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Investigation of olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix

Marianne McKay*, Florian F. Bauer, Valeria Panzeri, Astrid Buica

Department of Viticulture and Oenology, Stellenbosch University, South Africa

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ABSTRACT

The qualitative sensory perception of individual and of complex mixtures of five compounds, guaiacol ('burnt note'), *o*-cresol ('phenolic/tar'), 4-ethylphenol (4-EP, 'leather/barnyard'), 2-*iso*-butyl-3-methoxypyrazine (IBMP, 'green pepper/herbaceous'), and 2,4,6-trichloroanisole (TCA, 'cork taint/ mouldy') were tested in a partially de-aromatised red wine matrix using descriptive analysis by a trained panel of eleven judges. Compounds were characterised at peri- and sub-threshold concentrations using a partial D-optimal statistical design and response surface methodology. Results indicated that complex mixtures in red wine elicit an olfactory response that could not be predicted from the attributes or descriptors of single compounds. Positive sweet/fruity attributes were more intense in solutions containing fewer off-flavour compounds. Novel findings of this study include that IBMP at sub- and *peri*-threshold levels shows perceptual interaction with volatile phenols at the same levels, and samples containing combinations of these compounds manifested herbaceous and burnt characteristics. Olfactory interactions of this many off-flavour compounds have not been investigated previously in one study. The findings have direct implications for wines made from cultivars that are known to contain these compounds, and add to the understanding of the behaviour and impact of very low levels (peri- and sub-threshold) of volatile phenols, IBMP, and TCA derived from various sources during winemaking.

1. Introduction

Wine faults, such as those caused by microbial contamination, and lack of typicality of style are often detected by olfaction (Perry & Hayes, 2016). Frequently used indicators of the potency of aroma compounds associated with aroma in wine and many other matrices include odour detection threshold (ODT) (Czerny, Brueckner, Kirchhoff, Schmitt, & Buettner, 2011), odour activity value (OAV) measurements (Audouin, Bonnet, Vickers, & Reineccius, 2009), and, more recently, "aroma vectors", a combination between OAV and groups of descriptors (De-la-Fuente-Blanco, Sáenz-Navajas, Valentin, & Ferreira, 2020; Ferreira et al., 2016). In practice, a large number of issues influence the perception of the compounds involved, including matrix effects (McKay, 2019; Perry & Hayes, 2016; Pozo-Bayón, Pérez-Jiménez, Muñoz-González, Jiménez-Girón, & Esteban-Fernández, 2017) and the so-called 'sensory interaction' of compounds even at sub-threshold (infra-threshold) levels (McKay, 2019; Tempere, Schaaper, Cuzange, de Revel, & Sicard, 2017) which affect their perception by judges.

Interactions between odorous components may impact the evaluation of sensory quality of food stuff. In wine, some olfactory antagonistic effects have been studied (Lapalus, 2016; Tempere et al., 2016; Wilson, Brand, du Toit, & Buica, 2018) and several specific effects have been reported. For example acetic acid and ethyl phenols mask fruity notes (Atanasova et al., 2005; Campo, Ferreira, Escudero, & Cacho, 2005), while TCA even at sub-threshold levels decreases the perception of fruitiness and overall intensity of a Merlot wine (Tempere et al., 2017).

In wine, volatile phenols are frequently reported as being present. These compounds can be derived from storage in oak barrels (Chatonnet, Dubourdie, Boidron, & Pons, 1992) which have undergone toasting/firing during production or from smoke events near vineyards (Kennison, Gibberd, Pollnitz, & Wilkinson, 2008), and elevated levels can also be associated with the presence of *Brettanomyces* yeast infections (Botha, 2010; Curtin, Bramley, Cowey, Holdstock, Lattey, Coulter, & Godden, 2008). Although the attributes and odour detection thresholds of a number of VPs are discussed in the literature (Czerny et al., 2011; McKay, Bauer, Panzeri, & Buica, 2018; Tempere et al., 2017), the characteristics of specific VPs in combination with each other or other compounds are largely unexplored. Individually, guaiacol is known to impart a smoky, burnt character and exhibits the lowest aroma detection threshold of the VPs (Spillman, Iland, & Sefton, 1998). Guaiacol is generally the most abundant of the VPs detected in smoke-affected

* Corresponding author at: P/Bag X1, Matieland 7602, Stellenbosch, South Africa.
E-mail address: marianne@sun.ac.za (M. McKay).

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wines (Kennison, Wilkinson, Pollnitz, Williams, & Gibberd, 2011; Ristic et al., 2011), and is considered to be an important indicator for smoke-related taints. Although the impact of 'woodiness' (Atanasova et al., 2005) and 'bretty character' (ethyl phenols even at sub-threshold levels) (Tempere et al., 2016) on the perception of fruit odour in wine has been investigated there are no studies, to our knowledge, that combine volatile phenols with the classic 'green descriptor' 2-isobutyl-3-methoxypyrazine (IBMP) and the compound largely responsible for 'cork taint' (2,4,6-trichloroanisole, TCA).

It has been shown that binary mixtures of volatile phenols and IBMP in red wine did not give olfactory results that can be predicted from the attributes of the single compounds in the matrix (McKay, 2019). Perception of odour mixtures containing more than two components is far more complicated due to interactions arising from the complex chemical signal encoding and processing within the olfactory system (Kaeppler & Mueller, 2013; Laing, Eddy, & John Best, 1994). In the case of complex mixtures, the odour quality of the mixture is more frequently different to the quality of odorants in the mixture, and it has been noted that complex mixtures are more likely to induce the perception of a new odour (Ferreira, 2012; Livermore & Laing, 1998).

The rationale for this study was to investigate whether a range of negative descriptors (for example, 'tar', 'burnt rubber', 'earthy', 'animal', 'dusty') that have on occasion been attributed to red wines can be explained by the interactions between volatile phenols (VPs), IBMP, and TCA. One hypothesis to explain the perception of these descriptors is that the combination of such compounds could lead to the formation of new odour precepts/odour objects as outlined by Thomas-Danguin et al. (2014). Previous research by Panzeri (2013) and McKay (2019) showed olfactory perceptual effects when VPs were combined in red wine, in particular combinations involving 4-ethylphenol (4-EP) and *o*-cresol.

In the current study, descriptive analysis (DA) was chosen to evaluate mixtures of volatile phenols and IBMP and TCA. DA is the most recognised sensory methodology, is quantitative, and can be used to describe differences between products and the main sensory drivers (whether positive or negative) (O'Sullivan, Kerry, & Byrne, 2011). Five odour-active compounds were selected that had been associated with specific taint issues in wine aroma (Table 1), namely guaiacol, *ortho*-cresol (*o*-cresol), 4-ethylphenol (4-EP), 3-isobutyl-2-methoxypyrazine (IBMP) and 2,4,6-trichloranisole (TCA). Aroma descriptors for these compounds cover a continuum from 'burnt, smoky' and 'medicinal' to the 'herbaceous, green' aromas associated with IBMP (Allen, Lacey, & Boyd, 1996). When working with a large number of compounds, the appropriate experimental design is of outmost importance in reducing the number of samples to be assessed, as a full factorial design is practically impossible to implement (Yu, Low, & Zhou, 2018). The advantage of DA in this case is that the panel is trained and tested for their calibration on the intensity of the attributes; as a consequence, results from more than one session can be combined and a larger than usual number of samples can be evaluated. Even so, a design including samples spiked with five compounds at three levels (control/zero, sub-, and *peri*-threshold) has not been yet reported in one sensory experiment.

Therefore, the aim of this study was to investigate the sensory effects of the five taint-related compounds listed above in a partially dearomatised red wine matrix, in order to elucidate whether attributes associated with red wines, including 'burnt rubber', 'tar', and 'herbaceousness', might result from 'perceptual blending' and new odour-object formation resulting from complex mixtures of odorants. In addition to the academic value of the work brought on by the number of compounds and the experimental design, the results of the work could assist in explaining the findings of other researchers that are trying to reduce the negative sensory impact of volatile phenols in red wines using fining agents (Filipe-Ribeiro, Cosme, & Nunes, 2018; Milheiro, Filipe-ribeiro, Cosme, & Nunes, 2017) or evaluating the effects certain winemaking techniques have on the volatile phenol composition of wine (Kelly, Zerihun, Hayasaka, & Gibberd, 2014; Sommer, Wegmann-Herr, Wacker, & Fischer, 2018).

2. Materials and methods

To achieve the aims of the study, it was important to work with a matrix as relevant as possible to a real case scenario, but one that did not show any overt sensory characteristics (Tempere et al., 2017; Wilson, Brand, du Toit, & Buica, 2019). As the study included five compounds at sub- and *peri*-threshold odour levels and a large number of combinations, it was important that the sensory method chosen had been previously demonstrated to be sensitive enough to detect low intensities of a range of attributes. The results were evaluated based on qualitative and quantitative criteria. Qualitatively, attributes from simple to complex mixtures were described, while the perceptual impact of these combinations was quantified using appropriate statistical tools such as univariate and multivariate analyses.

2.1. Base wine

An unwooded 2016 Shiraz wine (300 L, pH 3.6, alcohol 13% v/v) was supplied by a local wine producer (Koelenhof Cellar Ltd, Simonsberg, South Africa) and stored at 4 °C in 25 L food-grade plastic containers under nitrogen at the Department of Viticulture and Oenology, Stellenbosch University, South Africa. A benchtop screening by five experienced sensory judges with tested sensitivity for the aroma compounds to be used in the study confirmed that the base wine was free of any form of 'mouldy' or 'herbaceous' odours that might have been associated with IBMP, and also any 'mouldy' or 'cork-taint' issues associated with TCA. The wine had an odour profile that was dominated strongly by fruit and berry aromas, which warranted partial dearomatization with activated charcoal powder (Merck, Darmstadt, Germany) following the method outlined by Wilson et al. (2018) prior to the wine being used in investigations into sub-threshold olfactory interactions. In a screening session, the expert panel chose a blend of 50:50 charcoal-treated wine to untreated wine which yielded a neutral wine base with low aromatic intensity. Analysis of volatile phenols in the wine was performed by gas chromatography-mass spectrometry (GC–MS) following the method described by de Vries, Mokwena, Buica, and McKay (2016) to determine the levels of the VPs present in the partially dearomatised base wine. The guaiacol level was 1.37 μg/L, *o*-cresol was 0.08 μg/L and 4-ethylphenol concentration was 1.4 μg/L.

Table 1
Odour Detection Thresholds (ODTs) in μg/L (unless otherwise indicated) in red wine and descriptors for compounds used in this study.

Compound	ODT	Descriptors	Reference
guaiacol	23 (red)	burnt, smoky, toasty, phenolic	Parker et al. (2012)
o-cresol	62 (red)	burnt, smoky, medicinal, tar	Parker et al. (2012)
4-EP	605 (red)	leather, bacon, medicinal, horse	Chatonnet et al. (1992)
IBMP	15 (red) ng/L	green, herbaceous, bell pepper	Roujou De Boubée, Van Leeuwen, & Dubourdieu (2000)
TCA	3.7 ng/L*	mouldy, musty, damp cardboard	Prescott, Norris, Kunst, & Kim (2005)

* Consumer rejection threshold.

Table 2
Spiking regime for 36 samples with five compounds in dearomatised red wine. The sample names are coded in the following order: (position 1: guaiacol), (position 2: *o*-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5: TCA) at 0, peri- (A) and subthreshold (B) levels. Control sample (00000) is shaded.

sample	D-Opt code	Guaiacol (µg/ L)	*o*-cresol (µg/L)	4-EP (µg/L)	IBMP (ng/L)	TCA (ng/L)
1	0AB0B	0	62	400	0	2
2	A0AAA	23	0	605	10	4
3	00A00	0	0	605	0	0
4	BBAA0	15	40	605	10	0
5	BB000	15	40	0	0	0
6	0ABA0	0	62	400	10	0
7	B00AA	15	0	0	10	4
8	ABBB0	23	40	400	7	0
9	AAA00	23	62	605	0	0
10	A0A00	23	0	605	0	0
11	0B0AB	0	40	0	10	2
12	A000B	23	0	0	0	2
13	AAAA0	23	62	605	10	0
14	00AA0	0	0	605	10	0
15	0BA0A	0	40	605	0	4
16	0AAAA	0	62	605	10	4
17	AB0BA	23	40	0	7	4
18	0AA00	0	62	605	0	0
19	AAA0A	23	62	605	0	4
20	BA0A0	15	62	0	10	0
21	AA00A	23	62	0	0	4
22	B0B0B	15	0	400	0	2
23	0A0AA	0	62	0	10	4
24	00,000	0	0	0	0	0
25	0A00A	0	62	0	0	4
26	00ABA	0	0	605	7	4
27	AA0AB	23	62	0	10	2
28	BAABB	15	62	605	7	2
29	A00A0	23	0	0	10	0
30	000B0	0	0	0	7	0
31	A0A0A	23	0	605	0	4
32	0000A	0	0	0	0	4
33	A0AAB	23	0	605	10	2
34	AABAA	23	62	400	10	4
35	AA000	23	62	0	0	0
36	00BAA	0	0	400	10	4

2.2. Experimental design

As DA is a time-consuming, fatiguing and expensive sensory method and generates a large amount of complex data, a D-optimal statistical design was chosen before practical implementation (Yu et al., 2018). Wine samples were spiked with combinations of the five compounds at three levels each, following the partial D-optimal design (Table 2) constructed with Statistica 12.

This design is ideal for multi-factor experiments with both quantitative and qualitative factors, at a mixed number of levels, as in this study. Combined with surface response methodology, this design generates contour plots by linear or quadratic effects of the key variables, and complex interactions can be visually represented. The D-optimal design is known to minimise the generalised variance of estimated regression coefficients (NCSS Statistical Software, 2010), and is useful when it is not possible to run a fully replicated factorial design, or there are budget and time constraints (such as those associated with running a sensory panel).

2.3. Preparation of spiked samples

Stock solutions of 1000 mg/L of the four compounds were prepared in 99.5% ethanol (Merck Darmstadt, Germany). Guaiacol (99.3% purity), 4-EP (99.5% purity), *o*-cresol (99%), IBMP (99%) and TCA (99%) were obtained from Merck, (Darmstadt, Germany). The compounds were dissolved in ethanol (10 mL) and then made up to volume with ultra-pure distilled water (Millipore, Bedford, MA, USA) to the concentrations required for spiking, *i.e.*: 100 mg/L for *o*-cresol and guaiacol; 1000 mg/L for 4-EP; and 5 µg/L for IBMP. Base wine was then spiked with an appropriate volume of stock solution to achieve the concentrations of each volatile compound required for detection threshold determinations. These solutions were used to produce wine samples spiked with the desired concentrations of each compound. For threshold levels, guaiacol concentration was 23 µg/L, *o*-cresol 62 µg/L, 4-EP 605 µg/L, IBMP 10 ng/L, and TCA 4 ng/L, respectively. For the subthreshold levels, a benchtop pre-screening by a focus group of experienced researchers established that 60–70% of the ODT did not add distinguishable attributes to the base wine. Base wine was spiked within 24 h of sensory analysis and stored at 5 °C in the dark. All levels used were subjected to extensive sensory pre-screening in order to determine whether they adhered to the sensory criteria set.

2.4. Panel selection

The panel consisted of 11 judges, all non-smoking females between the ages of 24 and 60. Judges had previous experience in the use of descriptive analysis, and experience in smoke taint evaluation in wine. Most of the panellists also took part in the determination of odour detection thresholds for these compounds and therefore already had some familiarity with the compounds under investigation.

2.5. Sensory training

A combination of consensus and ballot training was conducted before testing in six two-hour sessions over a period of two weeks. Twelve samples from the 36-sample D-optimal design (Table 2) as well as two clean controls were discussed in two consecutive sessions. Reference standards were presented in 50 mL amber glass bottles (Consol glass, RSA) following formulations adapted from Noble et al. (1987). For the first 30 min of each training session, panellists were asked to re-familiarise themselves with the specific aromas. The spiked wines were then profiled using descriptive analysis (DA) according to the general descriptive method (Lawless & Heymann, 2010). These discussions generated a comprehensive list of descriptors that included the familiar attributes, but also new attributes that were unique to the wines under study. The panel were also asked to rate the intensity of the various aromatic attributes. A final attribute list for testing was confirmed after the final training session. The twenty two attributes, agreed upon through consensus by the panel, included: 'dark berries', 'red berries', 'floral/ violets', 'prunes/raisins', 'vanilla/caramel', 'tobacco', 'pencil shavings', 'herbaceous/green', 'cooked veg', 'leather/barnyard', 'earthy/dusty/potato skin', 'smoky', 'ashtray', 'medicinal/Elastoplast™', 'mouldy/musty', 'black pepper', 'liquorice', 'tar/burnt rubber (BR)', 'soy sauce', 'rubber/chemical', 'acetone (nail varnish)' and 'alcohol'.

2.6. Sensory testing

Sensory testing took place in six consecutive sessions over two weeks. Wines 1–18 in the D-optimal design were tested in the first three sessions (in triplicate), with wines 19–36 replicated over the next three sessions. Although the panel was experienced in volatile phenol and smoke taint related sensory analysis, in this project, 18 (3 flights of six) samples per testing session was considered the maximum that the panel would be able to cope with without becoming fatigued. Testing was carried out in a sensory laboratory equipped with individual booths with standard artificial daylight lighting and temperature control at 20 ± 1 °C. Coded wines were presented to judges in black ISO 3591:1977 standard glasses and covered with plastic lids. The order of samples was 'counterbalanced' across individuals, by changing the presentation order, as recommended by Lawless and Heymann (2010). All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. Judges were asked to evaluate samples orthonasally (i.e., by sniffing). Communication was not allowed between the judges for the duration of the test.

2.7. Data analysis

Sensory data produced during DA was analysed with Statistica, Version 12 (StatSoft, USA) to generate one-way and two-way ANOVAs, and Pareto charts of standardised effects combined with fitted Surface Response plots which were able to evaluate relative significance of several treatment factors in the presence of complex interactions. Least Squares Means (LSM) diagrams for attributes and compounds were produced from the two-way ANOVAS to determine significant differences between attributes and compounds, and to account for judge effects (different letters denoting significant differences at $p \leq 0.05$). Data were also examined for trends between attributes using Windows Excel (Microsoft Corp, Redmond, WA, USA). Principal Component Analysis (PCA) biplots were compiled from datasets for individual compound samples, and binary samples using PanelCheck to help explain variance (Lawless & Heymann, 2010). All quoted uncertainty is the standard deviation of three replicates of one treatment.

3. Results and discussion

The results are presented according to the methodology. The initial exploration of the qualitative nature of the attributes generated from simple to complex mixtures is followed by the quantitative overview of the impact of the number of components added. Additionally, the effect of single compounds was confirmed in the study matrix. The perceptual interactions of mixtures were explored from binary to a higher order of complexity.

3.1. General overview of effects of interactions

The PCA biplot generated from the sensory results of the individual compounds spiked at their ODTs (A) - and subthreshold (B) levels in the partially dearomatised Shiraz wine is shown in Fig. 1. Attributes associated with five compounds (guaiacol (position 1), *o*-cresol (position 2), 4-EP (position 3), IBMP (position 4) and TCA (position 5)) separate approximately according to fruity/sweet-associated and earthy/herbaceous / burnt attributes, and the concentration of samples with a higher number of compounds can be seen on the biplot (yellow, orange and red colours).

The first two principal components explain 68% of the variance in the data, which is considered satisfactory for complex sensory datasets such as this one. The low levels (sub- and *peri*-threshold) and high number (five) of aroma compounds used in the study, the nature of the matrix (red wine) as well as the spiking regime could have contributed to the explained variance. In total, 36 samples were evaluated and the differences at a higher order of complexity were expected to be subtle. For example, between two quaternary mixtures, three compounds could be the same and only the fourth might have varied at sub-threshold level. However, inspection of the PCA shows a tendency for more complex samples (> 3 components) to be associated with the burnt/chemical and earthy/dusty negative attributes aligned on the positive side of PC1, and samples with fewer attributes (1–3 components - green and blue coloured) associated with the more positive sweet/fruity attributes on the negative side of PC1. It is notable that samples containing TCA (position 5) are not associated with the 'mouldy/musty' attribute, as is generally accepted to be the case. There does not seem to be a difference between peri- (A) and subthreshold (B) values on olfactory perception for the volatile phenols (first three positions in the sample code). There was an association between higher levels of IBMP and TCA with the 'earthy/dusty', 'cooked veg' and ashtray' attributes. Samples without IBMP and TCA do seem to position closer to the sweet-fruity 'tobacco', 'plums' and 'vanilla/caramel' attributes. The complexity of the PCA prompts an investigation of the sensory data to clarify effects of interactions of pairs of individual compounds.

3.2. Frequency of citation for five compounds

Attributes were subjected to a post-hoc examination of trends within the frequencies of citation. The frequency of citation (FC) of perceived attributes associated with control (clean) base wines ($n = 108$), wine spiked with single compounds ($n = 108$), binary ($n = 288$), tertiary ($n = 408$), and quaternary mixtures ($n = 322$) was assessed, as well as those associated with mixtures of all five compounds ($n = 72$). Attributes were counted regardless of their intensity, and all attributes were allocated an equal weight in the frequency of citation, whether they were intense, or present at very low levels.

As can be seen from Fig. 2, the FC of fruity/sweet-associated attributes generally decreased with increases in number of VPs or IBMP and TCA spiked into solution. This indicates that the panel's perception of the presence of these attributes gradually decreased as the mixture became more complex. This was independent of the nature of the attribute, and is consistent with the idea that a mixture of odorants could induce a note different from the one carried by its components as put forward by Barkat, Le Berre, Coureaud, Sicard, and Thomas-Danguin (2012). Positive 'sweet/fruity' attributes that are markedly affected by the presence of spiked compounds, and FC decreases as the complexity of the mixture increases include the 'prunes/raisins', both 'berry' attributes, 'plums', 'floral violets' and 'vanilla/caramel' attributes. Fig. 3A shows that the 'tobacco' attribute is also cited less frequently in wine spiked with mixtures of volatile phenols, IBMP and TCA.

The FC of various attributes demonstrates that if mixtures were more complex, and contained greater numbers of volatile phenols, the fruity attributes were cited less frequently and attributes generally perceived as negative to wine quality, for example: green-associated attributes like 'herbaceous' and 'cooked veg', were perceived more often. This would be expected if IBMP was involved, but the FC is unrelated to whether samples did or did not contain IBMP. This trend is also seen in other attributes, specifically 'earthy/dusty/potato skin', 'mouldy/ musty', and to a lesser degree, 'leather/barnyard'.

Fig. 3B illustrates the effects of increasing complexity of spiking regime on the 'chemical'- and 'burnt'- associated attributes. Here also, there seem to be some trends between perception of an attribute and increasing number of spiked compounds. The control sample (0) appears to have had some' smoky', 'ashtray' and' tobacco' attributes, but it is clear that the number of judges perceiving 'tar/BR, 'medicinal/Elastoplast^{TM}', 'rubber/chemical' and 'ashtray' attributes are increased if more compounds are present. Wilson and Stevenson (2010) noted that the more complex an odorant mixture is, and the more features of olfactory receptor activation overlap, the more difficult the task of perceptual grouping. It is not possible to gauge whether the panel would have created new odour-objects (as hypothesised in literature concerning olfactory perception) to describe the more complex solutions if they had been presented with these solutions for the first time, and were able to freely assign descriptors. It may be that the increases in frequency of citation are merely a representation of the 'halo effect' where attributes are assigned to the closest descriptor, losing some of the rich blending/interaction information, as suggested by Barkat et al. (2012) and that DA is not the ideal way to test for interaction effects.

3.3. Interaction effects between compounds

The information on significant effects per attribute was extracted from the Pareto chart of standardised effect estimates for each attribute (Table 3). Pareto charts plot ANOVA effect estimates, or standardised effect estimates in decreasing order of relative frequency sorted by size/absolute value, with a vertical line to indicate the minimum magnitude of statistically significant effects, given the current model and choice of error term (TIBCO, 2017). Quadratic ('Q') effects denote statistical significance when the response surface contains curvature and the

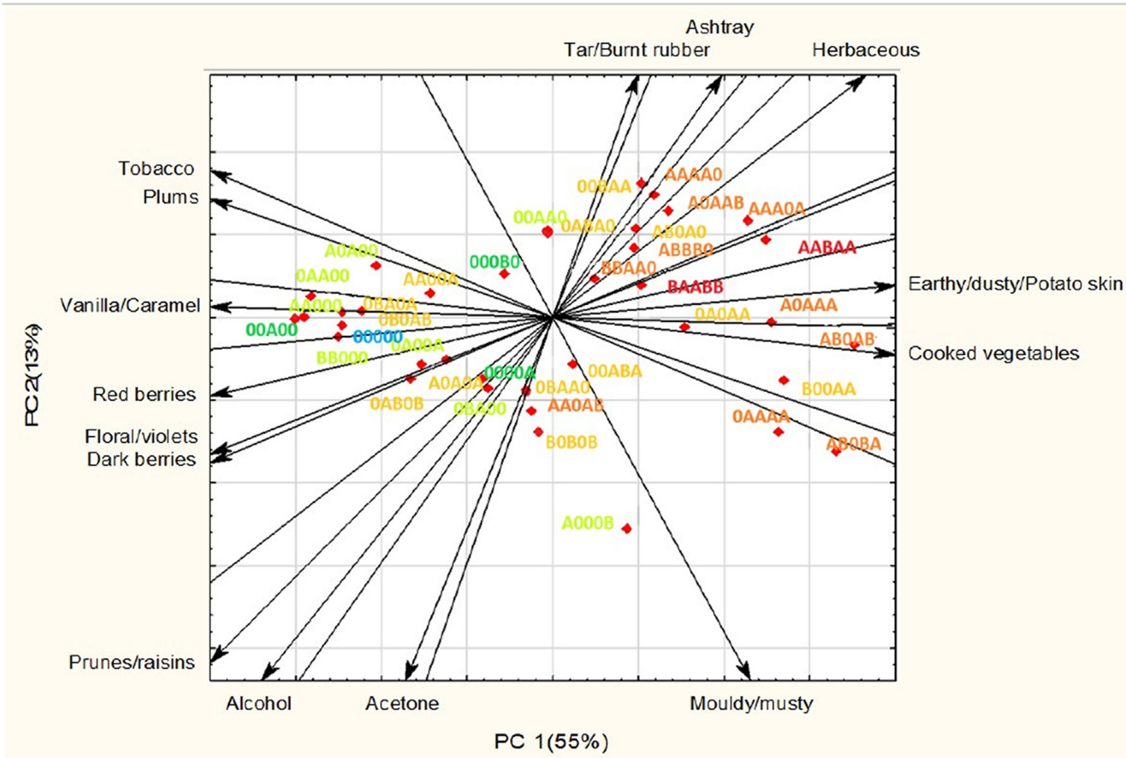

Fig. 1. PCA biplot of the general sensory results of attributes of samples spiked with combinations of the three VPs, IBMP and TCA in dearomatised Shiraz wine in the following order: (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5: TCA) at 0, peri- (A) and subthreshold (B) levels. Control sample (zero spike) in blue, single compound spikes in green, two compound spikes in light green, three compound spikes in gold, four compound spikes in orange and five compound spikes in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

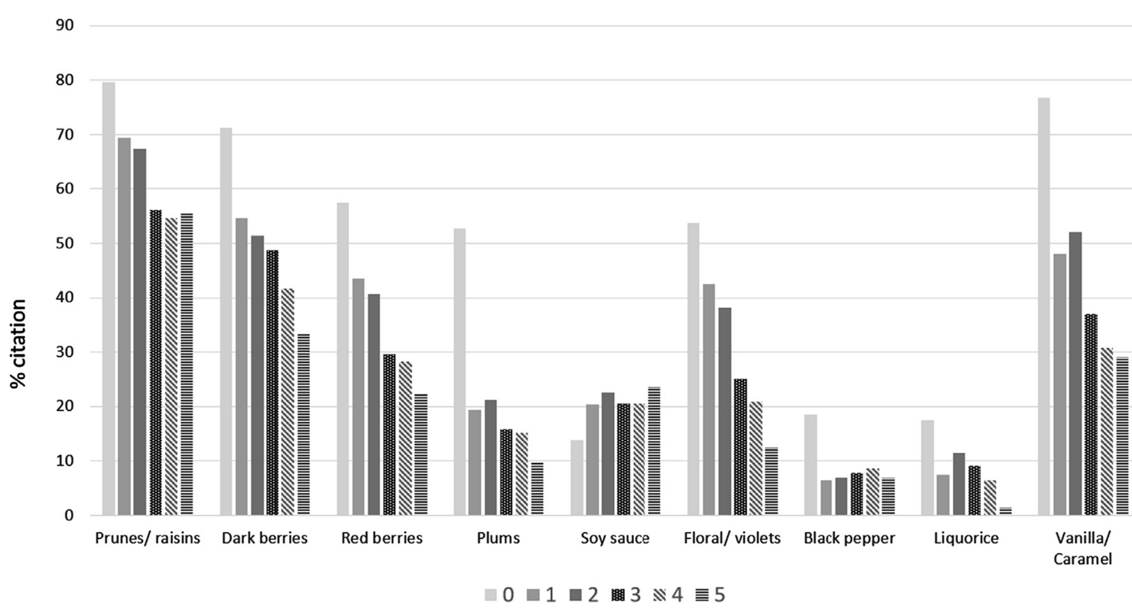

Fig. 2. Bar graph showing the effect of increasing number of spiked compounds (0–5) on frequency of citation (% of maximum possible counts of each attribute) of fruity / spicy attributes perceived in solution.

association is not just linear, 'L' denotes linear effect, and 'L by L' the linear interaction effects of pairs of variables/factors on the system. Changes in these factors are associated with changes in the response variable. If an interaction effect between two factors is significant, changes in each factor are associated with changes in the response variable, but the effects depend on the other factor. In this study, standardised effects estimates were illustrated, and $p = 0.05$ was used as the criterion of statistical significance. Table 3 shows standardised effects for the study ($p < 0.05$) which estimate linear (L), quadratic (Q), and linear interactions (L by L) between factors. Although a great many smaller interactions and effects were observed as a result of the addition of the compounds to the dearomatised red wine matrix in this study, only the most significant effects on perception of attributes will be discussed per compound, and then according to interactions between pairs of compounds.

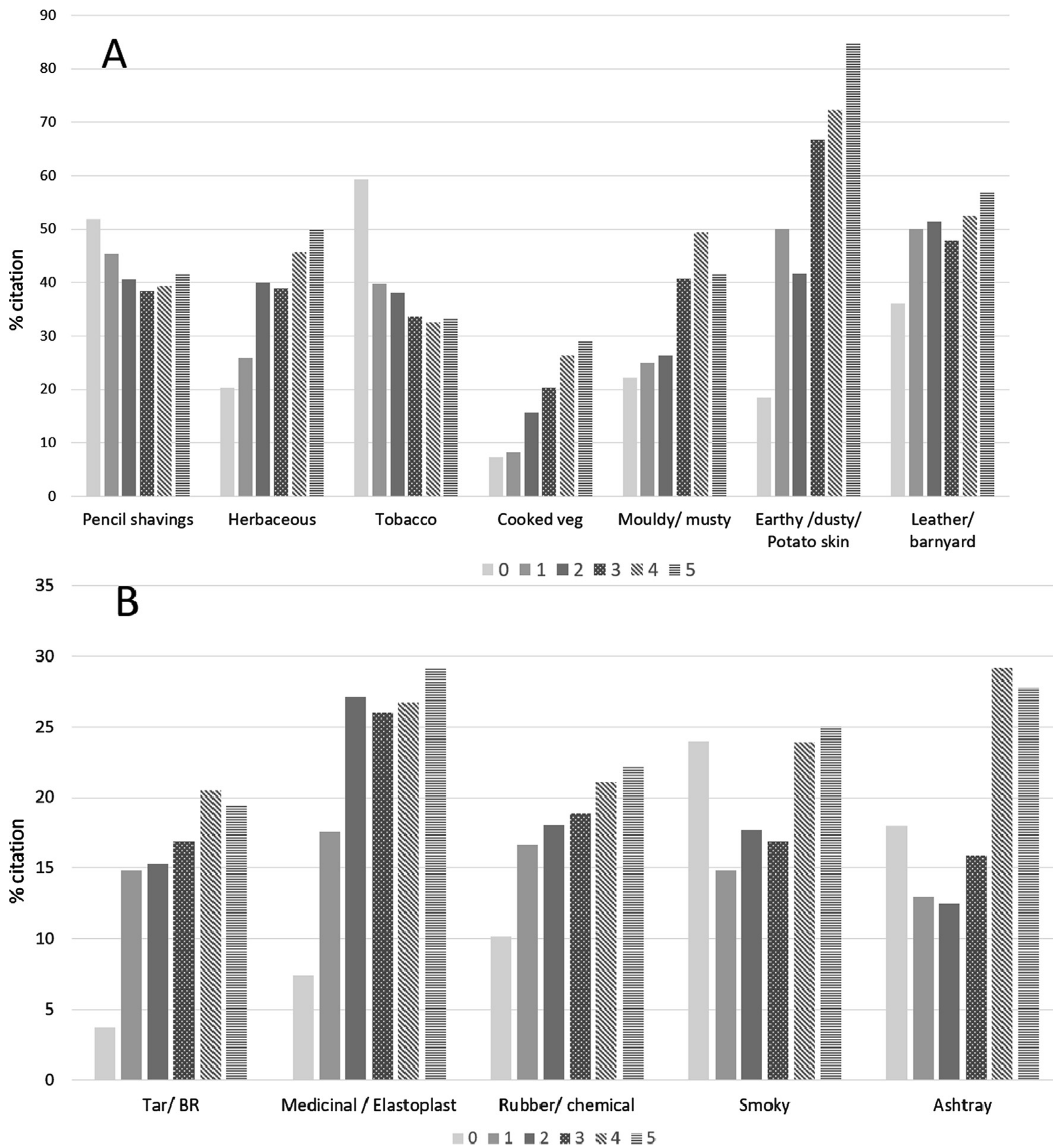

Fig. 3. Bar graph showing the effect of increasing number of spiked compounds (0–5) on frequency of citation (% of maximum possible count of each attributes) of (A) woody and earthy attributes (B) chemical/burnt attributes perceived in solution.

3.3.1. *Effects per single compound*

Three of the study attributes were not significantly affected by any of the five compounds at either level (sub- or *peri*-threshold), or any combination of compounds. These were 'soy sauce', 'alcohol' and 'acetone'.

Guaiacol on its own significantly decreased ($p < 0.05$) perception of the attribute 'plums' as spiking levels increased across the linear range 0, 15, and 23 μg/L. The attributes 'smoky', 'ashtray' and 'medicinal/Elastoplast™' were decreased in a quadratic (non-linear) effect across increasing levels of guaiacol. The presence of guaiacol alone does not explain the increases in smokiness found with increasing complexity of added compounds, so clearly the other compounds had an effect on this descriptor, as will be seen in the ensuing discussion of interactions between compounds to produce various attributes. When *o*-cresol was present on its own in solution, the perception of 'dark berries' was decreased significantly relative to the control, 'mouldy/ musty', 'herbaceous' and 'earthy/dusty/ potato skin' attributes were perceived to increase. This has not been shown previously in the limited number of studies that investigate the effects of this compound, where *o*-cresol has been associated with 'burnt' and 'phenolic' attributes (Table 1). It is interesting that sub- and *peri*-thresholds of *o*-cresol may be linked to herbaceousness and earthiness in red wine, even without the presence of methoxypyrazines. 4-EP, the compound normally responsible for perception of 'medicinal/ Elastoplast™' and 'leather/ barnyard' attributes in previous studies (Chatonnet et al., 1992; Romano, Perello, Lonvaud-Funel, Sicard, & de Revel, 2009), did not have a significant effect on 'medicinal/Elastoplast™' in this experiment, and was perceived to decrease the 'leather/barnyard' attribute significantly, and the effect was quadratic. The attributes 'cooked veg', 'herbaceous' and 'mouldy/musty' attributes were also decreased by the addition of 4-EP on its own in solution. Perception of the 'smoky', 'ashtray' and 'vanilla/caramel' attributes were increased significantly compared to the control across the range of additions. The two non-phenolic compounds, IBMP and TCA, had quite complex effects as single compounds in solution on various attributes, sometimes exhibiting different results in their linear and quadratic conditions

Table 3
Standardised effects ($p < 0.05$) per attribute extracted from Pareto's charts to estimate the linear (L), quadratic (Q), and linear interaction (L by L) between factors (Factor 1 = guaiacol, 2 = *o*-cresol, 3 = 4-EP, 4 = IBMP, 5 = TCA) (ns = not significant at p = 0.05).

Attribute	Pure error	Increased by	Standardised effect	Decreased by	Standardised effect
Dark berries	51.65	4Q	4.02	3L by 4L	−2.81
		4L by 5L	2.59	4L by 4L	−2.37
		2L by 3L	2.04	1L by 5L	−2.28
		5Q	2.02	2Q	−2.29
Red berries	40	5Q	1.9	5L	−2.65
				4L	−2.4
Plums	20.81	5Q	2.01	1L	−2.01
				2L by 5L	−1.95
Floral/violets	27.8	5Q	3.13	5L	−3.54
		4Q	3.16	4L	−2.82
				3Lx4L	−2.60
Prunes raisins	38.6	5Q	2.13	4L	−4.36
		4Q	2.06		
		1L by 2L	2.01		
Vanilla caramel	40.85	5Q	3.35	4L	−4.08
		4Q	3.21		
		3Q	2.19		
Tobacco	26.22	4Q	2.59		
Pencil shavings	38.92			3L by 5L	−2.66
Herbaceous	28.02	2L by 5L	3.12	4Q	−5.19
		3L by 4L	3.00	3Q	−3.75
		1L by 5L	2.77	4L by 5L	−3.41
		4L	2.29	2L by 3L	−2.43
		2L	1.96		
Cooked veg	21.64	4L	3.90	4Q	−4.80
		3L by 4L	3.33	3Q	−3.37
		2L by 4L	2.23	1L by 2L	−3.14
		4L by 5L	1.95		
Leather / barnyard	42.5			3Q	−2.35
				4L by 5L	−2.14
Earthy/dusty/ potato	63	4L	4.92	5Q	−5.06
		5L	3.71	4Q	−4.95
		2L	1.75		
Smoky	27.8				
		3L by 4L	2.77	4Q	−3.3
		3L	2.39	1Q	−2.33
		5Q	1.96		
Ashtray	24.6	3L	2.82	4Q	−3.37
		2Q	2.5	1Q	−2.02
		1L by 3L	1.38 (ns)		
		2L by 5L	1.38 (ns)		
Medicinal/Elastoplast	28.57	1L by 4L	2.43	1Q	−2.26
		1L by 3L	1.82 (ns)	2L by 4L	−2.24
				3L by 5L	−2.16
				5L	−1.99
Mouldy/ Musty	79.87	3L	2.48	5Q	−4.24
		4L	2.26	1L by 2L	−3.79
		2Q	2.19	3Q	−2.44
				2L by 5L	−1.76 (ns)
Tar/BR	25.42	3L by 4L	2.40	2L by 3L	−2.58
		1L by 4L	1.60 (ns)	4L by 5L	−2.34
Rubber/ chemical	21.75			3L by 4L	−2.32
				2L by 5L	−2.07

(Table 3).

IBMP had the most significant effect on its own in solution on the perception of a wide range of attributes in the study. Decreases (linear effect) were observed for sweet and fruity attributes, including 'floral/violets', 'red berries', 'prunes raisins', and 'vanilla/caramel'. Increases in perception of 'herbaceous', 'cooked veg', 'earthy/dusty/potato skin' and 'mouldy/musty' attributes were observed for increasing spike levels of IBMP. More subtle, quadratic effects of the single compound could been seen in its effect on the increases in perceptions of 'dark berries' and 'tobacco', and decreases in perception of 'smoky' and 'ashtray' attributes. TCA, reported frequently in the literature to be associated with 'cork taint' and 'mouldy/musty' aromas was seen to decrease perception of 'earthy/dusty/potato skin' and 'mouldy/musty' in quadratic effect as a single compound in this study. Perception of the 'dark berry', 'floral/violets' and 'medicinal/Elastoplast™' attributes were decreased in linear effect, but a number of sweet /fruity attributes of the wine seemed to be affected positively ($p > 0.05$) by this compound, which is counter-intuitive given its description as 'mouldy/damp cardboard'. These included 'red berries', 'plums', 'prunes /raisins', 'vanilla/caramel' and 'smoky' (Table 3). This result seems to be at least partly in contradiction to the literature, which reports that TCA even at values below ODT contributes to a reduction of the fruity note in wine (Tempere et al., 2017). The differences could be due to the cultivar used (Merlot in the literature *vs* Shiraz in this case), levels of spiking, and/or the methodology used (six point scale and triangle test *vs* DA).

3.3.2. Perceptual interactions of pairs of compounds

Guaiacol interaction with o-cresol (1L by 2L): Although not significant at the $p < 0.05$ level, the 'ashtray' attribute showed an interesting trend to increase when these two phenols were in combination. The

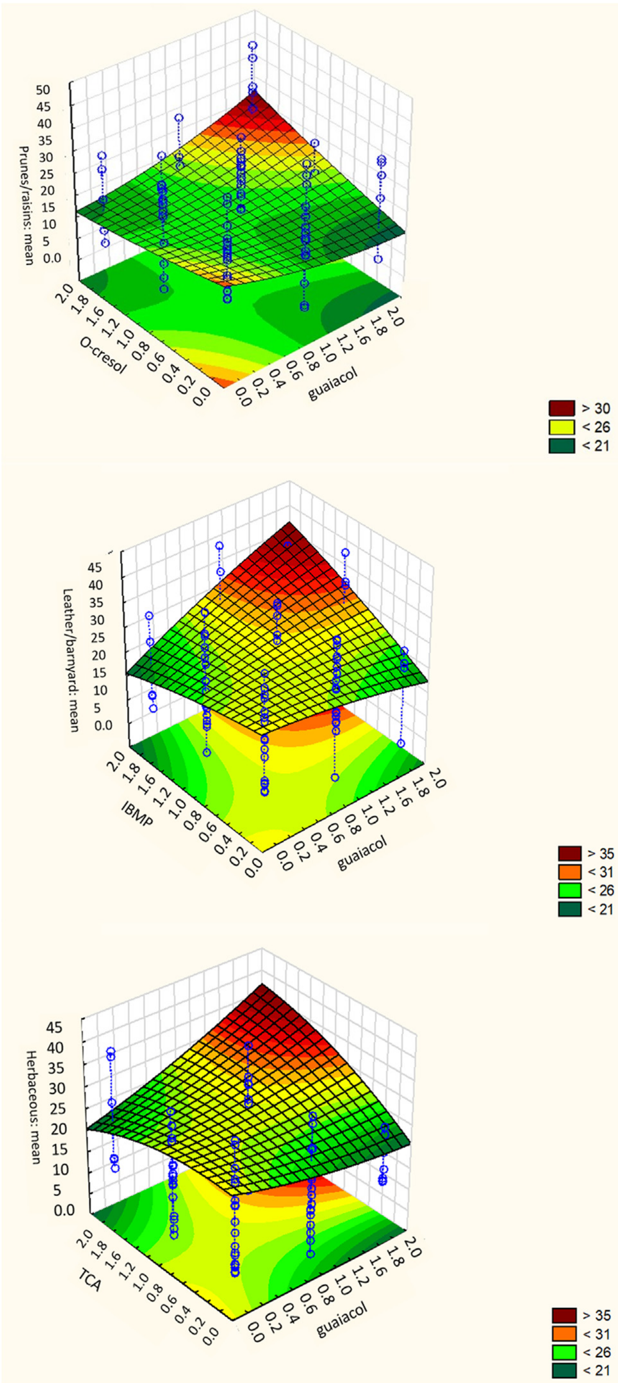

Fig. 4. Fitted surface plot showing interactions between guaiacol and *o*-cresol, IBMP, and TCA, for 'prunes/raisins', 'leather/barnyard', and 'herbaceous' attribute means, respectively (n = 108).

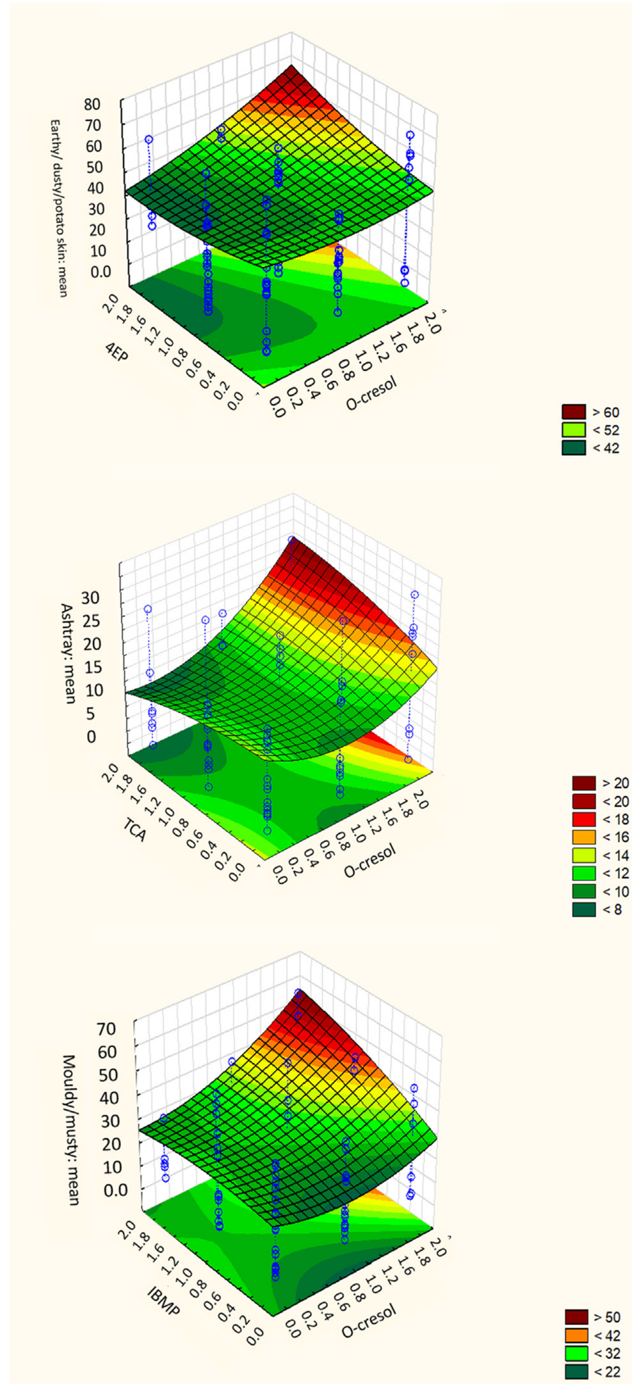

Fig. 5. Fitted surface plot for interaction effect of *o*-cresol with 4-EP, TCA, and IBMP on the 'earthy/dusty/potato skin', 'mouldy/musty', and 'ashtray' attribute means, respectively (n = 108).

attribute 'prunes/raisins' was also significantly increased (Fig. 4) by this interaction, which is also not explained by the descriptors for the compounds on their own in red wine. The attributes 'cooked veg' and 'mouldy musty' were significantly decreased ($p < 0.05$) by the interaction effect of guaiacol and *o*-cresol.

Guaiacol interaction with 4-EP (1L by 3L): Again, the combination of two volatile phenols guaiacol and 4-EP led to a perception of the attribute 'ashtray' and 'medicinal/Elastoplast™' increasing, although this was not significant at the $p = 0.05$ level.

Guaiacol interaction with IBMP (1L by 4L): Perception of the 'medicinal/Elastoplast™' attribute was significantly increased ($p < 0.05$) by the interaction of guaiacol with IBMP. 'Tar/BR' was increased, as was 'leather/barnyard' (Fig. 4) but these were not significant at $p < 0.05$.

Guaiacol interaction with TCA (1L by 5L): The perception of the 'dark berries' attribute was decreased significantly ($p < 0.05$) by the interaction of guaiacol with TCA compared to the control and single compound samples, but the perception of 'herbaceous' was increased significantly (Fig. 4).

o-Cresol interaction with 4-EP (2L by 3L): The interaction of *o*-cresol with 4-EP lead to significant increases in perception ($p < 0.05$) in 'leather/barnyard', 'dark berry' and 'earthy/dusty/potato skin' (Fig. 5) attributes compared to control and single compound samples. The attributes 'herbaceous' and 'tar/BR' were perceived to decrease significantly, which is interesting given the findings of Panzeri (2013), that 4-EP in combination with other phenols caused increases in olfactory perception of this attribute. This may be due to matrix effects, which will be addressed in subsequent work (McKay, 2019).

o-Cresol interaction with IBMP (2L by 4L): Perception of the attributes 'cooked veg' and 'mouldy/musty' (Fig. 5) increased significantly ($p < 0.05$) as a result of the interaction between IBMP and *o*-cresol. IBMP was shown to increase 'mouldy/musty' perception on its own, but it appears that the addition of *o*-cresol enhances the perception of this attribute. The perception of 'medicinal/Elastoplast™' was decreased by this interaction.

o-Cresol interaction with TCA (2L by 5L): The interaction of *o*-cresol and TCA causes an increase in the perception of 'herbaceous' ($p < 0.05$) and 'ashtray' (Fig. 5) attributes and a decrease in the perception of 'plums', 'rubber/chemical and 'mouldy/musty' (all $p < 0.05$).

4-EP interaction with IBMP (3L by 4L): Perception of the attributes 'dark berries', 'rubber/chemical' and 'floral /violets' was decreased significantly ($p = 0.05$) by the interaction of IBMP and 4-EP. The attributes 'cooked veg', 'herbaceous', 'smoky' and 'tar/BR' (Fig. 6) were seen to increase as a result of this interaction, which has implications for winemaking. In the event that cultivars (for example, Cabernet Sauvignon and Merlot Noir) are known to contain IBMP as part of their primary aroma profile (Allen et al., 1996), producers should be alert to the fact that the presence of volatile phenols, even at low levels, may enhance herbaceous, cooked veg, smoky and tar/BR attributes, which may be perceived as off-flavours.

4-EP interaction with TCA (3L by 5L): The interaction of 4-EP with TCA caused significant decreases in the perceptions of 'pencil shavings, and 'medicinal/Elastoplast™' compared to the original base wine.

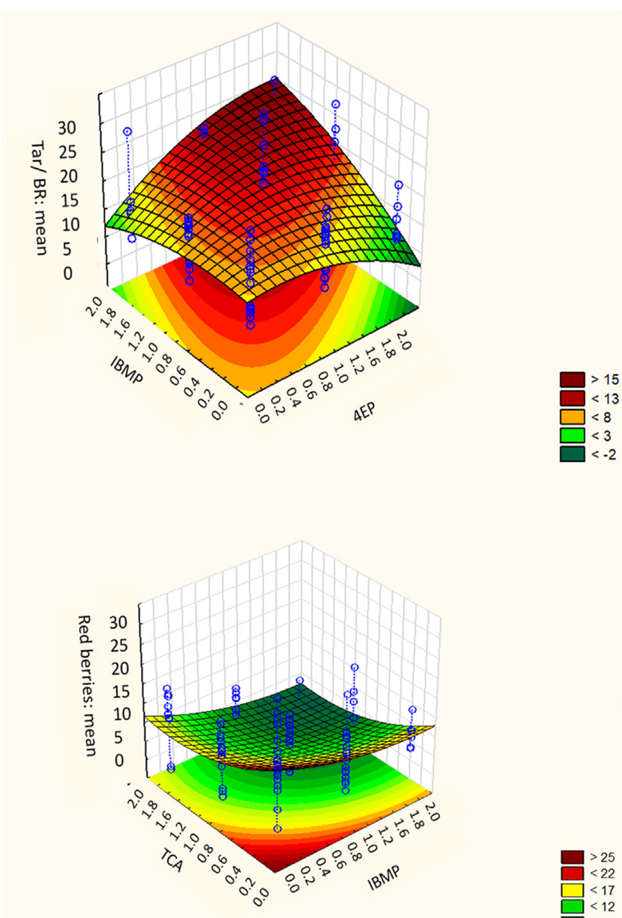

Fig. 6. Fitted surface plot for interaction effect of IBMP with 4-EP and TCA on the 'tar/BR' and 'red berries' attribute means ($n = 108$).

IBMP interaction with TCA (4L by 5L). The two non-phenolic compounds in the study showed some interesting interactions in combination. Unexpectedly, the perception of 'dark berries' was significantly increased ($p < 0.05$), as was 'red berries' (Fig. 6). The 'herbaceous' 'leather /barnyard' and 'tar/BR' attributes decreased, but 'cooked veg' increased. These interactions indicate that perception between seemingly related attributes like 'herbaceous' and 'cooked veg' (both viewed as 'green') can be complex, and depend on additional factors such as matrix effects, fatigue and experience of the panel.

3.3.3. Perceptual interactions between three or more compounds

A study of the Least Squares Means diagrams (Supplementary Figs. 1–14) for all the wines reveals that perceptions of increases in positive (fruity, sweet-associated) attributes, are caused mainly by single or binary combinations of VPs, but decreases are associated with higher numbers of compounds in solution. The increase in perception of 'floral /violets' (Supplementary Fig. 1) shows the caused by the combination of *o*-cresol and 4-EP in sample 0AA00, as well as the significant decreases compared to the control in samples containing IBMP and TCA (for example, 00ABA, 00BAA, 0B0AB). The 'prunes', 'red berries', 'vanilla/caramel' and 'dark berries' attributes (Supplementary Figs. 2–5) are also affected by combinations of samples containing VPs with IBMP and TCA, for example, 0B0AB, A0AAA, AB0BA and BAABB).

It is predominantly the earthy and chemical/burnt related descriptors that seem to be increased in samples with three or more added compounds. The 'leather/barnyard' attribute (Supplementary Fig. 6) is perceived to significantly increase over levels perceived in the control in the case of samples containing 4-EP at peri- and sub-threshold levels in combination with TCA and IBMP (samples 00BAA, 0BA0A and A0A0A). Even though a number of these perceptions were not significant, there are some notable trends, for example, the 'earthy/dusty/potato skin' attribute (Fig. 7), which shows increases compared to the control (sample 00000), in mixtures 0AAAA (*peri*-threshold levels of *o*-cresol, 4-EP, IBMP and TCA), 0B0AB (subthreshold levels of *o*-cresol and TCA, and *peri*-threshold levels of IBMP, AB0BA (*peri*-threshold levels of guaiacol and TCA, and subthreshold levels of *o*-cresol and IBMP. The 'cooked veg' attribute also shows some interesting increases in perception in combination compounds (Supplementary Fig. 8), indicating that samples with VPs and both IBMP and TCA are affected (samples 0B0AB, A0AAA and AABAA, for example).

The 'mouldy/ musty' attribute shows some interesting trends in samples containing combinations of TCA and VPs. When TCA was present as a single compounds, the effect on this attribute was not significant- indeed, TCA was seen to enhance some of the fruity aspects as a single compound. However, in combination with VPs, the 'mouldy/musty' attribute is significantly increased ($p < 0.001$) compared to the control (00000). This can be seen in Fig. 14, particularly in samples 0AAAA, 0AB0B, 0BA0A, A0AAA, AB0BA, and B00AA.

Although the attribute was present at a low level, the impact of combinations of compounds can also be seen in the LSM diagram for the 'ashy/ashtray' attribute (Fig. 7). When *o*-cresol or guaiacol are present in combination with TCA and/or IBMP, this characteristic seems to be enhanced. The 'smoky' attribute is enhanced when 4-EP is in combination with IBMP (Supplementary Fig. 13). This enhancement also affects the perception of the 'herbaceous' attribute (increased when IBMP or TCA are in combination with volatile phenols, as in samples 0AAAA, A0AAA, AABAA, 0BA0A and AAAA0).

This trend also affects the attribute 'tar/BR' which is likewise increased (not significantly) when volatile phenols and either TCA or IBMP are present, as in sample 00BAA, AAAA0 and A0AAB.

4. Conclusions

In order to explore interactions between five taint compounds

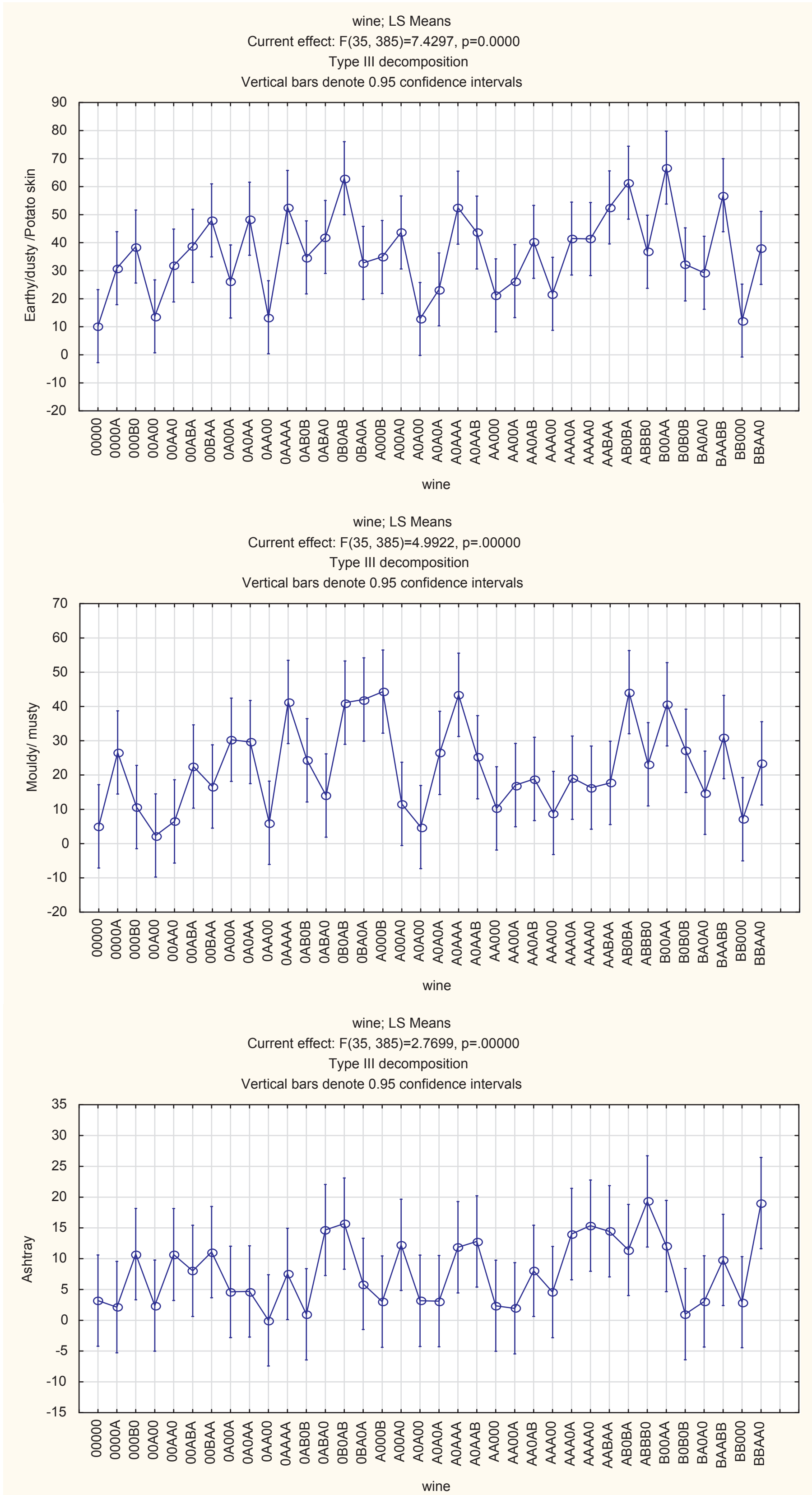

Fig. 7. Least Squares Means (Type III decomposition) diagram for 'Earthy/dusty/potato skin' attribute (p = 0.055). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5: TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals.

(guaiacol, *o*-cresol, 4-EP, TCA and IBMP) in red wine, base wine was spiked with low levels (*peri*-threshold and subthreshold) for each compound in a partial D-optimal design. The univariate and multivariate analyses of the sensory results demonstrated new odour-object formation. Generally negative attributes were not strongly associated with the descriptors assigned by the panel to the solutions containing single components, and did not always agree with expectations created by previous studies. For example, samples containing only TCA at low levels were not associated with the 'mouldy/musty' attribute until in combination with VPs and IBMP. In samples containing IBMP, the attributes 'cooked veg', 'herbaceous', 'smoky' and 'tar/BR' increased as a result of interactions at sub- and *peri*-threshold levels, which has implications for winemaking. Red cultivars (for example, Cabernet Sauvignon and Merlot Noir) that are known to contain IBMP as part of aroma profile the presence of volatile phenols, even at low levels, may manifest green characteristics such as herbaceousness and grassiness even if IBMP levels are very low.

This work also showed that positive characteristics in wine like fruity and sweet attributes are reduced significantly as a result of interactions of low levels of phenols, IBMP and TCA. In future work, a sensory strategy other than DA may lead to richer results and different descriptors for complex solutions.

Credit authorship contribution statement

Marianne McKay: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Florian F. Bauer:** Writing - review & editing, Supervision. **Valeria Panzeri:** Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing. **Astrid Buica:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Fig. 8. Least Squares Means (Type III decomposition) diagram for 'mouldy/musty' attribute ($p < 0.001$). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5: TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals. Fig. 9. Least Squares Means (Type III decomposition) diagram for 'Ashy' attribute ($p < 0.001$). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5: TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals. Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108878.

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