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Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions

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Abstract

Background and Aims: This study aimed to identify the minimal concentration of higher alcohols (HA) (isoamyl alcohol/isobutanol) that causes readily discernible changes in aroma properties and preferences according to a group of Spanish wine experts.

Methods and Results: A group of 16 Spanish wine experts carried out several series of two-alternative forced-choice sensory tests on three wine models (WMs) with specific aroma properties (fruity, woody and animal). Control WMs were contrasted to samples containing an increasing concentration of HA. Levels of 299 and 288 mg/L of HA were able to confer their spirit-like characteristic aroma to fruity and woody-like WMs and to suppress red fruit (299 mg/L) and woody (281 mg/L) attributes, respectively. A concentration of 365 and 375 mg/L of HA increased the animal aroma generated by ethyl phenols and the spirit-like nuances of HA, respectively. The presence of HA at a concentration ranging from 284 to 358 mg/L caused a decrease in the preference. In woody WMs, highly sensitive participants were able to detect and perceive as negative an addition of HA as little as 17 and 22 mg/L.

Conclusions: This is the first work estimating the concentration of HA able to affect aroma properties and hedonic character of red wines.

Significance of the Study: These results can assist while making decisions mainly during the fermentation stage to control HA production.

Keywords: *2-AFC, aroma, higher alcohols, model wine, preference*

Introduction

The aroma of complex mixtures is the result of interactions between the different volatile components taking part in the aroma formulation. Understanding how the single stimuli are processed in the brain is a challenge for flavour chemists and for the flavour industry. The basic stimuli of the different aroma components interact through diverse levels of additive, competitive or creative effects [for a complete review, see Ferreira (2012a,b)] to form the final aroma elicited by the product (Ferreira et al. 2016).

The volatile fraction of wine is a good example of a complex matrix. This makes it an interesting and widely studied product in terms of aroma formation. Most studies have focused on binary interactions between relatively simple stimuli present in wine, such as those occurring between whisky lactone (woody aroma) and ethyl butyrate (fruity aroma) (Atanasova et al. 2005a,b) or among fruity esters and acetates involved in the perception of wine fruitiness (Pineau et al. 2009, Lytra et al. 2012, 2013, Carmeleyre et al. 2015). Being a necessary starting point, those investigations ignored the effects of the numerous other stimuli present in the volatile fraction of wines. This can be particularly relevant because the mixture of major compounds present in wine have been described as displaying aroma buffering properties (Escudero et al. 2004). One such mixture includes higher alcohols (HA) and their acetates, volatile fatty acids and their ethyl esters, branched fatty acids and their ethyl esters, acetoin, diacetyl and acetaldehyde and, of course, ethanol. This complex group of molecules has a characteristic vinous or fermented aroma, in which the specific aroma nuances of single compounds cannot be distinguished, even if they are at a concentration well above their sensory threshold. This, together with the fact that the omission or addition of many single aroma compounds hardly affects the sensory properties of the mixture, is what led researchers to state that such a mixture has aroma buffering properties (Guth 1997, Ferreira et al. 2002,). The importance of HA such as isobutanol and isoamyl alcohol in those buffering characteristics has been recently confirmed (de-la-Fuente-Blanco et al. 2016), while the contribution of other HAs such as methionol and β-phenylethanol has been shown to be negligible. That research observed how, in poor aromatic contexts, which only present the vinous aroma, elicited by base compounds present in all wines, the sensory effects of the pair isobutanol and isoamyl alcohol were not significant. However, in contexts in which specific aroma notes were clearly perceived (i.e. woody, fruity, animal and humidity aroma nuances), their sensory effects became clearly noticeable. This pair of HA was found to suppress odour notes with either positive (fruity and woody) or negative hedonic tone (humidity), while specifically enhancing the animal aroma. In contrast, the suppression effect is a general trend observed in mixtures of dissimilar odorants (Kurtz et al. 2009) and has been suggested to take place either at the central system (Boyle et al. 2009) or at the periphery of the olfactory system (Chaput et al. 2012, Takeuchi et al. 2013). Then again, the integration of responses observed for the animal nuance of ethyl phenols and the spirit-like aroma of HA could be the result of either perceptual interactions (Laing et al. 1994) or synergistic mechanisms (Gottfried 2010).

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The levels of isobutanol and isoamyl alcohol in wines are mainly dependent on the content of amino acids in must, valine and leucine, and on yeast metabolism (Amerine et al. 1980, Giudici et al. 1990). The use of different strains, such as non-*Saccharomyces* employed for the reduction of alcohol concentration in wine, has been reported to induce the production of significant levels of HA (Contreras et al. 2014).

An old claim made in a proceedings paper by Rapp and Versini (1991) suggests that a total concentration of HA below 300 mg/L could contribute positively to the aromatic profile of wines, while above 400 mg/L would exert a negative effect. This claim, however, is not backed by documented research. Even if these compounds are usually considered important factors influencing wine flavour, there is a lack of empirical evidence, illustrating how the level of HA causes a readily discernible change in wine sensory properties or wine preference. This issue can be studied by determining the two-alternative forced-choice (2-AFC) sensory tests using constant stimuli as the control sample (Lawless and Heymann 2010). By means of these tests, the amount of change in a physical stimulus (concentration of target odourants) necessary to produce a perceptible sensory difference can be calculated. This methodology has been used in different complex matrices, such as model beverages (Camacho et al. 2015), pungent solutions (Orellana-Escobedo et al. 2012) or enriched foodstuff for the elderly (Kremer et al. 2007). Little research has dealt with wine components studied in simple solutions (Le Berre et al. 2008). There is also an absence of studies in more complex solutions similar to wine. Most probably because the construction of complex models displaying similar aroma properties to real wine samples has been achieved only recently (Ferreira et al. 2016, de-la-Fuente-Blanco et al. 2016).

In this context, the present work aimed to determine the minimal concentration of HA (isoamyl alcohol/isobutanol) able to cause readily discernible changes in aroma properties and preferences on three wine models (WMs) (fruity, woody and animal-like) according to a group of 16 Spanish wine experts. The rationale behind the choice of wine experts as participants was twofold. First, their opinion exerts an important influence on the wine market, and they tend to generate quality and/or preference prototypes among wine consumers. Second, most winemakers base their decisions during the winemaking processes on the information provided by other wine experts. This occurs mainly because they appear to have common wine prototypes memorised, especially within the same production area (Torri et al. 2013, Hopfer and Heymann 2014). This appears to be the contrary in less experienced consumers (Urdapilleta et al. 2011). Thus, specific data related to the minimal concentration of HA, which causes readily discernible changes and to their influence on preference based on expert's judgements, could be valuable information for winemakers when making decisions during winemaking.

Materials and methods

Chemicals and standards

Solvents. Ethanol of LiChrosolv quality, diethyl ether and *n*-pentane were purchased from Merck (Darmstadt, Germany); isooctane was supplied by Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

Standards. Chemical standards were supplied by Sigma-Aldrich (Gillingham, England) and Firmenich (Geneva, Switzerland).

Reagents. Tartaric acid and NaOH were supplied by Panreac.

Preparation of wine models

Isolation and purification of alcohols. Isobutanol and isoamyl alcohol standards were isolated and purified as described by de-la-Fuente-Blanco et al. (2016). In short, two steps were necessary. The first involved a reactive solid–liquid extraction with a carbonyl scavenger fixed to a solid support. Each solution of commercial standard was mixed with 2 g/10 mL of sulfonyl hydrazine polymer-bound (Sigma-Aldrich); the mixture was shaken for 4 h and filtered through a 0.45-μm nylon syringe filter. The percolate was analysed by GC with an ion trap MS detector to confirm the absence of aldehydes. The second step involved the confirmation of the sensory purity of standards by GC-olfactometry (O). For this, 1 μL of each standard diluted at 1% in pure dichloromethane was injected in splitless mode (60 s splitless time) in a Trace GC gas chromatograph (ThermoQuest, Milan, Italy) with a flame ionisation detector and a sniffing port ODO-I from SGE (Ringwood, Vic., Australia). The capillary column used was a DB-WAX (polyethylene glycol) supplied by J&W (Folsom, CA, USA), 30 m × 0.32 mm i.d. × 0.5 mm film thickness, and a deactivated precolumn (3 m × 0.32 mm i.d.) from Supelco (Bellefonte, PA, USA). Hydrogen was the carrier gas at a constant flow rate of 3.5 mL/min. Injector and detector temperature was 250°C. The temperature program used for standard analysis was 40°C for 5 min, increased by 4°C/min to 100°C and then 6°C/min to 220°C, maintaining this temperature for 10 min. A panel of six trained judges (two men and four women, ranging from 26 to 29 years of age, average age 26) sniffed the extracts. Participants were asked to provide a descriptor to characterise the eluted odour and to rate its intensity using a seven-point scale (0, no odour; 1, weak odour, low intensity; 2, clear perception of odour, strong intensity; 3, extremely strong intensity of odour; intermediate values of 0.5, 1.5 and 2.5 were allowed). Data were processed as proportion of modified frequency (%MF), which was calculated according to Dravnieks (1985): $MF(\%) = \sqrt{F\,(\%)\,x\,I\,(\%)}$, F (%) being the detection frequency of an odourant expressed as proportion of the total number of judges (n = 6), and I (%) the average intensity expressed as proportion of the maximum intensity.

Wine model preparation. Wine models were prepared by mixing a pool of common wine compounds (volatiles and non-volatiles) with one of the following aroma vectors: fruity (F), woody (W) or animal (A) for obtaining three different Control WMs (Table 1). Control samples contained 110 and 20 mg/L of the targeted HA: isoamyl alcohol and isobutanol, respectively. These values are the lowest concentration found in commercial red wines (San Juan et al. 2012). For evaluating the minimal concentration of isoamyl alcohol/isobutanol causing discernible changes on the orthonasal aroma properties of the models, each of the three Control WMs (F0) was spiked with eight different levels (F1–F8) of both HA (Table 2) covering the concentration ranges found in real red wines (San Juan et al. 2012). Final ethanol concentration was adjusted to 12% (*v*/v) and pH to 3.5. Isoamyl alcohol and isobutanol were added together, as a vector, at a 5:1 ratio in view of quantitative data reported for commercial wines inoculated with *Saccharomyces* yeasts (Yoshizaw 1966, Amerine and Joslyn 1970, Lee and Cooley 1981).

Table 1. Aroma vectors and composition of wine models used in the study.

		Compounds	Concentration (mg/L)
Pool of compounds conforming the common aroma base	Volatile	β- Phenylethanol	15
		Acetic acid	150
		Ethyl acetate	50
		hexanoic acid	2.0
		3-Methylbutanoic acid	0.30
		2,3-Butanedione	0.40
		Ethyl hexanoate	1.0
		Isoamyl acetate	1.0
		Ethyl 2-methylbutanoate	0.12
		Ethyl vanillate	0.25
		Vanillin	0.070
		γ-Nonalactone	0.020
		Guaiacol	0.010
		β-Damascenone	0.0040
		β-Ionone	0.00030
	Non-volatile	Tartaric acid	5000
		Glycerol	10000
		Tannic acid	50
		Quinine	7.0
		Arabic gum	75
Vector			
	Fruity (F)	2,3-Butanedione	14
		Isoamyl acetate	5.5
		Ethyl acetate	50
		Ethyl cinnamate	0.12
		β-Damascenone	0.0030
	Woody (W)	Whisky lactone	0.30
		Vanillin	0.10
		Eugenol	0.015
		Guaiacol	0.015
		4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	0.10
	Animal (A)	4-Ethylphenol	100
		4-Ethylguaiacol	13

Table 2. Concentration of isoamyl alcohol and isobutanol in the samples used to determine the minimal concentration able to induce changes in aroma perception and preferences.

Codification	Isoamyl alcohol (mg/L)	Isobutanol (mg/L)
F0	110	20
F1	132	24
F2	158	29
F3	190	35
F4	228	41
F5	274	50
F6	328	60
F7	394	72
F8	473	86

Sensory analysis

Participants. Sixteen participants (four men and 12 women, ranging from 24 to 60 years of age, average age 33 years), belonging to the category of established winemakers, wine-science researchers and teaching staff regularly involved in winemaking and/or wine evaluation, undertook the task involving fruity and animal WMs. For the woody WM, because of availability reasons, 14 out of 16 could complete all tasks. They were all wine experts according to Ballester et al. (2008), Parr et al. (2002) and Melcher and Schooler (1996).

Procedure. The sensory task comprised three parts. The first part consisted of two 15-min sessions (held on different days). It aimed at (i) familiarising participants with the aroma vectors and (ii) qualifying participants. In the first session, participants familiarised with the spirit-like aroma of the isoamyl alcohol/isobutanol vector and with the other three aroma vectors differing among WMs (Table 1): red fruity, woody and animal vectors. For that, they were presented with the four pure aroma vectors individually at high concentration. In the second session, four series, each containing one vector at three different concentration levels (level 0, absence; level 1, low concentration and level 2, high concentration), were presented (Table 3). All participants qualified as they were able to correctly identify the isolated vectors and rank them according to their intensity.

In the second part, participants attended six sessions (held on different days) of about 15 min each (one session in duplicate for each one of the three WMs). During each session, participants carried out one series of nine comparison tests according to 2-AFC staircase technique for calculating the minimal concentration of HA inducing sensory differences. Each comparison test consisted of two wines: Control (F0) and

Table 3. Composition of the vectors presented in the familiarisation phase.

Vector	Compounds of the vector	Level 1, low concentration (mg/L)	Level 2, high concentration (mg/L)
Fruity (F)	2,3-Butanedione	0.4	14
	Isoamyl acetate	1	5.5
	Ethyl acetate	50	50
	Ethyl cinnamate	–	0.12
	β-Damascenone	0.04	0.0030
Woody (W)	Whisky lactone	0.15	0.30
	Vanilla	0.050	0.10
	Eugenol	0.0075	0.015
	Guaiacol	0.0075	0.015
	4-Hydroxy-2, 5-dimethyl-3 (2*H*)-furanone	0.050	0.10
Animal (A)	4-Ethylphenol	35	100
	4-Ethylguaiacol	4.5	13

spiked sample (from F1 to F8), which differed exclusively in the concentration of the isoamyl alcohol/isobutanol vector. As an aid in the recognition of attributes, at the beginning of each session and before carrying out the 2-AFC tasks, participants were forced to smell the pure aroma vector of HA and the vector predominating in each WM (red fruity, woody or animal vectors). Then, they were asked to orthonasally evaluate each pair of samples (Control and spiked) and indicate which sample of the pair was more intense in two attributes: spirit-like aroma and in the predominant attribute corresponding to each WM (red fruity, woody or animal).

Similar to second part, the third part consisted of six sessions (held in different days) of about 15 min (one session in duplicate for each WM: red fruity, woody and animal). Participants were presented with the same samples and tests as in the second part. For each pair of samples (Control and spiked), they had to note which sample they preferred.

In second and third parts, presentation order of the Control wine within each pair (left or right) was randomised and different for each participant. Spiked samples were presented in ascending order of concentration, from F1 to F8, and participants received a new pair of samples every 2 min. Water was available as a rinsing agent. In all tests, 15 mL of samples (20 ± 1°C) were presented in dark ISO-approved wine glasses labelled with three-digit random codes and covered with plastic Petri dishes. The samples were served 5 min before the beginning of the sensory task. All the responses were collected on paper ballots.

Data analysis

For each concentration of the HA vector (F1–F8), the number of times that the Control was considered more intense than (second part of sensory analysis) or preferred over (third part of sensory analysis) spiked sample was counted. Duplicate 2-AFC tests were considered independently. Both types of responses (more intense than the Control or preferred over Control) were expressed as a proportion and plotted against the total concentration of HA (isoamyl alcohol + isobutanol). Criterion for significance ($P < 0.05$) was based on the binomial distribution table for one-tailed paired comparison test. The concentration of HA causing readily discernible changes in aroma properties was calculated by extrapolating in the graph (percentage of responses selecting Control as more intense vs total concentration of HA) the percentage of responses that reached the criterion of significance. The minimal concentration of HA inducing differences in preference was calculated following the same criterion.

Results and discussion

Standard purity

The purity of standards employed in the construction of WMs was evaluated by GC-O and by target quantitative analysis of the expected impurities. Both strategies showed that in the case of HA, even recently opened chemical standards of the maximum available chemical purity, they contained a significant level of their corresponding powerful smelling aldehydes. This result highlights the importance of evaluating olfactory impurities present in standards that can ruin the experiment and bias the conclusions. The strategy proposed in the experimental section and described elsewhere (de-la-Fuente-Blanco et al. 2016) was employed to remove impurities.

Sensory analysis

In this paper, the minimal amount of stimulus required to either perceive a difference or generate a change in preferences according to a group of Spanish wine experts was determined. The strategy followed consisted of a series of 2-AFC tests in which participants were given a Control sample, displaying a specific odour nuance (fruity, woody or animal-like), and a second one containing the same odour nuance yet spiked with an increasing amount of HA. They had to choose which one of the two samples was more intense in the target nuance and in the spirit-like aroma elicited by HA. With the same samples but in independent tasks, participants had to indicate which sample of the pair was preferred in terms of aroma. To ensure that participants were able to associate terms/descriptors to the specific odour nuances to be evaluated, they followed a familiarisation task with the four aroma nuances (fruity, woody, animal and spirit-like aromas) prior the 2-AFC tests. This step was found necessary because wine experts have shown to be better at recognising the wine-relevant smells than non-experienced participants. Their verbal skills (odour identification and naming consistency), however, have shown to be similar to those of novice judges (Parr et al. 2002, 2004).

Fruity wine model. Figures 1 and 2 illustrate results for the fruity model. Figure 1 shows the proportion of responses of participants selecting the Control WM (contains just 130 mg/L of HA) as more intense in red fruit and spirit-like attributes than fruity models containing an increasing concentration of HA (156–559 mg/L). The participant responses, $n = 31$, were derived from 16 participants in duplicate: one response is missing because one participant could not carry out the task in duplicate. Models containing at least 299 mg/L of HA were perceived significantly ($P < 0.05$) lower and higher in red fruit and spirit-like aromas, respectively, than the Control WM. The Control fruity model represented a wine with the minimal concentration of HA (130 mg/L) reported in real commercial red wines (San Juan et al. 2012). The minimal concentration which causes readily discernible sensory changes can be defined as the point that represents the quantity of additional stimulus necessary for perceiving a difference; thus, this minimal concentration for fruity models can be estimated as 169 mg/L. This decrease and increase of the fruity and spirit-like aromas, respectively, are well in line with results derived from descriptive tasks, in which the concentration of the isobutanol/isoamyl alcohol vector was increased from 130 to

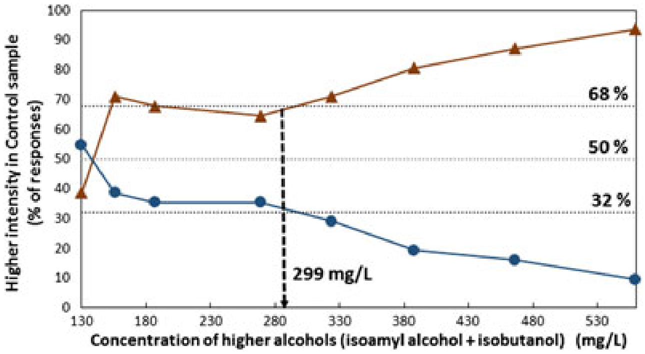

Figure 1. Proportion of responses (n = 31) selecting Control samples of the fruity wine model as more intense in red fruit (▲) and spirit-like (●) attributes than spiked samples. Lower and upper dotted lines (superior: 68% and inferior: 32%) indicate the 5% significance criterion for paired tests.

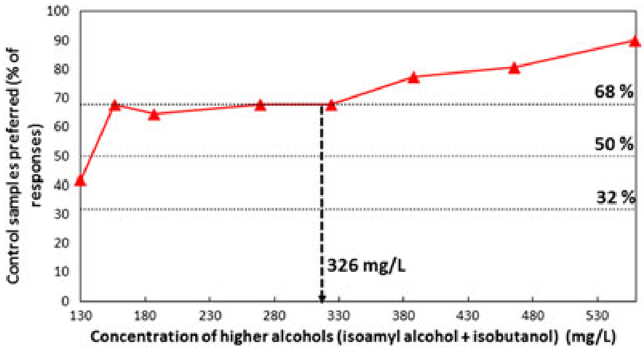

Figure 2. Proportion of responses (n = 31) selecting Control samples of the fruity wine model as preferred over spiked samples. Lower and upper dotted lines (superior: 68% and inferior: 32%) indicate the 5% significance criterion for paired tests.

430 mg/L (de-la-Fuente-Blanco et al. 2016). The result is also in close agreement with the numerical results recently presented by Carmeleyre et al. (2015). These authors observed by means of descriptive analysis that the addition of 131 mg/L of isobutanol and isoamyl alcohol (88 and 43 mg/L, respectively) to simple aroma mixtures of fruity esters and acetates generated a significant decrease in the intensity of fresh and jammy fruit attributes and increased the spirit-like aroma intensity of the mixture.

Independent series of tests were performed to evaluate aroma preferences for fruity WMs containing different levels of HA (Figure 2). Fruity aromas have been repeatedly reported to increase wine quality and/or preference based on the judgements of experts from different countries (Varela and Gambaro 2006, Lattey et al. 2010, Saenz-Navajas et al. 2013, Hopfer and Heymann 2014). Taking into account that liking has shown to be highly correated to quality perception for wine experts (Hopfer and Heymann 2014), it could be hypothesised that the presence of HA, which are able to mask the red fruit aroma, would induce a decrease in the preference of samples for this group of experts. Results of the present study indicate that the Control (containing 130 mg/L of HA) was significantly (P < 0.05) preferred over samples containing at least 326 mg/L of HA, which confirmed the initial hypothesis. Thus, the addition of at least 196 mg/L of HA to the Control fruity WM caused a decrease in preference. This threshold was close to, but slightly higher than (+30 mg/L) the minimal concentration inducing sensory changes (169 mg/L). Interestingly, this suggested that samples containing 299 mg/L of HA already displayed a slight spirit-like aroma conferred by these compounds; however, this concentration was not enough to generate a significant rejection of samples. This could support the idea that experts´ preferences would be more related to the aroma harmony reached by the mixture of different sensory attributes than by the presence of a predominant aroma note in wine (Sáenz-Navajas et al. 2012).

Woody wine models. Figure 3a shows the proportion of participant responses, which indicated woody WM to be higher in woody and spirit-like aromas than the same WM spiked with HA. The responses, n = 27, were derived from 14 participants in duplicate: one response is missing because one participant could not carry out the task in duplicate. Results showed that WMs containing a total of 150 mg/L of HA were perceived significantly lower in the woody attribute than the Control WM (130 mg/L of HA). This indicates that the addition of just 20 mg/L of HA (15% increment in the stimulus) caused a readily discernible sensory decrease of the woody nuance. Nevertheless, more than five times of HA (115 mg/L) had to be added (final concentration in spiked samples of 245 mg/L) to observe a significant sensory increase in the spirit-like aroma

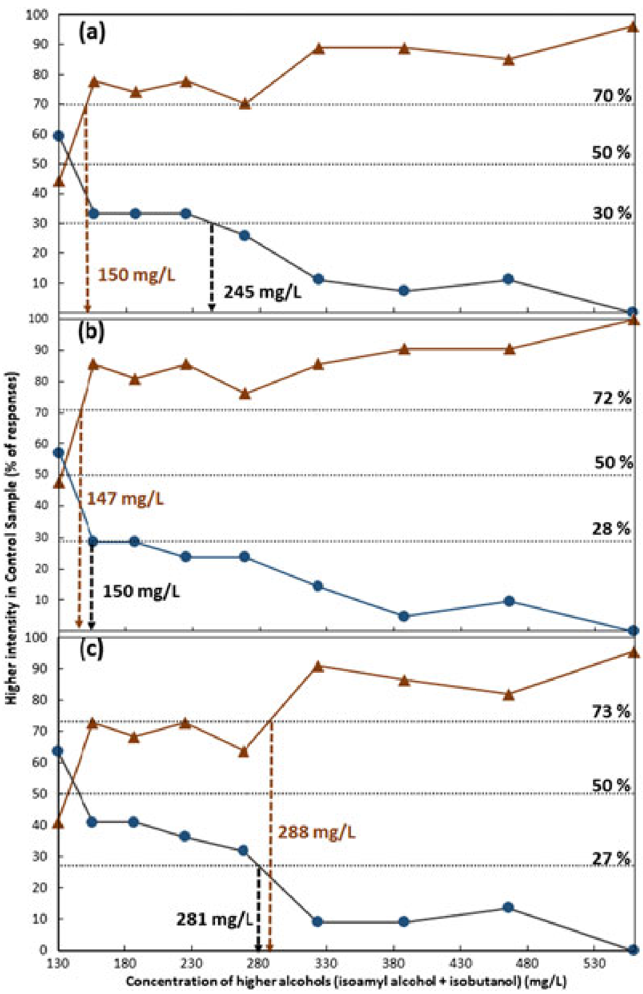

Figure 3. Proportion of responses: (a) n = 27, all the panellists; (b) n = 21, without less sensitive panellists; (c) n = 22, without more sensitive panellists, selecting Control samples of the woody wine model as more intense in woody (▲) and spirit-like (●) attributes than spiked samples. Lower and upper dotted lines [superior: (a) 70%; (b) 72%; and (c) 73% and inferior: (a) 30%; (b) 28%; and (c) 27%] indicate the 5% significance criterion for paired tests.

conferred by HA. When individual data were analysed in detail, two different behaviours were observed. On one hand, three out of the 14 participants performing this task (21% of experts) were not able to detect the spirit-like aroma in woody WMs with low HA concentration, which suggests that they were not especially sensitive to the changes generated by HA in this woody wine. In contrast, three different participants (21% of the total) were able to detect the suppression of the woody aroma in all tests. They were especially sensitive to the masking effect caused by HA on woody aroma. The result is quantitatively surprising even if it can be considered normal in terms of physiological inter-individual differences in sensitivity to specific odour nuances usually found in sensory panels (Lawless and Heymann 2010). The effect of both different sensitivities on the minimal concentration inducing sensory changes in woody WMs was studied. First, setting aside the responses of the three less sensitive members, samples containing 147 and 150 mg/L of HA were able to significantly decrease woody and increase spirit-like aromas, respectively (Figure 3b). All the responses, n = 21, were derived from 11 participants in duplicate: one response is missing because one participant could not carry out the task in duplicate. The minimal concentration, which causes sensory changes, based on the responses of this reduced panel (11 participants with 21 responses, n = 21), can therefore be estimated as 17 and 20 mg/L for woody and spirit-like attributes, respectively. Interestingly, the minimal concentration for the spirit-like aroma was lower (twice lower: 150 vs 299 mg/L) in this woody WM than in the fruity WM.

Setting aside the more sensitive participants, the concentration at which the participants perceived the Control WM significantly different in terms of woody and spirit-like attributes reached 288 and 281 mg/L, respectively (Figure 3c). All the responses, n = 22, were derived from 11 participants carrying out the tasks in duplicate. The minimal concentration generating sensory differences in this last case could be estimated as 158 and 151 mg/L for woody and spirit-like attributes, respectively. These concentration values are comparable with that found for the fruity WM (169 mg/L). These results confirm the existence of important differences in sensitivity for the spirit-like aroma conferred by HA in woody WMs.

With regards to aroma appreciation, the three highly sensitive participants preferred in all cases the Control, which were perceived higher in woody and lower in spirit-like aromas than WM spiked with barely 20 mg/L of HA. This made the preference threshold for the whole panel as low as 184 mg/L (Figure 4, woody model 1, n = 27). When the data of the least sensitive participants were removed, the concentration of HA inducing changes in preferences decreased approximately 30 mg/L, until 152 mg/L (Figure 4, woody model 3, n = 21). When the data from the three highly sensitive panellists were left out, however, the concentration of HA necessary to significantly change wine aroma appreciation increased 100 mg/L to 284 mg/L (Figure 4, woody model 2, n = 22), which is similar to the value of 288 mg/L found in Figure 3c for the fruity WM. These results reflect the strength of the highly sensitive panellists, who were capable of decreasing the concentration of HA inducing differences in preference. In any case, participants preferred Control wines over spiked samples at a high concentration of HA (>284 mg/L in any case). The fact that HA mask woody character and decrease appreciation is in agreement with previous studies that associate woody aromas to red wine acceptance and quality perceived by wine professionals (Lattey et al. 2010, Hopfer and Heymann 2014,).

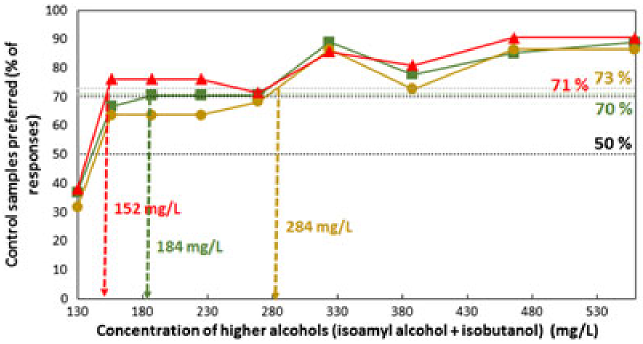

Figure 4. Proportion of responses of three subgroups of participants with different levels of sensitivity to higher alcohols [model 1 (■), n = 27; model 2 (▲), n = 21; model 3 (●), n = 22] preferring Control samples of the woody wine model over spiked samples. Lower and upper dotted lines (superior: 70% for n = 27, 71% for n = 21 and 73% for n = 22) indicate the 5% significance criterion for paired tests.

Animal wine model. For the animal WM, Figure 5 shows that samples containing 365 or 375 mg/L of HA were perceived significantly higher in both animal and spirit-like aromas than the Control, respectively. This result is in essential agreement with a previous one (de-la-Fuente-Blanco et al. 2016), in which the presence of 430 mg/L of the isoamyl alcohol/isobutanol caused a non-significant enhancement of the animal/leather/ink attribute and a significant increase of the spirit/alcoholic/solvent note. In the present approach, the panel appeared to be more sensitive to the detection of the first nuance and a little less to the latter. This apparent disagreement could be explained in terms of the nature of the approaches used. In discriminant tests, as the one used in the present work (2-AFC), participants are forced to find the different sample within a two-sample trial. This task appears to favour the ability to discern among samples with similar profiles rather than analytical methods using rating scales (Barylko-Pikielna et al. 2004, Villanueva et al. 2005) such as the one used in the previous study (de-la-Fuente-Blanco et al. 2016). In addition, in the rating task (de-la-Fuente-Blanco et al. 2016), the intensity of the animal nuance was closer to the higher end of the scale, which could have hampered the quantitative assessment. Data in Figure 5 additionally revealed that judges find progressively more difficulty in detecting the spirit-like character while they find it easier to detect the animal nuance. This should be the outcome of sensory interactions (Laing et al. 1994, Prescott 2015), resulting in sensory integration (Lawless

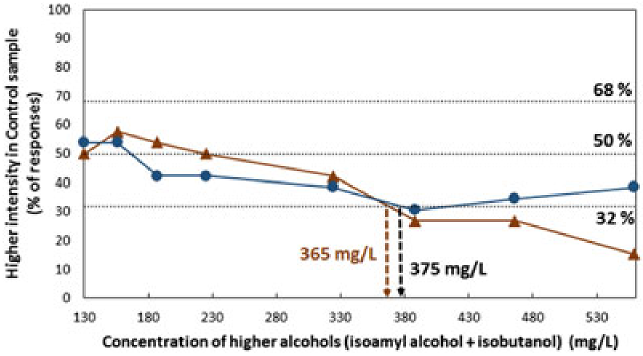

Figure 5. Proportion of responses (n = 31) selecting Control samples of the animal wine model as more intense in animal (▲) and spirit-like (●) attributes than spiked samples. Lower and upper dotted lines (superior: 68% and inferior: 32%) indicate the 5% significance criterion for paired tests.

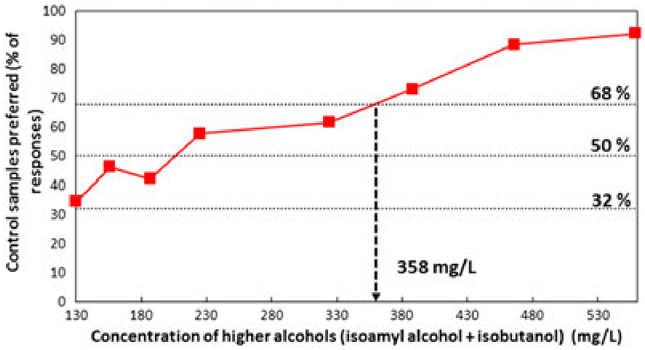

Figure 6. Proportion of responses (n = 31) selecting Control samples of the animal wine model as preferred over spiked samples. Lower and upper dotted lines (superior: 68% and inferior: 32%) indicate the 5% significance criterion for paired tests.

1995) of attributes sharing a hedonic category (i.e. with negative or positive valence). Most probably, at these concentration levels, participants integrated the signal of both spirit-like and animal aromas in a unique unpleasant signal mostly identified as animal (Figure 6). This was supported by Prescott (2015), who pointed out that the main aim of sensory integration is to confer hedonic valence (positive or negative) to flavour objects rather than identification.

With regard to the concentration of HA inducing differences in aroma preference, samples containing at least 358 mg/L of HA were significantly less preferred than the Control, which was close to the previously determined concentration thresholds (365 and 375 mg/L). These results are in accordance with the fact that the animal character is considered a major cause of rejection of samples by wine professionals (Frost and Noble 2002, Torri et al. 2013, Saenz-Navajas et al. 2015), thus increasing the intensity of this off-odour would be detrimental for wine quality and thus preference. It is noteworthy that these thresholds were much higher than those found for the fruity and woody models, confirming how the negative hedonic tone of HA was much easier to identify in pleasant fruity or woody contexts than in unpleasant contexts as was already suggested by Prescott (2015).

Conclusion

In summary, the results show that levels of approximately 281 mg/L to 299 of HA were able to confer their characteristic spirit-like aroma to fruity and woody-like WMs as well as to suppress red fruit (299 mg/L) and woody (288 mg/L) attributes, respectively. A concentration of 365 and 375 mg/L of HA increased the animal aroma generated by ethyl phenols and the spirit-like nuances of HA, respectively. Spirit-like and animal attributes were suggested to share a common unpleasant category, leading to sensory integration of both signals.

The presence of HA at a concentration ranging from 284 to 358 mg/L caused a decrease in preference of the WMs studied, which demonstrated that isobutanol and isoamyl alcohol are essentially detrimental to wine quality according to the panel of wine experts that carried out the present work. In the context of woody mixtures, highly sensitive panellists were able to detect and perceive as negative additions of HA as little as 17 and 22 mg/L, respectively.

These results are valuable for the wine industry as they provide an estimation of the concentration of HA able to affect the quality of model red wines, which can help in making decisions during the winemaking processes, especially at the alcoholic fermentation stage (e.g. yeast selection). Further research with wines of different sensory properties and estimating the minimal concentration generating changes in aroma perception and preferences for large consumer groups from different regions or countries and with different levels of expertise should be carried out. An apparent potential limitation of the current study could be the use of model wines instead of real wines. This approach is, however, the only way to control perfectly compositional variables, which are essential for understanding wine aroma formation and, further, reach general conclusions.

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