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Consumer rejection threshold of ethyl phenylacetate and phenylacetic acid, compounds responsible for the sweet-like off odour in wines made from sour rotten grapes

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Abstract

Backgrounds and Aims: This study aimed to determine a consumer rejection threshold (CRT) for ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA) in wine. These compounds have recently been reported to be responsible for sweet-like, honey off odours in wine made from sour rotten grapes.

Methods and Results: Non-expert wine consumers ($n = 35$) received pairs of samples comprising a control wine against a spiked wine with an ascending concentration of the target compounds and were asked to indicate which sample they preferred. Results estimated a conjoint CRT for EPhA and PhAA of 140 and 700 μg/L, respectively. Wines spiked with a EPhA and PhAA concentration around the CRT evoked intense 'dried fruit' aromas that led to a decrease of the general aroma quality; these wines are significantly rejected by consumers.

Conclusions: The measured CRT provides an initial estimation of the risk concentration for EPhA and PhAA in red wine, as they represent a 'taint' for regular wine consumers.

Significance of the Study: These data allow wine producers to predict if a given wine will be disliked by consumers or to help guide 'blending away' of such wines.

Keywords: *consumer rejection threshold (CRT), ethyl phenylacetate (EPhA), honey, phenylacetic acid (PhAA), sour rot, sweet-like off odour, wine*

Introduction

Grape sour rot, appearing 3–4 weeks before ripening, is caused by the combined action of several factors (insects, mildew, bacteria, yeast and other microorganisms) that damage the grape skin and cause mechanical and physiological injuries to the berry. Sour rot affects both crop yield and wine quality (Bisiach et al. 1982). It is commonly accepted by winemakers that the vinification of damaged grapes is associated with low-quality wines with poor storage/ageing potential (Loinger et al. 1977).

The organisms responsible for sour rot are known to alter fruit composition as a result of the production of a high concentration of a wide range of metabolites, including acetic acid, glycerol, ethyl acetate, ethanol, acetaldehyde, galacturonic and gluconic acids (Marchetti et al. 1984). In the late 1970s, Loinger et al. (1977) clarified for the first time the consequences of sour rot for wine quality, investigating the effect of the disease on the sensory characteristics of wines from Semillon grapes. Bunches with 20–40% rotten berries resulted in a clear reduction in wine quality, whereas wines produced from bunches with 80% rot were rejected.

A recent study (Barata et al. 2011) examined the sensory profile and volatile chemical composition of experimental wines produced from the Portuguese red grape variety Trincadeira affected by several levels of sour rot infection. Sensory descriptive analysis was combined with gas chromatography–olfactometry and quantification of relevant compounds by gas chromatography with mass spectrometry detection in order to identify the volatile (aromatic) compounds most likely related to this grape disease. Wines produced from damaged berries exhibited honey-like notes not evoked by healthy samples. Ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA), both presenting sweet honey-like notes, emerged as key aroma compounds. The concentration of these compounds in wines elaborated from infected berries was one order of magnitude above that found in control (healthy) samples, reaching a maximal concentration of 304 and 1668 μg/L of EPhA and PhAA, respectively. Phenylacetic acid is a plant-growth regulator (Wightman and Lighty 1982) so it is hypothesized (Barata et al. 2011) that this compound may be produced as a plant response to an alteration of the grape surface. Phenylacetic acid could then be extracted to the must if a maceration process is used and be further converted to its corresponding ethyl ester (EPhA) during fermentation or bottle ageing.

Ethyl phenylacetate has been suggested as being responsible for the 'sweet-like' off odour usually affecting Italian Aglianico del Vulture wines (Tat et al. 2007). It is not clear when the problem originates, but local producers suspect that the origin of this defect could be related to fungal diseases affecting late-ripened grape bunches. A strong empirical observation is that the 'sweet-like' off odour is not present in Aglianico wines without skin contact, which supports the theory that EPhA is formed from PhAA present in the surface of damaged berries.

A relatively recent approach to estimate the 'risk' concentration of off odorants affecting consumer preference is to calculate the so-called 'consumer rejection threshold' (CRT). CRT has been successfully estimated for common wine off odorants such as TCA (responsible for 'cork taint') (Prescott et al. 2005)

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or 1,8-cineole (responsible for 'eucalyptus' odours) (Saliba et al. 2009). More recently, CRT has been used to evaluate consumer acceptance of 'sweetness' in Semillon wines (Blackman et al. 2010) and of wines functionally enhanced with catechin extracts (Yoo et al. 2012). The CRT method is based on a series of paired comparison tests (spiked vs control wine) in which consumers are asked to indicate which of the two wines they prefer. In these studies, between 25 and 58 non-expert wine consumers were recruited to evaluate between five and eight concentrations of spiked wines against a control. Results have shown that the point at which consumers significantly reject a product above chance ($P < 0.05$) represents a threshold helpful for winemakers to assess the impact of target off odorants on consumer populations.

The main aim of the present work is to calculate a CRT for EPhA and PhAA in wine. A CRT of EPhA individually and of a conjoint threshold for EPhA and PhAA together will be estimated because both compounds exhibit honey-like odours and therefore an additive effect may be expected. Additionally, in an attempt to receive consumer feedback about the sensory characteristics driving preference, the group of consumers recruited was trained to undertake sensory descriptive tasks. The training phase aimed to familiarize consumers with wine vocabulary and to make them taste a variety of wine products (and not only those they normally taste), providing them with a common background before the measurement tests.

Materials and methods

Panellists

Thirty-five wine consumers were recruited through an informative email at the University of Zaragoza. Participants were selected only if they drank red and/or white wine at least twice a week. The sample comprised of 16 women and 19 men, ranging in age from 24 to 69 years (average = 36; standard deviation = 9). In a screening questionnaire, 3% identified themselves as 'knowledgeable wine drinkers', 80% as 'interested in wines' and 17% as 'novice drinkers'. Half of them (48%) had attended short tasting sessions offered by wineries who cater for tourists or local retailers.

Descriptive sensory training

Prior to measurements, panellists undertook training in descriptive analysis according to the frequency citation method (Campo et al. 2010). The training consisted of six weekly 1-h sessions. Panellists were provided with an initial list (Table 1) of 79 terms arranged by general odour families: white fruits, yellow fruits, citrus fruits, red fruits, black fruits, dried fruits, exotic fruits, nuts, floral, spices, toasty, woody, vegetal, undergrowth, animal and others. Special attention was paid to include not more technical or chemical terms in order to work with a list containing terms for common odours.

During training, reference standards representative of aroma terms were presented. Standards were either commercially available odorants from 'Le Nez du Vin' (Jean Lenoir, Provence, France) and Firmenich (Switzerland) or natural products (fruits, juices, spices, vegetables, etc.) prepared at the beginning of the day as explained elsewhere (Campo et al. 2010; Saenz-Navajas et al. 2010). Each of the six training sessions was divided into two parts. In the first part (30 min), panellists became familiar with the specific vocabulary of the list and smelled different aroma references. In the second part (30 min), they evaluated six to eight different wines and described their odour properties with the aid of the list. The session ended in a discussion in which the panel leader highlighted the terms most frequently cited by consumers to describe each wine. Consumers became familiar with the terms of the list and learned to label the sensory attributes they perceived in a variety of wines. The wines selected for the training phase presented a variety of odour properties and included wines from different styles, grape varieties and origins. Consumers were also instructed to recognize common wine faults, such as oxidation, reduction and cork taint, but special attention was made by the panel leader not to mention an eventual sweet-like off odour in any of the training sessions.

Table 1. List of terms employed for sensory descriptive analysis.

WHITE FRUITS	**EXOTIC FRUITS**	**FLORAL**	**WOOD**	**OTHERS**
Apple	Lychee	Orange blossom	Fresh oak	Alcohol
Banana	Mango	Rose	Old oak	Cauliflower
Pear	Passion fruit	Violet		Olive juice
	Pineapple	White flower	**HERBAL**	Cocoa
YELLOW FRUITS			Mint	Dust
Melon	**DRIED FRUITS**	**HONEY**	Tobacco	Ink
Peach	Fig			Lactic
	Prune	**SPICY**	**VEGETAL**	Butter
CITRUS FRUITS	Raisin	Anise	Asparagus	Sulphur
Lemon		Black pepper	Green beans	Yeast
Grapefruit	**NUTS**	Cinnamon	Pepper/earthy	
	Almond	Clove		
RED FRUITS	Walnut	Licorice	**UNDERGROWTH**	
Cherry		Vanilla	Humus	
Raspberry	**CANDIED FRUITS**		Mushroom	
Strawberry		**TOASTY**	Wet	
	MUSCAT	Caramel		
BLACK FRUITS		Coffee	**ANIMAL**	
Blackberry	**CIDER**	Smoky	Leather	
Blueberry		Toasted bread	Transpiration	
Redcurrant			Wet dog	

CRT determination and descriptive analysis

Stimuli. The base wine was a popular medium-priced Spanish red wine from the 2009 vintage made from Grenache and Shiraz. It was selected for three reasons: (i) it contained a trace of the target compounds (EPhA and PHAA) according to GC-MS analysis (Lopez et al. 2002); (ii) the aroma was neutral and was presumed to be generally liked by consumers; and (iii) it was sealed with a plastic stopper so that the risk of cork taint was minimized. Analysis of the wine (WineScan™ FT 120, FOSS®, Barcelona, Spain) revealed a pH of 3.45, acetic acid of 0.41 g/L, reducing sugars of 1.90 g/L and alcohol content of 13.4% (v/v).

Wine was presented unspiked (control) or spiked with EPhA either alone or with PhAA (both supplied by Sigma, St. Louis, MO, USA). The wine was spiked with EPhA at 5, 10, 20, 40, 80, 160, 320, 160 and 1280 μg/L and PhAA at 25, 50, 100, 200, 400, 800, 1600, 3200 and 6400 μg/L. Wine samples with both EPhA and PhAA always presented a concentration ratio of 1:5 in view of previous quantitative results obtained in our laboratory and the literature (Tat et al. 2007, Barata et al. 2011).

Both control and spiked wines were opened approximately 24 h before testing, and the appropriate amount of standard solution containing the target compound(s) was added directly to 1 L of wine with a micropipette. The volume of such additions did not cause a significant modification of the alcohol content of the wine. Control and spiked wines were stored in the dark at 5°C.

Procedure. Three 45-min sessions were conducted with participants. The first two sessions were used for CRT measurements (test A and B) and the final session for the evaluation of hedonic rating and sensory descriptive analysis of selected samples. A summary of the samples submitted to each test is presented in Table 2. The experiment was conducted in a sensory laboratory, where each panellist was seated in an individual booth separated from other participants by a partition. Each consumer was randomly allocated a panellist number. Samples (18 ± 1°C) were presented in dark ISO (ISO NORM 3591, I. N. 1977)-approved wineglasses labelled with three-digit random codes and covered by plastic petri dishes according to a random order for each panellist.

CRT. Test A measured the CRT of samples spiked with the ester (EPhA), whereas test B measured CRT in samples spiked with both EPhA and PhAA. The procedure followed that of Prescott et al. (2005). Accordingly, nine paired comparison tests, one for each EPhA or EPhA + PhAA concentration, were carried out. Each pair consisted of a sample of the control wine alone and a sample of the control wine spiked with the target compound(s). In each test, panellists were asked to evaluate both samples orthonasally and indicate on a score sheet which sample of the pair they preferred. Presentation order of the control wine within each pair (left or right) was randomized. Wines were presented in ascending order of concentrations, and panellists received a new pair of samples every 2 min. A 15-min break was enforced in the middle of the session to limit fatigue. Participants were told that the experiment aimed to determine preference for a given wine and were not told that the experiment was investigating a 'sweet-like' off odour. Similarly, they were instructed to complete the task according to their own criteria and that there were no right or wrong answers. In light of preliminary results from the CRT test, six samples spiked with both EPhA and PhAA were selected for further sensory tests (see Table 2).

Hedonic rating. Panellists rated how much they liked each wine on a nine-point interval scale from 'dislike extremely' to 'like extremely'.

Table 2. Summary of sensory tests performed in the different samples.

Sample	Concentration (μg/L) of EPhA or EPhA + PhAA added to control wine	Test A	Test B	Hedonic rating	Descriptive analysis
1	Control wine			X	X
2	5	X			
3	5 + 25		X		
4	10	X			
5	10 + 50		X		
6	20	X			
7	20 + 100		X	X	X
8	40	X			
9	40 + 200		X		
10	80	X			
11	80 + 400		X		
12	160	X			
13	160 + 800		X	X	X
14	320	X			
15	320 + 1600		X	X	X
16	640	X			
17	640 + 3200 (replicate 1)		X	X	X
18	640 + 3200 (replicate 2)			X	X
19	1280	X			
20	1280 + 6400		X	X	X

EPhA, ethylphenyl acetate; PhAA, phenylacetic acid.

Descriptive analysis. Panellists were asked to evaluate each of the six selected wines orthonasally and to describe their odour by choosing a maximum of four attributes from the list (Table 1). This number was selected as a compromise between two factors; the possibility of letting panellists express their perceptions with a relatively high number of terms and the known limited capacity of humans to discriminate odour qualities in a mixture (Laing and Glemarec 1992). The sessions lasted approximately 45 min. A 10-min break was enforced in the middle of the session to limit fatigue.

Data analysis. CRT. Criteria for significant detection of product rejection as a function of the concentration of either EPhA alone or combined with PhAA was based on the binomial distribution table for paired comparison test (Roessler et al. 1956).

Hedonic rating. The preference scores were subjected to a two-way analysis of variance (ANOVA) model (panellists and products as factors) using the SPSS statistical package (version 15.0, SPSS, Inc. Chicago, IL, USA). Multiple mean comparisons were also performed with a Duncan test at $P < 0.05$ to complete analysis of products.

Descriptive analysis. Terms present in the list were ranked according to their citation frequency to identify the most relevant descriptors of each wine. Only descriptors cited by a minimum of six panellists (more than 15% of the panel) in, at least, one wine were considered for subsequent analysis (Campo et al. 2010). The most discriminant terms were identified with a chi-square (χ^2) analysis of the citation frequency of each term. A contingency table (wines in rows and descriptors in columns) containing the average citation frequency was constructed and submitted to correspondence analysis (CA). In order to incorporate panellists' preference in the sample CA map, the average hedonic score of each sample was considered as a supplementary variable in the CA dataset. This implies that this variable did not participate in the construction of the CA factors, but panellists' preferences were projected in the sample descriptive space by calculating their correlation coefficients with the CA factors. All analyses were conducted with SPAD software (version 5.5, CISIA-CESRESTA, Montreuil, France).

Results

CRT

Figure 1 illustrates the proportion of panellists who preferred the control wine to the spiked wine at each concentration of EPhA (test A) or of EPhA + PhAA (test B). The control wine was preferred by more than 50% of the panel over samples containing at least 40 μg/L of EPhA (with or without PhAA). According

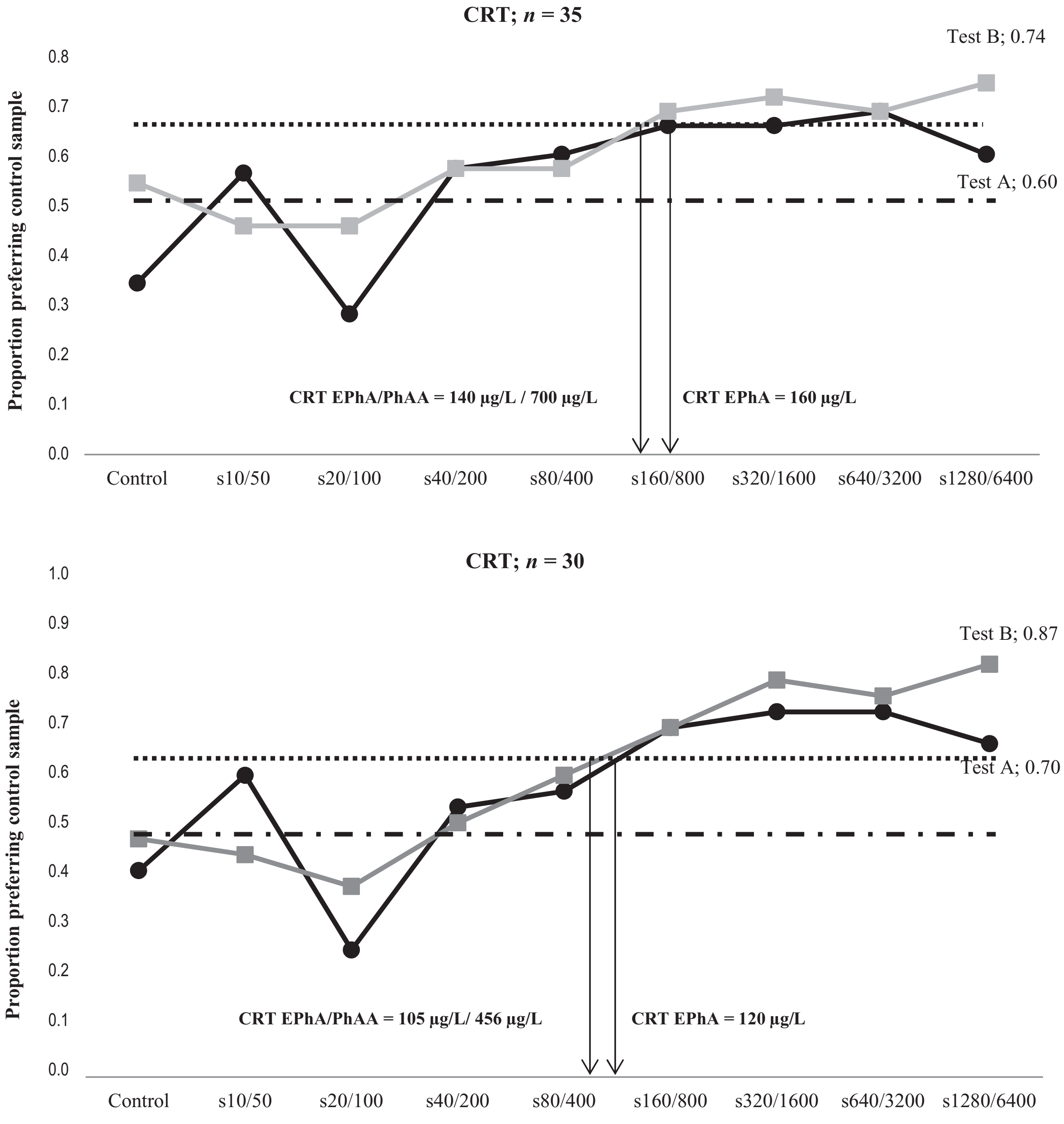

Figure 1. Proportion of consumers ($n = 35$ and $n = 30$) preferring the control wine at each ethyl phenylacetate (EPhA) (test A, ●) and EPhA/phenylacetic acid (PhAA) (test B, ■) concentration. The dashed line (0.5) represents no preference, while the dotted line indicates the minimum proportion of agreeing consumers necessary to establish preference ($n = 24$ and $n = 21$; $\alpha = 0.05$) using the one-tailed binomial distribution for paired comparison tests. (s indicates spiked sample). CRT, consumer rejection threshold.

to the 0.05 confidence level for one-tailed paired comparison tests (Roessler et al. 1956), the minimum agreeing judgments necessary to establish significant preference between samples (control or spiked) is 23 out of 35 possible responses, which corresponds to 0.66. The CRT was determined from the point at which the theoretical preference line intersects with the chance line (dotted line in Figure 1). For samples containing both EPhA and PhAA CRT was 140 and 700 µg/L, respectively. For EPhA alone, it was estimated at 160 µg/L. An interesting result to comment on is that the control was significantly preferred to samples containing from 160 to 640 µg/L of EPhA but not to the sample with the maximal concentration of EPhA (60% of agreeing panellists choosing the control). This behaviour is unexpected and difficult to interpret. A possible explanation would be that as samples in the test were presented in ascending order, some of the panellists may have 'failed to reject' this sample because of a possible saturation effect at the odour receptors level.

If the odour evoked by the presence of EPhA and PhAA was universally disliked, then it might be expected that an increasing concentration of these compounds in wine would eventually result in 100% of all respondents choosing the wine without EPhA and PhAA. Above 160/800 µg/L, however, the proportion of panellists preferring the control wine remained between 70 and 75% (Figure 1), which meant that almost a third of the participants still preferred the spiked wine over the control wine. Exploring individual data in detail, we realized in both tests that 5 out of the 35 participants (14%) systematically choose as preferred the spiked wine in all the pairs with the concentration of EPhA and PhAA above 160/800 µg/L. Results suggest that these individuals do not find the odour objectionable and actually think that wines with EPA and PhAA are more pleasant.

The proportion of panellists preferring the control wine over the spiked wine in a subpopulation not including these five individuals ($n = 30$) was determined (Figure 1). Above 160/800 µg/L, this group significantly preferred the control wine as points are above the dotted line. Above this concentration a greater proportion (70–90% of panellists) prefers the control compared to when 35 individuals are considered. Some of the participants, however, still declared preference for the spiked sample at the highest concentration because the point at which 100% of panellists preferred the control was not reached. Extrapolating from the point at which the proportion of panellists rejecting the spiked sample reached the criterion for significance (0.67) gave a calculated CRT value of 120 µg/L for EPhA alone, and 105 and 456 µg/L for EPhA and PhAA, respectively, when both compounds are present in the wine.

Hedonic rating

According to preliminary results from the CRT estimations, the six samples selected for sensory analysis included two samples below the CRT (control and spiked wine with 20 µg/L of EPhA + 100 µg/L of PhAA) and four samples at and above the CRT. One of the samples (640/3200 µg/L) was presented in duplicate to the panellists. Table 3 shows the average rating of samples for the total population ($n = 35$) and the subpopulation of 30 individuals (excluding those systematically liking spiked samples). For the global population, significant differences were found in panellists' overall liking ($P < 0.05$ for the product effect in the ANOVA), confirming that samples caused different reactions among the panellists. The hedonic rating of the first four samples (from control wine to 320/1600 µg/L) were positioned in the middle of the scale corresponding to products that were classified as indistinct or slightly disliked. The two samples with the highest concentration of EPhA + PhAA were significantly less preferred, reaching values of 4.07 and 3.61, respectively. Mean ratings were recalculated for the subpopulation of 30 panellists. The same pattern was observed with the two last samples, being significantly less preferred than the rest. On this occasion, however, the preference of the last sample (3.23) significantly decreased with respect to the preceding one (4.07), confirming that this subpopulation clearly disliked the wine spiked with the highest concentration of both compounds.

Table 3. Hedonic preference means for control and spiked wines (total population; $n = 35$ and subpopulation $n = 30$).

Sample	$n = 35$	$n = 30$
Control wine	5.17[a]	5.23[a]
20(EPhA)/100(PhAA)	4.88[a]	5.07[a]
160(EPhA)/800(PhAA)	4.83[a]	5.03[a]
320(EPhA)/1600(PhAA)	4.97[a]	5.00[a]
†640(EPhA)/3200(PhAA)	4.07[b]	4.07[b]
1280(EPhA)/6400(PhAA)	3.61[b]	3.23[c]

The concentration of EPhA and PhAA in wines is expressed as µg/L. Means marked with different letters are significantly different (Duncan test, $\alpha = 0.05$). †Calculated as the average of two replicates. EPhA, ethylphenyl acetate; PhAA, phenylacetic acid.

Table 4. Sensory profiles of control wine and spiked wines with EPhA/PhAA (µg/L) and the significance of chi-square (χ^2) parameter.

Descriptor	Control	20/100	160/800	320/1600	640/3200†	1280/6400	P (χ^2)‡
White fruits	8	5	2	3	1.5	0	**0.028**
Red fruits	3	6	6	6	6	1	0.313
Black Fruits	8	5	8	5	6.5	1	0.297
Exotic fruits	6	6	6	7	6	4	0.865
Dried fruits	1	4	11	9	10	11	**0.045**
Floral	6	4	4	6	4	5	0.770
Honey	0	0	1	2	2	14	**0.000**
Spicy	2	3	6	3	4.5	3	0.747
Toasty	6	4	3	5	3.5	7	0.848
Oak	10	15	8	10	11.5	11	0.315
Undergrowth	1	3	2	3	2.5	5	0.701

Descriptive data are expressed as absolute citation frequency ($n = 35$). †Average of two replicates. ‡Terms varying significantly among samples are highlighted in bold letters. EPhA, ethylphenyl acetate; PhAA, phenylacetic acid.

Sensory descriptive analysis

A total of 11 terms from the list reached a frequency of citation higher than 15% (noise threshold) in, at least, one sample. The absolute citation frequency of these terms ($n_{max} = 35$), together with the significance of the chi-square (χ^2) parameter, was determined (Table 4). The descriptors most frequently used to characterize the set of samples were those belonging to the 'fruity' family ('white, red, black and dried fruits') as well as the

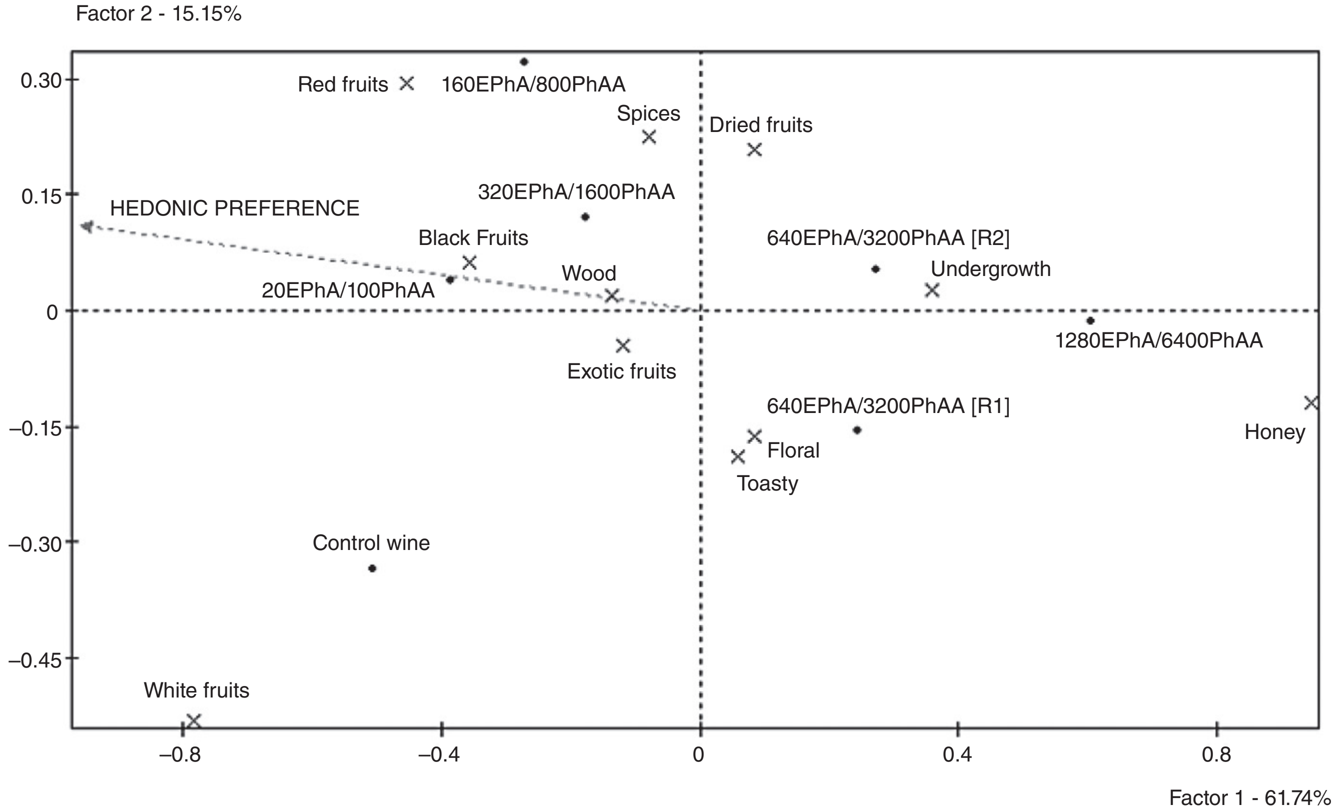

Figure 2. Projection of wines (●) and descriptors (×) in the correspondence analysis map (dimensions 1 and 2). Concentrations of wines for ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA) are expressed as µg/L. Wine liking (in grey) is projected as a supplementary variable.

'oak' term. Data in the table reveal that three terms varied significantly ($P < 0.05$): 'white fruits' ($P = 0.028$), 'dried fruits' ($P = 0.045$) and 'honey' ($P = 0.000$), which means that such descriptors were the most pertinent to characterize differences in aroma among samples.

The projection of wines and descriptors into a bidimensional CA map can be seen in Figure 2. The first dimension (F1) explains most of the variance, a total of 62%, whereas the second dimension explains 15% of the remaining variance. Looking in detail to the projection of samples on the F1, it can be seen that the latent information retained by F1 actually represents a gradient of EPhA/PhAA concentration from control wine (left extreme) to the sample with the highest concentration in the right extreme (1280 µg/L EPhA/6400 µg/L PhAA). This means that panellists were able to discern among samples with an increasing concentration of EPhA/PhAA from a sensory viewpoint. With respect to the hedonic scores, overall preference was clearly projected towards samples containing the lowest content in EPhA/PhAA. On the contrary, samples containing 640/3200 µg/L and above were clearly disliked by the panel. Control wine and samples up to 320/1600 µg/L were mainly described by 'fruity' terms ('white, red or black fruits'). The sample with the highest concentration evoked a clear 'honey' character, as 40% of the panel choose this term. Another important observation is the importance of the 'dried fruit' odour with increasing EPhA/PhAA concentration. This term is projected (Figure 2) close to samples 160/800, 320/1600 and 640/3200 µg/L (R2). Data in Table 4 show that 'dried fruit' is clearly perceived by almost a third of the panel in all samples from a concentration of 160/800 µg/L, whereas it is rarely perceived in the control or low-level spiked wine. This term increases from the control to a concentration around the CRT, where it stabilizes.

Discussion

The present work aimed to determine a CRT for EPhA alone and for EPhA combined with PhAA, as it is unclear at which concentration these compounds in wine may represent a 'taint' for non-expert wine consumers. Results show that spiked samples below 160 and 800 µg/L for EPhA + PhAA, respectively, were not significantly rejected by panellists. The small difference between the CRT for EPhA alone and EPhA in presence of PhAA (20 and 15 µg/L for $n = 35$ and $n = 30$, respectively) suggests that contrary to expectations, PhAA is not able to boost the sensory note of EPhA by an additive effect. This would confirm previous data reported by Tat et al. (2007), which pointed out EPhA as being mainly responsible for the sweet-like off odour. Initial estimates of EPhA threshold in red wine reported by these authors were around 75 µg/L, which is approximately half of the CRT calculated in this work. These authors calculated the threshold using a group of experienced oenologists; therefore, a gap appears to exist between the threshold concentration for wine professionals and that rendering a wine unacceptable for wine consumers. This is not surprising, however, because it is well known that experience increases odour sensitivity, and the use of professional panels leads to a risk concentration lower than at which the majority of wine consumers will detect a 'taint' (Prescott et al. 2005, Saliba et al. 2009).

Concerning hedonic data, preference tends to decrease with an increasing concentration of EPhA and PhAA (see Table 3). This trend is not significant, however, until a concentration well above the CRT. It is important to point out the apparent disagreement between the established CRT value and the concentration at which samples are significantly less appreciated according to hedonic rating, that is, a concentration as high as 640 and 3200 µg/L for EPhA + PhAA, respectively. This fact can be explained in terms of the nature of the approaches used for hedonic rating and CRT estimation. During the hedonic rating task, consumers evaluate all the wines (control and spiked samples) at the same time and are asked to give a score in a nine-point scale. Our data show that from the six samples evaluated, most of them were rated around the middle point of the scale, significant differences on scoring being observed only between the first four samples and the last two samples (see Figure 2). Additionally, it is well known that panellists avoid using extreme categories, reducing the nine-point scale to a seven-point scale and thus limiting the discriminative power of the test (Moskowitz 1980). The CRT approach, on the contrary, always presents the control wine against a spiked sample, forcing respondents to choose one product over the other. Various studies in sensory science give evidence that methods forcing trade-offs by respondents (i.e. paired comparison or ranking tests) provide greater discriminating power than methods based on absolute judgements (i.e. rating methods) (Villanueva et al. 2000, Barylko-Pikielna et al. 2004).

A secondary objective of this work was to evaluate the sensory attributes driving consumer preference. In an attempt to obtain some feedback about why samples with a concentration above the CRT were mostly rejected by our panel, we deliberately trained the panellists in descriptive analysis prior to measurements. According to the descriptions, the clue to explain why products at a concentration above the CRT are significantly disliked appears related to the 'dried fruits' character. Data in Table 4 show that wines with a concentration from CRT clearly evoke this aroma (while samples with a lower EPhA/PhAA concentration did not), which is undoubtedly linked to a rather sweet-like character of the samples. Results strongly support the idea that the perception of this new attribute involves a dramatic decrease of the general aroma quality of the wines, before that clear honey-like off odour could be perceived by consumers. The presence of an increasing concentration of EPhA + PhAA seems to induce a shift towards a different concept of fruitiness, as the perception of the 'white fruit' term significantly decreased in the most heavily spiked samples. The 'white fruit' term is defined as 'banana', 'pear' or 'apple' but not at a ripened state, thereby making the term an example of 'fresh fruit' character. The fine balance existing between the perceptions of these two fruity concepts – 'fresh fruit' versus 'dried fruit' – in relation to the off odorant methional has been recently pointed out (San Juan et al. 2011).

In summary, the present work provides a first estimation of the concentration of EPhA and PhAA in red wine that might be a cause of concern for wine producers thus acting as a guideline for quality control of wines made from sour rotten fruit. The differences in concentration between the odour detection threshold calculated by a group of wine experts (Tat et al. 2007) and the CRT estimated here point out the need to measure CRT for wine consumers and to not rely on professional wine judgments. A potential limitation of the current work is that the consumers used were trained and therefore may have become more 'critical'. Our approach, however, provided a compromise solution between consumers being able to label the perceived product attributes but not having the critical prejudgment of expert wine assessors towards the sweet-like off odour. More research is needed to calculate the CRT in wines from different grape varieties and styles, as well as to expand CRT estimations for larger consumer groups and/or specific target consumer segments.

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References

Barata, A., Campo, E., Malfeito-Ferreira, M., Loureiro, V., Cacho, J. and Ferreira, V. (2011) Analytical and sensorial characterization of the aroma of wines produced with sour rotten grapes using GC-O and GC-MS: identification of key aromacCompounds. Journal of Agricultural and Food Chemistry **59**, 2543–2553.

Barylko-Pikielna, N., Matuszewska, I., Jeruszka, M., Kozlowska, K., Brzozowska, A. and Roszkowski, W. (2004) Discriminability and appropriateness of category scaling versus ranking methods to study sensory preferences in elderly. Food Quality and Preference **15**, 167–175.

Bisiach, M., Minervini, G. and Salomone, M.C. (1982) Recherche expérimentales sur la pourriture acide de la grappe et sur ses rapports avec la pourriture grise. EPPO Bulletin **12**, 5–28.

Blackman, J., Saliba, A. and Schmidtke, L. (2010) Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines. Food Quality and Preference **21**, 679–683.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D. (2010) Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: an application to Burgundy Pinot noir wines. Food Quality and Preference **21**, 44–55.

ISO NORM 3591 (1977) Sensory analysis: apparatus wine tasting glass (International Organization for Standardization: Geneva, Switzerland).

Laing, D.G. and Glemarec, A. (1992) Selective attention and perceptual analysis of odor mixtures. Physiology & Behavior **52**, 1047–1053.

Loinger, C., Cohen, S., Dror, N. and Berlinger, M.J. (1977) Effect of grape cluster rot on wine quality. American Journal of Enology and Viticulture **28**, 196–199.

Lopez, R., Aznar, M., Cacho, J. and Ferreira, V. (2002) Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. Journal of Chromatography A **966**, 167–177.

Marchetti, R., Guerzoni, M. and Gentile, M. (1984) Recherche sur l'etiologie d'une nouvelle maladie de la grappe: la pourriture acide. Vitis **23**, 55–65.

Moskowitz, H.R. (1980) Psycometric evaluation of food preferences. Journal of Foodservice systems **1**, 149–167.

Prescott, J., Norris, L., Kunst, M. and Kim, S. (2005) Estimating a 'consumer rejection threshold' for cork taint in white wine. Food Quality and Preference **16**, 345–349.

Roessler, E.B., Baker, G.A. and Amerine, M.A. (1956) One-tailed and two-tailed tests in organoleptic comparisons. Journal of Food Science **21**, 117–121.

Saenz-Navajas, M.P., Campo, E., Cullere, L., Fernandez-Zurbano, P., Valentin, D. and Ferreira, V. (2010) Effects of the nonvolatile matrix on the aroma perception of wine. Journal of Agricultural and Food Chemistry **58**, 5574–5585.

Saliba, A.J., Bullock, J. and Hardie, W.J. (2009) Consumer rejection threshold for 1,8-cineole (eucalyptol) in Australian red wine. Food Quality and Preference **20**, 500–504.

San Juan, F., Ferreira, V., Cacho, J. and Escudero, A. (2011) Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of Spanish red wines can be predicted from their aroma-active chemical composition. Journal of Agricultural and Food Chemistry **59**, 7916–7924.

Tat, L., Comuzzo, P., Battistutta, F. and Zironi, R. (2007) Sweet-like off-flavor in aglianico del vulture wine: ethyl phenylacetate as the mainly involved compound. Journal of Agricultural and Food Chemistry **55**, 5205–5212.

Villanueva, N.D.M., Petenate, A.J. and Da Silva, M. (2000) Performance of three affective methods and diagnosis of the ANOVA model. Food Quality and Preference **11**, 363–370.

Wightman, F. and Lighty, D.L. (1982) Identification of phenylacetic acid as a natural auxin in the shoots of higher-plants. Physiologia Plantarum **55**, 17–24.

Yoo, Y.J., Saliba, A.J., Prenzler, P.D. and Ryan, D. (2012) Total Phenolic Content, Antioxidant Activity, and Cross-Cultural Consumer Rejection Threshold in White and Red Wines Functionally Enhanced with Catechin-Rich Extracts. Journal of Agricultural and Food Chemistry **60**, 388–393.

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