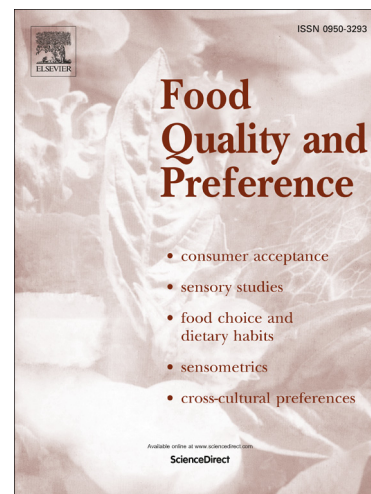
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Cross-modal interactions and effects of the level of expertise on the perception of bitterness and astringency of red wines

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Abstract

The present study investigates the role of sensory interactions and the level of expertise of consumers on the perception of bitterness and astringency in a red wine complex matrix.

In the first experiment, a commercial red wine was spiked with quinine sulphate to produce two levels of bitterness. Seven samples with high and seven with low bitterness were further spiked with one of the following aroma vectors: *vegetal, herbal, spicy, roasted, animal, dried fruit* or with tartaric acid to modulate sourness. In the second experiment, the wine was spiked with an astringent proanthocyanidin fraction to produce two levels of astringency and with one of the seven vectors used in the first experiment or with quinine sulphate to modulate bitterness. Fourteen and 16 spiked samples of experiments 1 and 2, respectively, were sensory evaluated together with control samples to assess the intensity of the six aroma vectors, sourness, bitterness and astringency. Evaluation was performed using a descriptive analysis technique based on intensity rating by three groups of participants with different levels of expertise. The effect of the addition of one aroma vector, sourness or bitterness on bitter or astringent intensity was evaluated by ANOVA.

No significant effect of aroma, sourness or bitterness was observed for astringent intensity, confirming that in these red wines, cross-modal interactions are not relevant in astringency. Bitter-aroma interactions were demonstrated for animal aroma just for less-experienced consumers (novices) attributed to a hedonic synergism. Significant decreases in bitterness due to sour vector were observed regardless consumer expertise. This could be explained in terms of interactions at both cognitive and receptor level.

Key words: sensory interactions; level of expertise; red wine; astringency; bitterness

1. Introduction

Food products contain a combination of volatile and non-volatile compounds able to impact the different sensory receptors. The flavour of the product is the result of the integration of all the sensations perceived in the mouth and nose cavities, including aroma, taste and tactile sensations, rather than the summation of individual discrete sensations (Prescott, 2012; Small & Prescott, 2005). Hence, understanding flavour integration is essential to understand consumer's choices. There is a broad range of scientific bibliography dealing with interactions between sensory modalities such as aroma-aroma (de-la-Fuente-Blanco, Sáenz-Navajas, & Ferreira, 2016), aroma-taste (Gaudette, Delwiche, & Pickering, 2016) or aroma-tactile (Ferrer-Gallego, Hernandez-Hierro, Rivas-Gonzalo, & Escribano-Bailon, 2014) sensations in different matrices. Most reports work mainly with simple solutions including sweet or acidic solutions (Prescott, Johnstone, & Francis, 2004; Stevenson, Prescott, & Boakes, 1999) and only a few deal with more complex matrices such as cider (Symoneaux, Guichard, Le Quere, Baron, & Chollet, 2015), olive oil (Caporale, Policastro, & Monteleone, 2004), dairy products (Tomaschunas, Hinrichs, Köhn, & Busch-Stockfisch, 2012) or wine, which is a good example of a complex matrix (Arvisenet, Guichard, & Ballester, 2016; Saenz-Navajas et al., 2012; Saenz-Navajas, Campo, Fernandez-Zurbano, Valentin, & Ferreira, 2010).

In the case of wine, bitterness and astringency are amongst the least understood perceptions. This can be due to a number of different reasons related to their complexity and multimodality, probably also because they induce fatigue generating great individual variability among consumer perception, but maybe also because most often previous research has neglected interactions with other stimuli such as aroma or taste.

A few reports have looked at aroma–bitterness and aroma–astringency interactions in wine matrices. Saenz-Navajas et al. (2010) demonstrated that the addition of volatile fruity extracts from a Chardonnay white wine to the de-aromatized non-volatile extracts of white and red wines brought about decreases in astringency and bitterness and an increase in sweetness. In a subsequent work (Saenz-Navajas et al., 2012), these authors demonstrated that the astringency

of red wines was mainly driven by non-volatile compounds, while odour played a secondary role.

Based on those results, different predictive models of sensory properties from chemical composition were derived. Some of these models (Hernandez, 2016; Saenz-Navajas, Avizcuri, Ferreira, & Fernandez-Zurbano, 2014; Saenz-Navajas et al., 2010) suggest that odour could modulate bitterness as well as astringency in red wines. In these models 1) astringency is positively correlated with bitterness and spiciness, and negatively correlated with roasted aroma; 2) bitterness is positively correlated with animal, vegetal and herbal aromas as well as with astringency and sourness, while is negatively correlated to spicy and dried fruit aromas. Although all models were statistically significant, they have not been properly validated and cannot be considered conclusive. Given the interest of understanding astringency and bitterness, the present work will check, using different experiments, the possible involvement of taste-aroma interactions as suggested by the models.

On the other hand, sensory perception (Hughson & Boakes, 2001) and, in particular, sensory interactions (Arvisenet et al., 2016) have been demonstrated to be influenced by the level of expertise of the consumer, as a consequence of the higher cognitive and perceptual expertise developed by experts in comparison with novices or less experienced consumers (Hughson & Boakes, 2001, 2002). Differences in the level of expertise of consumers lead to different strategies in the processing of flavour information. Castriota-Scanderbeg et al. (2005) showed different patterns of brain activation for non-expert and expert consumers when drinking a wine. Non-experts present activation of brain areas related to emotions and seem to automatically attribute a global hedonic valence, positive or negative, to the perceived flavour, which is the result of taste-aroma integration generated from associative learning (Prescott, 2015). This is well in agreement with the fact that non-experts tend to classify flavours in terms of hedonic quality, contrary to wine experts (Ballester, Patris, Symoneaux, & Valentin, 2008), who are more prone to use analytical terms (Chollet & Valentin, 2000) and are able to label and/or classify perceptual qualities separately (Ballester et al., 2008). The capacity of wine experts to

evaluate each perception (e.g. aroma and taste) individually lead them to follow a more analytical strategy than non-experts. In line with these results, Arvisenet et al. (2016) confirmed the use of different strategies (synthetic or analytic) dependent on the level of expertise. They concluded that in situations in which analytical strategies were possible, the more experienced participants (trained and experts) were able to reduce the degree of interactions between aroma and sweetness, contrary to untrained participants, who remain using a synthetic strategy in which aroma-sweet interactions were very strong.

In this context, the present study aims to investigate specific sensory interactions in wine as a function of expertise. More specifically, the present work seeks to evaluate:

- i) The capacity of sour taste and aroma (herbal, vegetal, spicy, roasted, dried fruit and animal) to modulate bitter perception.
- ii) The capacity of sourness, bitterness and aroma to modulate astringent perception.
- iii) The effect of the level of expertise (novices, trained participants and wine experts) on the aforementioned sensory interactions.

2. Experimental

Briefly, the experimental protocol included the preparation of wine models by adding 6 different chemical aroma vectors, taste and astringent stimuli eliciting unique aroma, taste and astringent properties. Further, aroma-bitter, sour-bitter, aroma-astringency, sour-astringency, and bitter-astringency interactions were measured by preparing wines combining aroma vectors and sour stimuli with bitter or astringent stimuli. Modulation of bitter and astringent intensities was assessed by three panels with different levels of expertise.

2.1. Chemicals and standards

Solvents. Ethanol of LiChrosolv quality were purchased from Merck (Darmstadt, Germany).

Standards. The chemical standards were supplied by Sigma - Aldrich (Gillingham, U.K.) Alfa Aesar (Karlsruhe, Germany) and Firmenich (Geneva, Switzerland).

2.2. Preparation of wine models

2.2.1. Preparation of astringency fraction

The astringent fraction added to commercial wine to obtain wines high in astringency (As) was obtained as described in Sáenz-Navajas et al. (2017). Briefly, a 200-mL sample of astringent wine in 500-mL rounded flasks was evaporated in the rotary evaporator for 30 min at 28 °C to remove ethanol. The resulting liquid was freeze-dried in a LyoQuest -85 freeze dryer system from Telstar (Terrassa, Spain). The dearomatised syrup was then dissolved in 20 mL of a 12 % hydroalcoholic solution (v/v), and fractionated using a TSK Toyopearl gel packed in a HW-50F Millipore (Bedford, MA) Vantage L column (120 mm x 36 mm i.d.) at atmospheric pressure. The flow rate was regulated at 4 mL min⁻¹ using a peristaltic pump. The first fraction (F1), containing mainly low-molecular weight polyphenols and anthocyanin-derivative pigments, was eluted with 720 mL of ethanol/water/formic acid (55:44:1, v/v/v). A second fraction (F2), containing low-astringent proanthocyanidins (PAs), was recovered by elution with 80 mL of 100 % acetone. Both fractions, F1 and F2, were discarded. A third fraction (F3), containing astringent proanthocyanidins (PAs), was eluted with 160 mL of acetone/water (60/40, v/v). Acetone was removed on the rotary evaporator during 15 min and further freeze-dried. The total absence of solvents was assessed by headspace solid phase micro extraction (Carboxen/PDMS 75 µmat 30 °C × 10 min) and GCMSQP2010 system (Shimadzu, Tokyo, Japan) with an overall system detection limit of 1 ng/sample.

Fraction F3 was added to wines at twice the concentration found in original wine to assure that astringent intensity was easily perceived by all participants.

2.2.2. Wine samples

A neutral (in terms of aroma and in-mouth properties) commercial young red wine was spiked or not with quinine to obtain a wine high (Bi) or low in bitterness (B). High (As) and low (B) levels of astringent wine was obtained by spiking or not the commercial wine with the PA-fraction (F3). These wines were further spiked with one aroma vector or taste stimuli (tartaric

acid for sourness or quinine sulphate for bitterness). A total of six aromatic vectors (animal -An, spicy - Sp, herbal -He, vegetal -Ve, roasted -Ro, and dried fruit -Df) and two taste stimuli (sourness - So, and bitterness - Bi) were used in this study. Samples and the range of possible concentrations of the vectors are summarised in Table 1. These concentrations assured that they were easily recognised by participants.

Concentrated aroma vectors were prepared in ethanol and added to wine to achieve desired concentrations (Table 1). Quinine sulphate, tartaric acid and proanthocyanidin fractions were directly dissolved in the base wine. Final concentration of ethanol in wines was adjusted to 13 % (v/v).

Training samples. Different wines were prepared for the training sessions: 1) 27 samples of the same commercial red wine spiked with one of the vectors at three levels of concentration (Table 1); and 2) samples with different combinations of aroma-taste vectors.

Evaluation samples. A total of 38 wines were prepared for the evaluation tasks. The concentration of vectors employed to spike the wines is marked in bold in Table 1. Sixteen samples were prepared to evaluate the effect of aroma and sourness on bitter perception (experiment 1). Therefore, twelve 750-mL bottles of the commercial red wine were mixed and the total volume (9 L) was divided in two parts of 4.5 L. One part was spiked with 100 mg L⁻¹ of quinine (labelled as Bi-high level of bitterness) and the other part was not spiked (labelled as B-low level of bitterness). Each wine (Bi and B) was divided into nine 500-mL samples. Seven of these samples were spiked with one of the aroma (animal -An, spicy -Sp, vegetal -Ve, roasted -Ro, herbal -He, dried fruit -Df) or taste (sourness -So) vectors. The 8th and 9th samples were control samples (Bi-high bitterness- and B-low bitterness in duplicate) and were not spiked with any vector. A total of sixteen different wines were prepared: eight with high bitterness (Bi, AnBi, SpBi, VeBi, RoBi, HeBi, DfBi, SoBi) and eight with low bitterness (B, An, Sp, Ve, Ro, He, Df, So).

Eighteen different samples were prepared to evaluate the effect of aroma, sourness or bitterness (Bi) on astringent perception (experiment 2). The same procedure described for bitterness was followed to obtain samples with high (As, AnAs, SpAs, VeAs, RoAs, HeAs, DfAs, SoAs, BiAs) and low (B, An, Sp, Ve, Ro, He, Df, So, Bi) levels of astringency.

2.2.3. *Sensory properties of aroma vectors and in-mouth stimuli*

An independent and specific descriptive sensory analysis was conducted to confirm that the aroma vectors as well as taste (bitter and sour) and astringency stimuli generate specific aroma/flavour differences and did not change others.

Participants. A total of 17 participants (60% women, aged between 22 and 54 years; mean age = 39 years) carried out the sensory description. They are part of the sensory panel of the Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño (La Rioja, Spain). They had participated in at least 17 training sessions over a period of three months, in which they were presented with a wide range of taste, astringency and aroma standards. During a typical training session panellists became familiar with the specific vocabulary of an initial list of 110 aroma descriptors (Sáenz-Navajas, Fernandez-Zurbano, Martin-Lopez, & Ferreira, 2011) and with the rating of four attributes evaluated in-mouth: sweetness, acidity, bitterness and astringency. In each session reference standards were presented as described elsewhere (Sáenz-Navajas, Fernandez-Zurbano, et al., 2011) to illustrate the aroma and in-mouth attributes. They were qualified when they were able to correctly identify at least 85% of references.

Samples. Ten samples were described exclusively for aroma and four for in-mouth properties.

Wines described for aroma were: 1) B (base wine), 2) Bi (base wine spiked with quinine sulphate), 3) As (base wine spiked with the fraction of proanthocyanidins), 4) So (base wine spiked with sourness vector), 5) To (based wine spiked with the toasted vector), 6) An (based wine spiked with animal vector), 7) Ve (based wine spiked with the vegetal vector), 8) He (based wine spiked with the herbal vector), 9) Sp (based wine spiked with the spicy vector) and 10) Df (based wine spiked with the dried fruit vector). Wines described for in-mouth properties were: 1) B, 2) Bi, 3) As, and 4) So.

Procedure. Trained panellists described wines following the procedure described in Sáenz-Navajas, Fernandez-Zurbano, et al. (2011). Twenty-mL wine samples were presented in dark ISO-approved wine glasses labelled with 3-digit random codes and covered with plastic Petri dishes according to a random arrangement and monadic sequential presentation. Each panellist completed one session comprising two parts (ca. 30 and 10 min each part, respectively) for the analysis of 10 and 4 samples in part 1 and 2, respectively. In the first part, panellists were asked to smell each wine, describe their odour by choosing a maximum of five attributes from the list of 113 according check-all-that-apply (CATA) methodology. Then, in the second part of the session, they were asked to taste four samples and rate sourness, bitterness, and astringency on a structured 10-cm scale. Trained panellists rated samples using the sip and spit protocol described by Colonna, Adams, and Noble (2004). All wines were served at room temperature and were evaluated in individual booths according to a monadic sequential presentation. Panellists were not informed about the nature of the samples to be evaluated.

2.3. Study of sensory interactions by descriptive analysis

2.3.1. Participants.

Three different panels with different levels of wine expertise participated in the study:

- i. Novices were all regular consumers of red wine (drink red wine at least once every two weeks), who had never participated in sensory studies and had no experience in the wine industry.
- ii. Trained panellists included staff from Instituto de Ciencias de la Vid y del Vino (ICVV) and students from Universidad de la Rioja. They were recruited on the basis of their interest and their availability during 6 weeks (two 60-min sessions per week) and were trained specifically for this study to score aroma and taste in wines.
- iii. Experts were all established winemakers and wine professionals, who had run wine-tasting classes and had relevant professional experience in winemaking (Parr, Heatherbell, & White, 2002).

A total of forty-nine participants (18 novices, 15 trained panellists and 16 experts) carried out both experiments 1 and 2. In experiment 1, only the responses of thirty-nine participants (51% women, aged between 22 and 62 years; mean age = 37 years) were considered based on their sensitivity to bitter perception (see section 2.3.2.): 16 novices, 9 trained and 14 experts. In experiment 2, the responses of the 49 participants (59% women, aged between 22 and 62 years; mean age = 37 years) were considered. The screening and selection process for the three panels ensured similar age and gender distributions among novices, trained panellists and experts.

Participants were all recruited based on their interest and their availability. Panellists were not paid for their participation.

2.3.2. Procedure

Sensory analysis consisted of three steps: step 1) *training* (only for trained panel) or *familiarisation sessions* (only for novices and experts); step 2) *evaluation sessions* (for the three panels) and step 3) *evaluation of sensitivity to bitterness* (for the three panels).

Step 1: Training or familiarisation

Training sessions. Fifteen participants attended six 60-min descriptive sensory training sessions over a period of three weeks. Panellists worked in subgroups of 7-9 people and were trained to evaluate in-mouth properties (sourness, bitterness, astringency) and aroma vectors (animal, spicy, vegetal, herbal, roast and dried fruit) in wines. Different levels of reference-spiked wines representative of taste and aroma vectors (concentration ranges in Table 1) were presented to help with the identification and intensity evaluation of attributes. Participants that were able to correctly identify attributes and order their intensity carried out the evaluation sessions.

Familiarisation session. Experts and novices participated in a familiarisation session (ca. 15 min) prior to the evaluation sessions, in which standard references for aroma (animal, spicy, vegetal, herbal, roasted and dried fruit), taste (sourness and bitterness) and astringency were presented.

Step 2: Evaluation sessions

Experiment 1: Participants attended two formal sessions for the characterisation of 18 wine samples. In session 1 they evaluated B, Bi, An, AnBi, He, HeBi, Df, DfBi, So, SoBi and in session 2: B, Bi, Sp, SpBi, Ve, VeBi, Ro, RoBi. They were asked to rate eight attributes: bitter, sour, animal, herbal, dried fruit, spicy, vegetal and roasted on an anchored non-structured 10-cm scale (0 = “absence”; 5 = “average”; and 10 = “very high”).

Experiment 2: Participants attended two formal sessions for the characterisation of 20 wine samples. In the first session they evaluated the following wines: B, As, An, AnAs, So, SoAs, Am, AmAs, Df, DfAs and in session 2: B, As, Sp, SpAs, Ve, VeAs, Ro, RoAs, He, HeAs. They were asked to rate nine attributes: bitter, sour, astringency, animal, herbal, dried fruit, spicy, vegetal and roasted on an anchored non-structured 10-cm scale (0 = “absence”; 5 = “average”; and 10 = “very high”).

Samples were prepared three hours before sensory sessions and stored at 10 °C. They were served 15 minutes before the evaluation task. In both experiments, ten millilitres wine samples (20 ± 1 °C) were presented in dark ISO approved wineglasses labelled with 3-digit random codes, covered with plastic Petri dishes according to a random order and different for each judge. Panellists were asked to expectorate samples and rinse with fresh apple (experiment 1), apple pectin solution and mineral water (experiments 1 and 2). Samples were monadically presented and a 15 minutes' break was imposed in each session with the aim avoiding carry-over effects. This made that they evaluated a maximum of 5 samples in each part of the session. All wines were served at room temperature and evaluated in individual booths. Panellists were not informed about the nature of the samples. Responses were recorded on paper ballots.

Step 3: Evaluation of sensitivity to bitterness.

This task was carried out once experiment 2 was finished to avoid participants focusing on bitterness during evaluation sessions. Nine series of three samples containing two unspiked (control) samples (mineral water) and one sample spiked with increasing concentrations of quinine ($1.56\text{-}400$ mg L⁻¹ in 1:2 dilution series) were presented. In each series, panellists were

asked to taste the three samples from left to right and to indicate the sample which was different from the other three. In all tests, 10 mL of samples (20 ± 1 °C) were presented in plastic cups labelled with 3-digit random codes. The presentation order of samples within each series was also randomised.

Only the data given by participants who correctly identified all spiked samples from series 2 (3.3 mg L^{-1} of quinine) up to series 9 (400 mg L^{-1}) were analysed. This assured that participants were able to detect bitterness in wines.

2.4. Data analysis

2.4.1. Sensory properties of aroma vectors and in-mouth stimuli

Aroma terms cited by at least 40% of participants in the ten wines (B, Bi, As, So, To, An, Ve, He, Sp, and Df). A contingency table, in which rows were the wines and columns were the terms, was submitted to correspondence analysis (CA).

Three two-way ANOVAs (judges as random and wines as fix factors) were calculated with sour, bitter and astringent intensity scored in the four wines (B, Bi, As, and So).

2.4.2. Study of sensory interactions by descriptive analysis

The effects of aroma and sourness on bitter intensity and of aroma, sourness and bitterness on astringent intensity were evaluated by three-way ANOVAs (participants as random factors and wine -W- and level of expertise -LE- as fixed factors) considering main effects (W and LE) and their interaction (W x LE). In each ANOVA, the spiked sample was compared to the control sample (B for low bitterness and astringency, Bi for high bitterness or As for high astringency) presented in the same session. For example, to evaluate the effect of Vegetal (Ve) aroma on the perception of bitterness in wines with low levels of bitterness (B), a three-way ANOVA was calculated with the scores of bitterness for samples Ve (wine with no addition of quinine or low level of bitterness and spiked with vegetal aroma vector) and its corresponding control sample (B: wine with no addition of quinine and unspiked with any vector).

3. Results

3.1. Sensory properties of aroma vectors and in-mouth stimuli

Figure 1 shows that the addition of tartaric acid, quinine sulphate or the PA fraction do not generate any important aroma change in wines, as they are plotted close to the base wine in the CA map. Besides, wine samples spiked with aroma vectors present distinctive aroma properties. Thus, the wines spiked with: roasted vector (Ro) is described with terms such as: toasted bread, caramel and toffee; animal vector (An) with animal and leather; vegetal vector (Ve) with green pepper, herbal vector (He) with herbal and tomato leaf; spicy vector with spices; and dried fruit vector (Df) with dried fruit, prune, caramel and toffee.

Concerning in-mouth attributes, Figure 2 shows that samples spiked with tartaric acid, quinine sulphate and the PA fraction (So, Bi and As, respectively) generate unique in-mouth properties corresponding to sourness, bitterness and astringency, respectively.

3.2. Study of sensory interactions by descriptive analysis

The ANOVAs showed no significant effect of any aroma vector (*vegetal, herbal, spicy, roasted, animal, dried fruit*) or tastes (*sour or bitter*) on astringency scores, regardless of the level of expertise of participants (data not shown).

Table 2 shows the significant effects of the addition of aroma vectors or taste stimuli on wines with low (B) or high (Bi) bitterness. It can be observed that the addition of the *animal* aroma vector to either B or Bi generates a significant effect on bitter scores. The significant effect of the interaction (level of expertise x wine) indicates that such effect depends on the group of participants evaluating wines. Figures 3 and 4 show that *animal* aroma generates a significant increase in bitter intensity but only for the group of less experienced consumers (novices), being not significant for more experienced participants (trained and experts).

A second significant effect is observed for sour stimuli (Table 2) but only in the case of wines with high bitterness (Bi), regardless the level of expertise of participants. Figure 5 shows that

the addition of the tartaric acid (sour stimuli) to bitter wines generates a decrease in bitterness for the three groups of participants.

5. Discussion

In the present work, a sensory experiment was designed in order to validate predictive models obtained in previous works (Hernandez, 2016; Saenz-Navajas et al., 2014; Saenz-Navajas et al., 2010), which suggested that bitterness and astringency could be modified by taste-taste (bitterness- sourness), taste - aroma (animal, spicy, vegetal, herbal, roasted or dried fruit with bitterness), taste - astringency (astringency – sourness or astringency – bitterness) or astringency - aroma interactions (animal, spicy, vegetal, herbal, roasted or dried fruit with astringency). Under the hypothesis that these sensory interactions could be modulated by the level of expertise of consumers, interactions perceived by novices, trained panellists and experts were investigated.

Results, however, revealed the existence of a quite limited number of sensory interactions affecting exclusively bitterness (bitterness-sourness and bitterness-animal), while confirmed that in the red wine context, astringency is driven almost exclusively by polyphenols and that it is not influenced by taste or aroma interactions, as previous results had suggested. Even if we had expected to observe more interactions, results are highly relevant, since to the best of our knowledge these are the first reported cases in complex matrices of suppression of bitterness by sour taste, regardless the level of expertise of participants, and of enhancement of bitterness by animal aroma, which in this case is strongly related to the consumer expertise.

Aroma-taste or taste-taste interactions are reported to be the result of three types of interactions: physicochemical interactions in the product itself, interactions at the receptor level or cognitive interactions (Small & John Prescott, 2005). Although physicochemical interactions have not been directly checked in the present work, they do not seem to be majorly responsible for the effects observed. This would be especially not likely for bitter-animal interactions: first, because such effect was observed exclusively for novices and not for the rest of participants; second, because this interaction occurs for both levels of bitterness: high (addition of quinine sulphate)

and low (no addition of sulphate quinine). The absence of quinine sulphate in wines with low bitter levels (not added in B and not naturally present in wine) implies that the physicochemical interaction of quinine sulphate with the animal aroma vector (composed by 4-ethyl phenol and isovaleric acid) is simply impossible.

Similarly, interactions at receptor (peripheral suppression) or cognitive levels (central suppression) could explain bitter-sour interactions observed for all participants, regardless their level of expertise. Some works have indicated that certain salts, especially sodium salts, can suppress the bitterness elicited by quinine (Keast, Breslin, & Beauchamp, 2001; Kroeze & Bartoshuk, 1985) due to affinity of salts and quinine for common receptor sites. This could be the case of tartaric acid and quinine sulphate used in the present study, because at wine pH tartaric acid exists in two main forms: fully protonated and associated conjugate base. This last form would be as sodium or potassium salt, which could compete with quinine receptors, generating bitter suppression. However, suppression at central level cannot be discarded as a function of either attentional deviation (inability of consumers to direct their attention to a specific attribute such as bitter when other sensory property such as sourness is perceived at higher intensity) or confusion of sour and bitter tastes (McAuliffe & Meiselman, 1974; O'Mahony, Goldenberg, Stedmon, & Alford, 1979) at cognitive levels. The present experiment cannot unequivocally explain the kind of interactions occurring between bitter and sour taste, even if it seems plausible to suggest that it is the result of interactions at both receptor and cognitive level as it was proposed for bitter suppression by other salts (Kroeze & Bartoshuk, 1985).

Concerning the synergism observed for animal aroma and bitter taste exclusively for less experienced consumers (novices), it seems to be the result of a cognitive interaction caused by their congruent negative hedonic tone. This idea is further supported by the fact that novices tend to find more difficulties in separating odour and taste in complex mixtures, and tend to classify flavours based on their hedonic quality (Ballester et al., 2008; Royet, Plailly, Saive, Veyrac, & Delon-Martin, 2013), contrary to more experienced consumers that are more prone to

discern perceptual qualities (e.g. aroma and taste). Thus, novices seem to adopt a synthetic perceptual strategy or integral holistic perception. They tend to value the different perceptions (aroma, taste and tactile sensations) as a whole, while trained and expert participants tend to act in a more analytical way.

Concerning aroma-astringency or taste-astringency interactions, no significant effect was observed in the present work. This result is in accordance with a previous work dealing with reconstituted wines formed by volatile and non-volatile fraction of highly astringent red wines (Saenz-Navajas et al., 2012). The absence of taste-astringency or aroma astringency interactions confirms that astringency, elicited by proanthocyanidins, was the main responsible for this tactile sensation perceived in wines, being the role of aroma, sourness and bitterness just secondary in its modulation. This is in apparent contradiction with other works, which demonstrated the capacity of aroma to modulate astringency (Ferrer-Gallego et al., 2014; Saenz-Navajas et al., 2010), but discrepancies could be the simple result of differences in the composition of wine matrices and in the experimental scope. The interactions observed by Ferrer-Gallego et al. (2014) were observed in a pH adjusted-aqueous matrix containing just one or a simple mixture of phenolic compounds and a single aroma compound. In the work by Saenz-Navajas et al. (2012) the effect on red wine astringency was just observed when the fruity aroma fraction extracted from a Chardonnay white wine was added to the red wine non-volatile matrixes. Therefore, results obtained in the present work confirm that aroma- and taste-astringency interactions are quantitatively not relevant in determining the astringency levels of normal red wines.

The present work increases the knowledge about sensory interactions in complex matrices and is valuable for researchers in the sensory field as well as for the food industry. Further research with wines with different levels of complexity in terms of both volatile and non-volatile composition should be carried out. An apparent potential limitation of the current study could be the use of quinine sulphate to induce different levels of bitterness, which is not naturally present

in wines. However, the lack of knowledge about compounds eliciting exclusively bitterness in red wines makes it essential to use this compound for understanding bitter interactions in wine.

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Figure captions

Figure 1. Projection of the aroma terms (cited by $\geq 40\%$ of panellists) and wine models on the first two factors of the correspondence analysis space based on orthonasal aroma description carried out by a trained panel.

Figure 2. Comparison of the mean sensory ratings of a) sourness, b) bitterness and c) astringency of B (base wine), Bi (base wine spiked with quinine sulphate), As (base wine spiked with the fraction of proanthocyanidins) and So (base wine spiked with sourness vector) wine samples. Different letters mean significant differences ($P < 0.05$) among wines. Error bars are mean standard errors.

Figure 3. Comparison of the mean sensory ratings (for novices, trained and experts) of wines with low bitter intensity with no addition of aroma vectors or taste stimuli (B) and with the addition of the animal aroma vector (An). Different letters within novices, trained or expert groups mean significant differences ($P < 0.05$) for bitter score between B and An; ns: no significant differences. Error bars are mean standard errors.

Figure 4. Comparison of the mean sensory ratings (for novices, trained and experts) of wines with high bitter intensity with no addition of aroma vectors or taste stimuli (Bi) and with the addition of the animal aroma vector (AnBi). Different letters within novices, trained or expert groups mean significant differences ($P < 0.05$) for bitter score between Bi and BiAn; ns: no significant differences. Error bars are mean standard errors.

Figure 5. Comparison of the mean sensory ratings (for novices, trained and experts) of wines with high bitter intensity with no addition of aroma vectors or taste stimuli (Bi) and with the addition of the sour stimuli (SoBi). Different letters within novices, trained or expert groups mean significant differences ($P < 0.05$) for bitter score between Bi and BiSo. Error bars are mean standard errors.

Table 1. Chemical composition of the wines spiked with different levels of aroma vectors or taste stimuli subjected to sensory analysis

		Concentration in wine ($\mu\text{g L}^{-1}$)		
		Level 1	Level 2	Level 3
ANIMAL (An)	4-Ethylphenol	42	125	250
	4-Ethylguaiacol	5.0	15	30
	3-Methylbutyric acid	0.21	0.63	1.3
SPICY (Sp)	2-Methoxyphenol	60	180	540
	Eugenol	600	1801	5403
	Ethyl vanillate	1500	4500	13500
HERBAL (He)	cis-3-Hexenal	90	270	810
VEGETAL (Ve)	2-Isobutyl-3-methoxypyrazine	0.023	0.068	0.20
ROASTY (Ro)	4-Hydroxy-2,5-dimethyl-3(2H)-Furanone	300	900	2700
	Furfuryl mercaptan	0.12	0.36	1.1
	L-Cysteine	600000	600000	600000
DRIED FRUIT (Df)	Isovaleraldehyde	470	940	1880
	Phenylacetaldehyde	340	680	1360
	Methional	50	100	200
	Furfural	22000	44000	88000
	β -Damascenone	50	100	200
	Ethyl Cyclohexanoate	0.13	0.25	0.50
	Ethyl hydrocinnamate	2.0	3.9	7.8
	2-Methoxyphenol	14	27	54
	Whisky lactone	123	245	490
	4,5-Dimethyl-3-hydroxy-2,5-Dihydrofuran-2-one	880	1760	3520
	2,3-Butanedione	6250	12500	25000
Linalool	29	57	114	
BITTERNESS (Bi)	Quinine hemisulphate monohydrate	50000	100000	200000
SOURNESS (So)	Tartaric acid	8.1×10^6	11.4×10^6	15.9×10^6
ASTRINGENCY (As)*	F3	0.5 fraction	1 fraction	2 fractions

The concentration of vectors employed to spike the wines for the evaluation task is marked in bold.

* One fraction of F3 corresponds to the content of proanthocyanidins originally present in the wine from which

it was obtained.

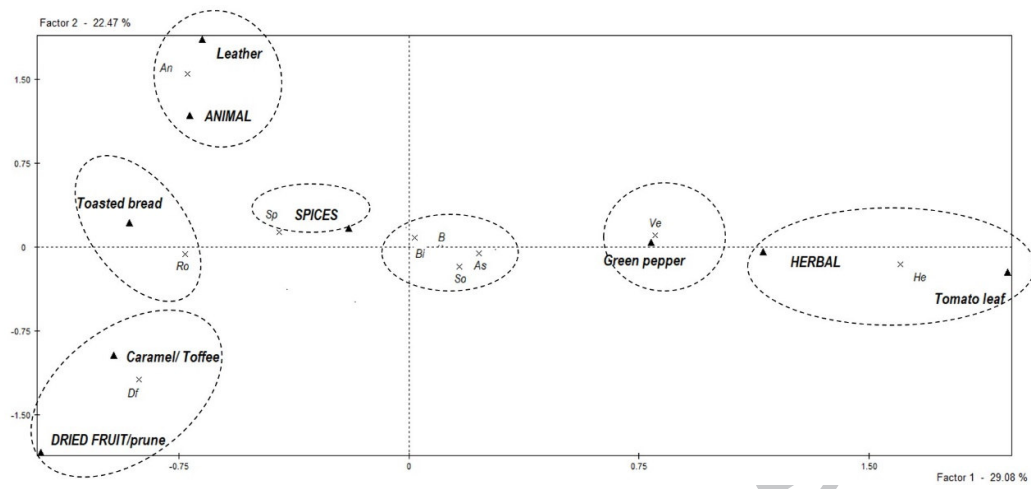
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Table 2. Results of the three-way ANOVA (participants as random factor and level of expertise and wine as fix factors) calculated to evaluate the effect of sour taste or of 6 aroma vectors on the intensity of bitterness perceived in wines high (Bi) and low (B) in bitterness. Only significant effects ($P < 0.05$) are included in the table; ns: non-significant

Factors	level of expertise		wine		level of expertise x wine		
	Vectors	F	P	F	P	F	P
<i>Sourness (Bi)</i>		0.21	ns	14.0	<0.001	0.315	ns
<i>Animal (Bi)</i>		1.85	ns	4.20	<0.05	3.45	<0.05
<i>Animal (B)</i>		0.76	ns	4.25	<0.05	4.36	<0.05

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Figure 1



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Figure 2

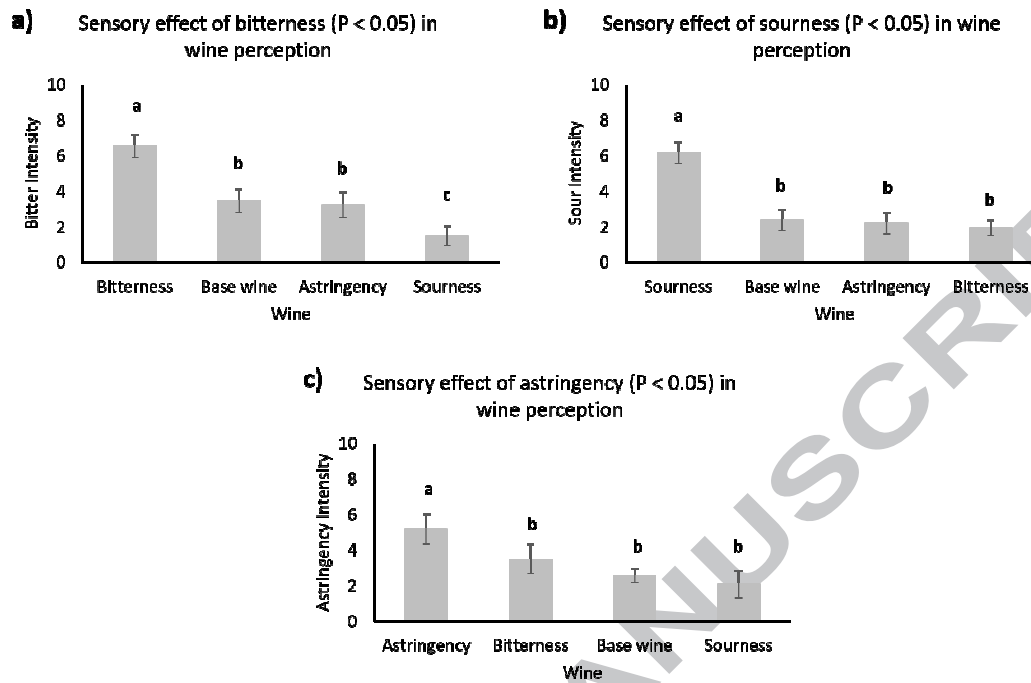
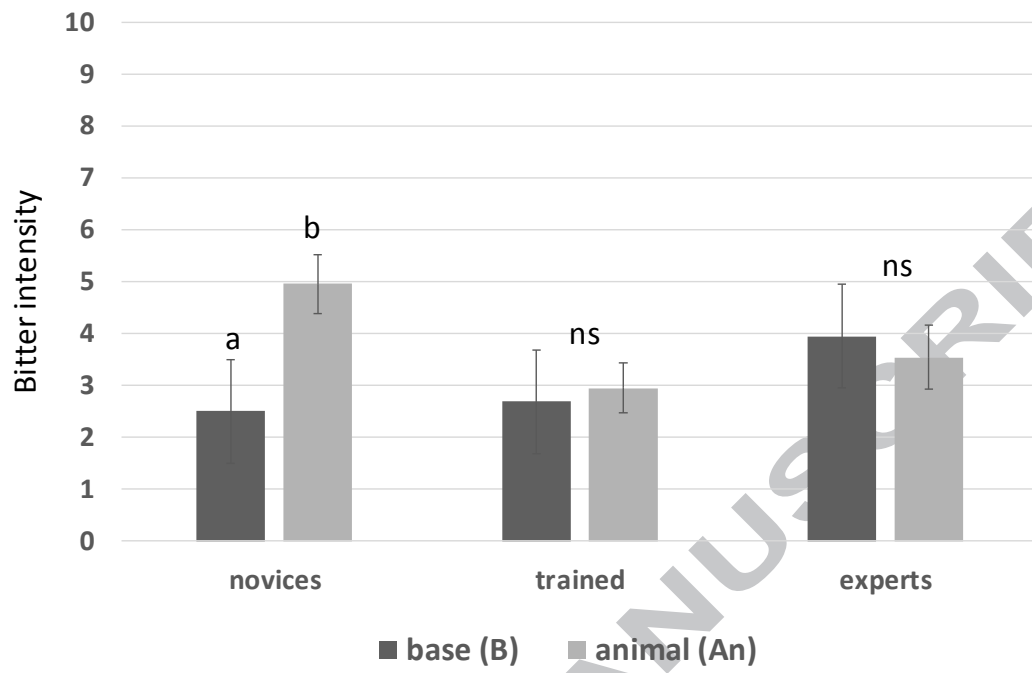


Figure 3



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Figure 4

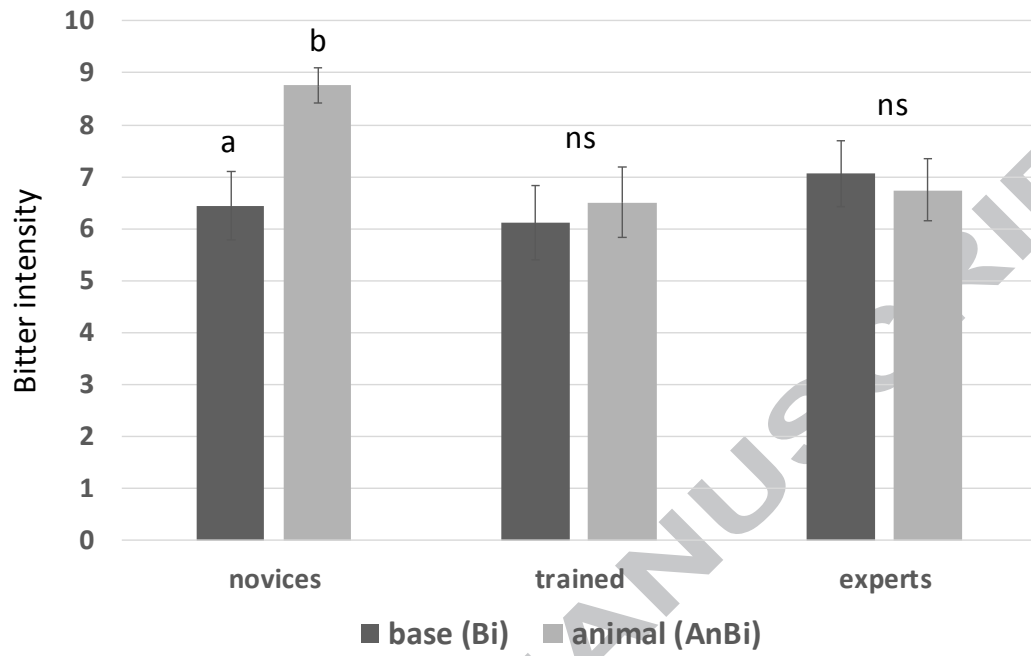
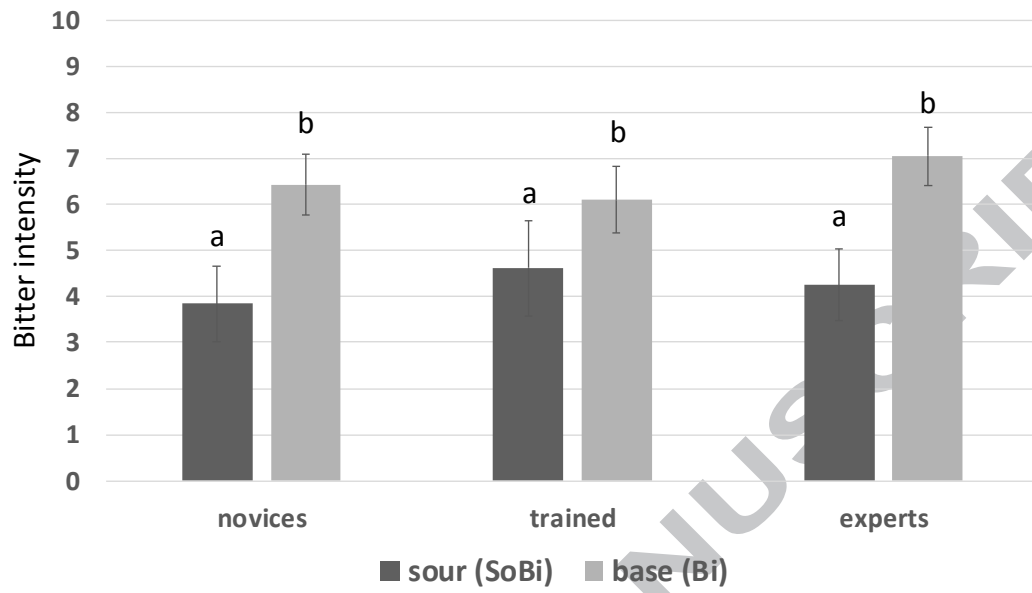


Figure 5



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HIGHLIGHTS

- Effect of sensory interactions on bitterness and astringency on red wine is studied
- The role of the level of consumer expertise on sensory interactions is investigated
- Bitter-animal aroma interactions are attributed to hedonic synergism for novices
- Decrease in bitterness due to sour taste is observed regardless consumer expertise
- Bitter-sour interactions could be explained at cognitive and receptor level

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