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PII: S0950-3293(18)30375-6

DOI: <https://doi.org/10.1016/j.foodqual.2018.07.001>

Reference: FQAP 3536

To appear in: *Food Quality and Preference*

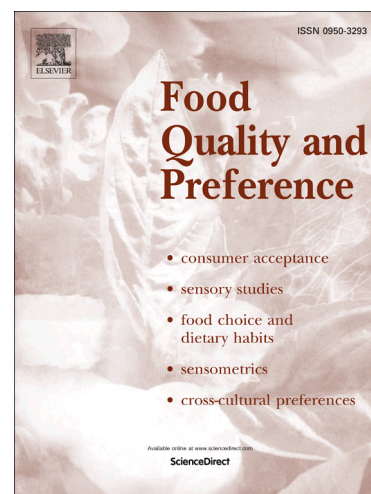
Received Date: 24 September 2017

Revised Date: 5 June 2018

Accepted Date: 2 July 2018

Please cite this article as: Arvisenet, G., Ballester, J., Ayed, C., Sémon, E., Andriot, I., Le Quere, J-L., Guichard, E., Effect of sugar and acid composition, aroma release and assessment conditions on aroma enhancement by taste in model wines, *Food Quality and Preference* (2018), doi: <https://doi.org/10.1016/j.foodqual.2018.07.001>

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**Effect of sugar and acid composition, aroma release and assessment conditions on  
aroma enhancement by taste in model wines**

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**Abstract**

Context. When congruent taste and retronasal aroma are perceived simultaneously, aroma can be enhanced by taste. Different explanations have been proposed: (i) physico-chemical interactions between tastants and aroma compounds, inducing a change of the aroma stimulus before it reaches the receptors, (ii) a contextual bias during sensory tests (dumping), when at least one relevant attribute is not proposed to the panelists to assess a product, (iii) a misunderstanding of the conceptual difference between aroma and taste, or (iv) a perceptual incapability of panelists to distinguish between two congruent percepts. This study was undertaken to better understand aroma enhancement by taste in model wines containing different sugar and acid concentrations but the same volatile composition.

Method. We used a twofold approach:

- (i) model wine retronasal aroma intensity was assessed twice by trained panelists. During the first session, panelists only assessed aroma intensity. During the second session, taste intensity was assessed before aroma intensity, to reduce dumping effects.
- (ii) *in-mouth* release of volatile compounds was measured by nosespace analysis with the same panelists.

Results. Acid concentration influenced aroma compounds release, but it did not impact perceived aroma intensity. Increasing sugar concentration delayed ethyl octanoate (EO) release after swallowing. When taste was not assessed, perceived aroma intensity was not explained by aroma compounds release, but it increased with sugar concentration, probably because of a dumping effect. When taste was assessed, aroma intensity also depended on sugar concentration, but it was significantly correlated to the time of release of EO. Our hypothesis is that when taste declined, late aroma was more easily individualized, and thus assessed with a higher intensity. This entails that panelists focused on aroma to individualize it from taste. We concluded that trained panelists understand the conceptual difference

between taste and aroma, but are not completely able to distinguish congruent and simultaneous taste and aroma percepts.

**Keywords**

Flavor, cross-modal interactions, PTR-MS, sensory analysis, *in vivo* aroma release, aroma enhancement by taste

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## 1. Introduction

It is now accepted that the aroma perceived in food products is not only determined by the odorant volatile compounds contained in the product. It also depends on other product properties, like taste and texture (King et al., 2006; Visschers, Jacobs, Boelrijk, Frasnelli, & Hummel, 2006), and on consumer experience and previous exposure to products (Chrea, Valentin, Sulmont-Rosse, Nguyen, & Abdi, 2005; Petit, Hollowood, Wulfert, & Hört, 2007). The literature reports numerous cases of tastant-dependent changes in perceived retronasal aroma intensity. For instance, the intensity of fruity aroma was shown to increase with sweetener content in model solutions (Dalton, Doolittle, Nagata, & Breslin, 2000; Fujimaru & Lim, 2013; Green, Nachtigal, Hammond, & Lim, 2012; Hort & Hollowood, 2004; Pfeiffer, Hollowood, Hort, & Taylor, 2005), orange juice (Bonnans & Noble, 1993) model dairy desserts (Lethuaut et al., 2005; Tournier, Sulmont-Rosse, Semon, Issanchou, & Guichard, 2009) and custard dessert (Green, et al., 2012).

Aroma enhancement by taste can be provoked by an increased release of the volatile compounds, due to the presence of tastants modifying the odorant stimulus before it reaches the olfactory receptors. It can also be linked to an artifact of the sensory task (“dumping”) or to other effects generally referred to as “perceptual”. It is still unclear whether these perceptual effects are provoked by confusion between sensations perceived by the senses of taste and smell, or are the result of a cognitive association of congruent aroma and taste. The way these mechanisms impact aroma perception is described below.

- Physico-chemical interactions between aroma compounds and tastants in food products can affect the quantity of aroma compounds available for perception, depending on the type of the tastant and its concentration (Rabe, Krings, & Berger, 2003). For instance, sugar concentration was shown to influence the release of aroma compounds and this change in

chemical input induced a change in sensory output (Piccone, Lonzarich, Navarini, Fusella, & Pittia, 2012).

- Dumping effect was studied in several sensory studies, where taste-aroma interactions were shown to be instruction-dependent. The number of attributes panelists had to score influenced the intensity of the perception they reported. Indeed, when a salient property of the product is not proposed to panelists as an attribute to be evaluated, panelists may transfer their judgement about that property of the product into another attribute they are asked to score. For example, when a single intensity estimate was requested in a multidimensional product, independent but congruent sensory dimensions combined to influence the response (Clark & Lawless, 1994; Frank, van Der Klaauw, & Schifferstein, 1993; Frank, Wessel, & Shaffer, 1990). This effect was sometimes interpreted as resulting from the impact of attributes on how attention is directed towards odor and taste (Prescott, 2012; van der Klaauw & Frank, 1996).

- Another interpretation of aroma enhancement by taste involves people's failure to understand what taste and orally perceived aroma are. This probably results from the fact that both stimuli occur when a food is placed in the mouth. Indeed, the more congruent a person perceives a couple of taste and retronasal odor, the more this person tends to locate this odor in the oral cavity (Lim, Fujimaru, & Linscott, 2014). Most people ignore that taste and aroma are perceived by different receptors. As a consequence they do not make distinction between for example, sweet taste and fruity aroma, both referred to as "taste" in everyday language (Spence, Smith, & Auvray, 2015). It can reasonably be thought that this conceptual distinction can be made by trained panelists.

- Having observed that even when their attention was directed to the salient attributes of a mixture, trained panelists experienced taste enhancement by a congruent odor, Stevenson (2001) interpreted this result as proof of the relative cognitive impenetrability of some associations of congruent retronasal aroma and taste. He considers this effect so strong that he

refers to the “binding” of different perceptual events in the brain, resulting in a unified flavor experience (Stevenson, 2016). This is consistent with studies showing that, after being conveyed by different sensory channels, taste, oral somatosensory and olfactory inputs combine in the central nervous system to form a unitary percept (Small, 2012; Small & Prescott, 2005). This phenomenon is thought to result from associative learning resulting from individuals’ previous experience of the association between the stimuli.

It is possible that several of these effects could co-exist, making it difficult to understand the mechanisms involved in retronasal aroma enhancement by taste. It can be difficult to prove that aroma enhancement by taste result from perceptual effects. Temporal measurement or devices using individualized solutions of tastants and aroma compounds have mostly been used to study perceptual effects. As an example, in a study of a model solution of menthone and sucrose, panelists’ perception of mint flavor was shown to follow sucrose release in saliva rather than menthone release (Davidson, Linforth, Hollowood, & Taylor, 1999), proving the perceptual nature of interactions. Fujimaru & Lim (2013) also succeeded in demonstrating that odor enhancement by sucrose originated from perceptual phenomenon; in their study, panelists inhaled citral in vapor phase via the mouth while tasting sucrose.

In complex products, physico-chemical interactions and perceptual effects often co-exist. In this case, physico-chemical interactions are easier to detect than perceptual effects, which can be particularly difficult to evidence. Comparing the scores of different panels, Arvisenet, Guichard & Ballester (2016) demonstrated the occurrence of perceptual effects in model wines, despite the presence of physico-chemical interactions. Retronasal aroma enhancement by sugar was assessed by three panels with different levels of training and product expertise. When panelists were provided with only one scale to score aroma and were not asked to score taste, all three panels experienced the same level of aroma enhancement by sweet taste. Yet, when they were asked to score both taste and aroma, trained panelists and wine experts were

able to reduce the intensity of aroma enhancement by taste, compared to untrained panelists. This result suggests that when conditions allow avoiding dumping effects, experts and trained panelists are more able than untrained panelists to make a distinction between taste and aroma. This proves that the aroma enhancement by taste was mostly due to a perceptual effect stronger in untrained panelists than in trained panelists and experts. The nature of this perceptual effect cannot easily be determined. Trained panelists and experts may know better than untrained panelists the difference between taste and aroma, and may be less likely to confuse taste and aroma. Thanks to their previous exposure to sweet wines and to descriptive tasks, trained panelists and experts might also have a higher capability than untrained panelists to disentangle the cognitive association between sweet taste and fruity aroma.

In this previous study, aroma release was only investigated by static *in vitro* measurements, in order to be compared to orthonasal odor. But retronasal aroma enhancement by taste would most probably result from tastant-induced change of in-mouth aroma release. Thus, this *in vivo* dynamic process has to be taken into account to better understand how panelists assess aroma intensity when taste changes.

The present study was carried out in order to gain further understanding about the mechanisms involved in aroma enhancement by taste by perceptual effects. To this end, we studied the correlations between the aroma scores assessed by trained panelists and the release of key aroma compounds over time. Two sensory tests conditions were tested, varying by the number of descriptors panelists were asked to assess: aromas only or tastes first and aromas afterwards. Our objective was to understand the factors involved in the assessment of aroma when taste varies.



## 2. Materials and methods

### 2.1. Model wines

Four model wines (8% v/v ethanol) were prepared, varying the tartaric acid content (2.5 or 5 g.L<sup>-1</sup>) and the sugar (mixture of fructose and glucose, 2:1) content (5 or 80 g.L<sup>-1</sup>) (Table 1). The concentrations of tartaric acid and sugars were chosen to cover the range of those found in Gewurztraminer wines and to produce sweetness and acidity intensities distinctively different from one model wine to another. The four studied model wines contained the same amount of glycerol, acetic acid, citric acid and sulfur dioxide. All these compounds were food grade and were purchased from Sigma-Aldrich (St Quentin Fallavier, France).

Model wines were flavored as described in Arvisenet et al. (2016) with exactly the same quantity of the same mixture of aroma compounds in each model wine (Table 2). These compounds and their concentrations were chosen based on previous research of the aroma of Gewurztraminer wines (Guth, 1997; Ong & Acree, 1999). Model wines containing this aroma composition could be taken for a real Gewurztraminer wine by untrained panelists (Arvisenet, et al., 2016). Those wines were freshly prepared on the day of testing for both sensory tests and nospace analyses.

### 2.2. Sensory analyses

#### 2.2.1. Sensory evaluation of the retronasal aroma of model wines varying in sugar and acid content

This study was approved by a human ethics committee “(Comité de Protection des Personnes EST I”, Reference: 2014/14 – ID RCB: 2013-A01767-38). Informed written consent was obtained from the subjects prior to their participation. Panelists were told they would participate in a study about Gewurztraminer wine aroma. They were not informed about the

real objective of the study or that the wines they would evaluate were artificial. Pregnant women were excluded from the study.

#### 2.2.1.1 Panel

The panel was composed of 19 intensively trained panelists and 14 wine experts who took part in our previous study (Arvisenet et al., 2016). Having observed in this previous study that trained panelists and experts had identical perception of taste-aroma interactions in model wines, we included them in the present study as a same group, referred to as “trained panelist” throughout this paper.

Seven panelists were selected from the group of 33, on the basis of their sensory results, to represent all the levels of aroma enhancement by taste observed in the group of 33 panelists: the smallest observed enhancement, the highest observed enhancement, and medium level of enhancement. This smaller group was composed of five men and two women. Two of them were under the age of 26, two were between 26 and 40 and three were over 40 years of age. These seven panelists participated in both the sensory evaluation and measurements of *in vivo* aroma release.

#### 2.2.1.2. Model wine evaluation

Tests took place in a sensory lab equipped with booths. Samples were served at ambient temperature (20°C). For each task, 20-mL samples were poured into standardized tasting glasses (ISO, 1977) that were opaque (black) to eliminate visual cues as sources of information. The glasses were coded with 3-digit numbers and covered with plastic Petri dishes. In order to limit carry-over effects and memory biases, all wine samples were presented in a different order specific to each participant for each task within each session, according to a Williams Latin square arrangement generated by FIZZ software (Biosystemes,

Courtenon, France). Panelists were asked to use water and unsalted crackers as palate cleansers between samples.

Structured-numbered 8-point scales were used with values ranging from 0 to 7 and intensity terms associated with extreme values: “none” to “very intense”.

Two sessions took place. During the first session, panelists were asked to score the intensity of two retronasal aroma descriptors (fruity and floral) and the global intensity of aroma for each sample of model wine, but were not asked to score the taste of the samples. In the second session, panelists were asked to score first sourness and sweetness and then, the intensity of fruity and floral aroma as well as the global intensity of aroma, in the same order as in the first session.

In the second session, we expected to limit the dumping effect that can occur when panelists are asked to score a small number of descriptors, compared to the number of sensory characteristics that can be easily perceived in the products.

In the two sessions, model wines were assessed according to a serial monadic protocol. Panelists were not allowed to re-taste previously assessed samples.

### 2.3. Nosespace analyses

The seven panelists performed a nosespace study conducted with a proton transfer reaction-mass spectrometer (PTR-MS) instrument equipped with a Time-of-Flight (ToF) analyzer (PTR-ToF 8000, Ionicon Analytik, Innsbruck, Austria). Parameters of the PTR-MS instrument were adjusted according to a previous work conducted on model wines, which allowed ethanol chemical ionization conditions while minimizing the protonated molecular ion fragmentation of the volatiles used in this study with no compromise in sensitivity for *in vivo* applications (Sémon, Arvisenet, Guichard, & Le-Quéré, 2018). All the measurements were carried out under the following drift tube conditions: drift pressure of 2.31 mbar, drift

temperature of 80°C, and drift voltage of 340 V, resulting in an E/N ratio of 80 Td. Data acquisition was performed at 1 mass spectrum ranging from m/z 0 to 250 per 0.108 second. Mass axis calibration and calculation of peak areas were done with the software PTR-MS Viewer version 3.1.0.28. Breath volatile concentrations were expressed as normalized cps (ncps), taking into account corrected transmission and normalization to the protonated water and the protonated ethanol (C<sub>2</sub>H<sub>5</sub>OH)H<sup>+</sup> monitored at their respective <sup>18</sup>O isotopic contributions found at m/z 21.022 [H<sub>3</sub><sup>18</sup>O<sup>+</sup>] and m/z 49.054 [C<sub>2</sub>H<sub>5</sub><sup>18</sup>OH<sub>2</sub><sup>+</sup>]. All the mass spectra were background-subtracted using the background signal measured before sample introduction into the mouth. A warm-up sample preceded the tested samples in order to introduce ethanol vapor in the inlet and the drift tube to pre-establish ethanol chemical ionization conditions and to familiarize subjects with the protocol and the nosepiece used for nosespace sampling. This ergonomic Teflon® nosepiece, delivering the sampled nosespace through both nostrils, was mounted on a light helmet that enabled the subject to move his/her head freely. It was connected to the transfer line of the PTR instrument by a flexible heated (75°C) PEEK tubing (85 cm long, 1mm internal diameter) (Schlich et al., 2015). Sampling was performed at a total flow rate of 60 mL/min with the transfer line maintained at 80°C.

Among the 12 aroma compounds present in the model wine (Table 2), only 5 could be easily detected by PTR-MS (Table 3). However, only isoamyl acetate and ethyl octanoate could be unequivocally detected. Although minimized with the PTR-MS conditions used, fragmentations of protonated molecular ions were not completely absent (Sémon, et al., 2018). 3-Methylbutan-1-ol (winey-brandy) was followed by its dehydrated protonated molecular ion [MH<sup>+</sup>-H<sub>2</sub>O] at m/z 71.086, which also bore a small contribution from the main fragment ion of isoamyl acetate; ethyl butanoate (fruity, buttery) was followed by its protonated molecular ion MH<sup>+</sup> at m/z 117.091, which bore a small contribution from the main fragment ion of ethyl hexanoate; isoamyl acetate (fruity, banana) was univocally

characterized by its protonated molecular ion  $MH^+$  at  $m/z$  131.107; ethyl hexanoate (fruity, apple) protonated molecular ion  $MH^+$  at  $m/z$  145.122 bore a small contribution from the main fragment ion of ethyl octanoate; and ethyl octanoate (fruity, apricot) itself was unequivocally followed by its protonated molecular ion  $MH^+$  at  $m/z$  173.154 (Table 3). These compounds all had a concentration higher than or equal to  $0.42 \text{ mg}\cdot\text{L}^{-1}$ .

Nosespace measurements were carried out in triplicate.

An example of a smoothed release curve in the nosespace of one individual is given in Figure 1 for the ion  $m/z$  71.086. All the release data were calculated from the breath concentration ncps data, using Microsoft Excel 2010. Ten parameters were extracted from the smoothed release curves (Boisard et al., 2014): maximal intensity before and after swallowing ( $I_{BS}$  and  $I_{AS}$ , respectively), time to reach maximal intensity before and after swallowing ( $T_{BS}$  and  $T_{AS}$ , respectively), area under the curve ( $A_{BS}$ : area before swallowing;  $A_{AS}$ : area after swallowing;  $A_T$ : total cumulative area) and time to reach 10% ( $T_{10}$ ), 50% ( $T_{50}$ ) and 90% ( $T_{90}$ ) of  $A_T$  (Figure 1).

#### 2.4. Statistical analysis

All statistical analyses were carried out with XLSTAT (2014.1.10).

##### 2.4.1. Analysis of sensory scores

The two sensory evaluation sessions cannot be considered as repetitions of the same experiment as they were not performed with the same protocol: the presence or absence of the taste rating was expected to impact the scores for retronasal aroma.

To know if the results could be interpreted separately within condition, we first compared the results obtained in both conditions by a 4-way ANOVA on retronasal aroma scores, with

condition, panelists, sugar concentration and acid concentration as factors ( $\alpha=5\%$ ). All first-order interactions were considered in this ANOVA model.

For the interpretation of each scoring condition separately, a 3-way ANOVA with panelists, sugar concentration and acid concentration as factors was carried out on retronasal aroma scores ( $\alpha=5\%$ ). All first-order interactions were considered in this ANOVA model.

#### 2.4.2. Analysis of in-mouth release parameters and correlation with sensory scores

Fifty parameters were extracted from the *in vivo* release curves (5 studied compounds  $\times$  10 parameters). Due to the high subject variability in these data, the data were standardized for each subject before being submitted to a 2-way ANOVA with acid concentration and sugar concentration as factors and including interactions. Given the exploratory nature of this experiment, the significance level was set at 10% in order not to rule out parameters that could potentially explain sensory scores from further analysis. Only the variables for which a significant effect of acid or sugar concentration was observed were kept for the next step and represented on a Pearson PCA, with nosespace parameters as variables and products as observations.

Finally, Pearson's correlation coefficients were calculated, using individual data, between the median of nosespace parameters (normalized data) and sensory descriptors (raw data). Only those parameters correlated to at least one sensory descriptor at  $\alpha=0.05$  were studied.

### 3. Results

#### 3.1. Sensory analyses

In both scoring conditions, floral aroma assessed by the 33 panelists did not significantly discriminate the four samples. Only the results related to global intensity of aroma and intensity of fruity aroma will be further studied.

The comparison of global aroma and fruity aroma obtained in each scoring condition showed a significant effect of the condition on global intensity of aroma ( $P_{\text{val}}=0.041$ ). More interestingly, the scoring condition tended to have different effects on fruity aroma scores depending on sugar and acid content ( $P_{\text{val}}(\text{sugar} \times \text{condition}) = 0.070$  and  $P_{\text{val}}(\text{acid} \times \text{condition}) = 0.054$ ). Results were thus interpreted within each condition.

Global intensity of aroma and intensity of fruity aroma, assessed by the 33 panelists were significantly enhanced when the concentration of sugar increased in the model wines, in both scoring conditions (Figure 2). These effects were also significant when considering only the subset of 7 panelists. When the 33 panelists assessed only aroma and not taste, there was a significant panelist  $\times$  sugar interaction on fruity aroma intensity ( $P_{\text{val}} = 0.026$ ). This interaction was due to the scores of three panelists who did not experience fruity aroma enhancement by sweetness in this assessment condition, contrary to the other 30 panelists. When taste was scored before aroma, there was no panelist  $\times$  sugar interaction effect on fruity aroma intensity, showing that in this assessment condition, the 33 panelists were in accordance with one another about the impact of sweetness on aroma. In the subset of 7 panelists, all panelists were in accordance about the influence of sugar concentration on fruity and global aroma intensity, in both conditions of assessment.

When panelists assessed only aroma and not taste, acid concentration had no effect on fruitiness intensity, nor on the global intensity of aroma, for both panels. With the 33 panelists, there was a significant panelist  $\times$  acid interaction for both aroma scores, ( $P_{\text{val}} = 0.012$  and  $0.030$  respectively, for fruity aroma and global intensity of aroma). It means that

panelists were not in accordance. Indeed, some of them perceived an aroma enhancement by sourness, others perceived an aroma decrease by sourness, but the majority of the panelists perceived the same aroma intensity with the two levels of sourness. When taste was scored before aroma, acid concentration had a significant effect on fruitiness intensity for the whole panel and a significant effect on the global intensity of aroma for the subset of 7 panelists. There was no panelist  $\times$  acid interaction effect on these scores, showing that the level of acid affected all the panelists in the same way, in this condition.

For the four retronasal aroma scores and for both panels, there was no sugar  $\times$  acid interaction.

### ***3.2. Relationship between nosespace parameters and tastant composition of matrices***

Among the 50 parameters extracted from the nosespace curves, 16 varied significantly with the concentration of acid and/or sugar in the products. For the 5 studied compounds,  $A_T$  was not affected by the concentration of tastants while  $I_{BS}$ ,  $A_{BS}$ ,  $T_{BS}$ ,  $I_{AS}$ ,  $A_{AS}$ ,  $T_{AS}$  and  $T_{10}$  were. When a parameter increased significantly with tastants concentration before swallowing, it tended to decrease significantly with tastants concentration after swallowing (Table S1). This shows that, while globally equivalent, the release of the aroma compounds was time-shifted under the effect of tastant concentration.

The 16 parameters varying with tastants concentration were represented in a normalized PCA (Figure 3). The first two principal components enable 95.6% of explained variance. The first axis (73.1% of the total variance) opposes nosespace parameters  $I_{BS}$  and  $A_{BS}$  on the one hand to  $I_{AS}$ ,  $A_{AS}$  and  $T_{10}$  on the other hand. The two acid concentrations are opposed according to this dimension. The maximal intensity before swallowing ( $I_{BS}$ ) significantly increased with acid content for ions 71.086, 117.091, 131.107 and 145.122, characterizing respectively



3-methylbutan-1-ol, ethyl butanoate, isoamyl acetate and ethyl hexanoate, the four most volatile aroma compounds in the products. The area under the curves before swallowing ( $A_{BS}$ ) followed the same pattern for ions 71.086, 117.091 and 131.107. The parameters before swallowing should be considered with caution for ion 71.086 (3-Methyl-1-butanol) because for this ion, the area under the curve before swallowing was negligible (8%) compared to the global area (before and after swallowing). For ions 117.091, 131.107 and 145.122,  $A_{BS}$  represented respectively 29%, 25% and 27% of the total area.

On the contrary,  $I_{AS}$  (for the ions 117.091, 131.107 and 145.122) and  $A_{AS}$  (for the ions 117.091 and 131.107), were significantly lower when acid concentration was high. This means that these volatiles were preferentially released during the in-mouth process than after swallowing, when acid concentration was high.

$T_{10}$  significantly decreased for ions 71.086, 117.091 and 131.107 when acid concentration increased, which means that an increase in acid concentration significantly shortened the time of release of 3-methylbutan-1-ol, ethyl butanoate and isoamyl acetate.  $T_{10}$  corresponds to the time at which 10% of the cumulated total area under the curve was reached. It occurred after swallowing for all the compounds. As no significant effect was observed for the total area under the release curve, an increase in acid concentration only modifies the dynamic of release of the volatile compounds.

The second axis of the PCA (22.5% of the total variance) is characterized by 71- $T_{10}$ . This axis evidences an interaction between sugar and aroma for the time to reach 10% of the cumulative area of this ion. At first sight, this time seems longer for the product containing low acid and low sugar concentration and the product containing high sugar and high acid concentration. But this result has to be taken with caution because 71- $T_{10}$  may not strongly contribute to perception. Indeed, for ion 71,  $T_{10}$  occurred just after swallowing and most of the release occurred after  $T_{10}$  (Figure 1).

173- $T_{AS}$  is opposed to the other parameters on axis 2. 173- $T_{AS}$  increased when sugar concentration increased. It evidences a delayed release or greater in-mouth duration of ethyl octanoate when sugar concentration increased. Ethyl octanoate was nearly exclusively released after swallowing (173- $T_{AS}$  represented 97% of the total area for ion 173.154).

### ***3.3. Relationship between nosespace parameters and sensory results***

Among the 17 parameters varying significantly with tastant composition of the products, 7 were significantly correlated to at least one sensory descriptor score (Table 4).

The maximal intensity before swallowing ( $I_{BS}$ ) of four ions (71.086, 117.091, 131.107 and 145.122) was negatively correlated to the retronasal aroma assessed when taste was not scored. As the total amount of aroma released did not vary with the model wine composition, these correlations mean that when these four volatile compounds were released earlier, the intensity of global and fruity aroma decreased. Thus, the perceived aroma seems to be explained more by the amount of aroma released after swallowing, which gives the last in-mouth sensation.

$T_{10}$  was positively correlated to global retronasal aroma only when it was assessed after taste assessment. This correlation was positive for ions 131.107 and 145.122 (isoamyl acetate and ethyl hexanoate, respectively). This indicates that, when taste and aroma were both assessed, perceived global intensity of aroma was higher when  $T_{10}$  occurred later, that is when isoamyl acetate and ethyl hexanoate were released later.

Finally, 173- $T_{AS}$  - the only parameter related to ethyl octanoate release influenced by tastant composition - was significantly correlated to global and fruity aromas scores obtained when taste and aroma were assessed. In this condition, when the release of ethyl octanoate compound was delayed, the fruity and global retronasal aroma perception increased.

#### 4. Discussion

In this study, we aimed to understand the impact of tastants concentration, *in vivo* aroma release and sensory assessment conditions on retronasal aroma enhancement by taste. We compared four wines with exactly the same volatile composition but different concentrations of tastants by nosespace and sensory analysis. Tartaric acid concentration seemed to have a low effect on retronasal aroma scores. When acid concentration increased, the subset of seven panelists perceived a higher global intensity of aroma, but this effect was not significant for the whole panel (n=33). Instead, the whole panel experienced a significant effect of acid concentration on fruitiness intensity. As a consequence, we can only consider that acid concentration tends to increase the perceived aroma, and that this tendency was only observed when taste was scored. It suggests that dumping effect was not involved in the observed enhancement, but we would need more information to confirm this.

As in our former study (Arvisenet, et al., 2016), retronasal aroma intensity was significantly enhanced by sugar, and despite the use of taste and aroma attributes in the same sensory session, enhancement of aroma by sweet taste occurred. This result is consistent with other studies (Bonnans & Noble, 1993). It suggests that, even if dumping effect occurred, it was probably not the only factor explaining the observed aroma enhancement by sweetness.

In order to try to understand other reasons for this enhancement, *in vivo* release and sensory scores were compared. Correlations were found between perceived aroma and aroma release parameters. The sensory scores correlated to aroma release parameters before swallowing, were different from those correlated to aroma release parameters after swallowing. So, these two steps will be further discussed separately.

##### ***4.1. Effect of tastants on perceived aroma before swallowing***

The increase of acid concentration in the model wines induced an increased in-mouth release of several compounds (3-methylbutan-1-ol, ethyl butanoate, isoamyl acetate and ethyl hexanoate) before swallowing. Many studies have shown that volatile compounds release from a solution can be either increased, decreased, or unchanged by the presence of solutes, depending on the nature of volatile compounds and solutes (Buettner & Beauchamp, 2010; Hewson, Hollowood, Chandra, & Hort, 2008). Indeed, the presence and the concentration of tastants can affect the partition properties of volatile compounds, by changing volatility, water activity, etc. Little modification of the headspace concentration at equilibrium was observed for these volatile compounds as a function of model wine composition (Arvisenet et al., 2016), suggesting negligible effect of the acid concentration on the partition coefficients. However this experiment was realized without addition of saliva. Neyraud et al. (2009) demonstrated that stimulations with increasing amounts of acid induced an increase in the salivary flow rate and in the total amount of proteins in saliva due to a parotid secretion. As the saliva composition differs between whole saliva and parotid saliva, the increase rate of release could thus be explained by a modification of the salivary protein composition, which could induce a different binding behavior between the volatile compounds and the proteins.

In our study, this increased aroma input before swallowing seems not to have been perceived by panelists. Indeed, when both taste and aroma were assessed, none of the aroma intensity scores correlated with the parameters of release of the studied aroma compounds. When panelists were required to assess only aroma, global intensity of aroma was paradoxically negatively correlated to  $I_{BS}$ , which demonstrates that panelists did not perceive the increase of the odorant stimulus in this test condition either. This negative correlation is difficult to explain. Our hypothesis is that a stronger concomitant phenomenon may have occurred, which provoked a decrease of perceived aroma simultaneously with the acid increase. It can reasonably be assumed that the increased perception of sourness due to increased acid

concentration provoked an impression of a drop in sweetness, because of the well-known sourness-sweetness perceptual interaction (Bonnans & Noble, 1993). Confusion between “more acid” and “less sweet” was all the more likely to occur when panelists did not have to score taste. In this sensory test condition, a decrease in perceived sweetness may have, in turn, provoked a decrease in retronasal aroma enhancement by sweetness. This is consistent with the high aroma enhancement by sweetness observed in the studied matrices, and with previous studies which showed that aroma enhancement by sweetness can be instruction dependent (Frank, 2002). This tentative explanation would need to be confirmed, and results should be considered with caution, as there is no evidence that the parameters measured before swallowing are relevant, especially with a liquid.

#### ***4.2. Effect of tastants on perceived aroma after swallowing***

*In vivo* release parameters after swallowing were affected by acid or sugar concentrations.

When acid concentration decreased, isoamyl acetate and ethyl hexanoate were released later, as suggested by the negative correlation between  $T_{10}$  of these compounds and acid concentration. But the influence of acid concentration and  $T_{10}$  on the global intensity of aroma was similar. This contradictory result could mean that there is little effect of acid concentration on aroma perception. This is in line with the results obtained with the group of 33 panelists, whose global intensity scores of aroma did not depend on acid concentration.

When sugar concentration increased, the after-swallowing intensity peak for ethyl octanoate occurred later, as shown by the positive correlation between sugar concentration and  $T_{AS}$  of ethyl octanoate. This later release of ethyl octanoate seems to have been perceived by panelists only when they were asked to assess taste and aroma. One tentative explanation is that sweetness perception is known to gradually decrease after swallowing (Monaco, Miele,

Volpe, Picone, & Cavella, 2014; Reyes, Castura, & Hayes, 2017). As a consequence, the later the aroma compounds are released after swallowing, the less taste and aroma percepts may overlap. In our study, when panelists were asked to score taste and aroma, they are supposed to have paid more attention to the differences between these two percepts. Indeed, the conditions of the tests and the order of presentation of scales - corresponding to the temporality of perceptions of taste, first, and then aroma - were meant to help panelists to distinguish between taste and aroma. In these conditions, when the aroma intensity peak occurred later, panelists probably better noticed taste decline and it helped them to better distinguish aroma and taste. In this situation, panelists seem to have taken into account more the time of release than the intensity of release in their intensity score. This result was not observed when the sensory test instructions were to score only aroma. One tentative explanation is that, when panelists were only required to assess aroma (and not required to assess taste), they may have considered the flavor as a whole in their aroma score. As a result, not only did aroma intensity increase with sugar concentration, but it was also not correlated to aroma compounds release. These results show that trained panelists understand the conceptual difference between taste and aroma. Nevertheless, their aroma scores are highly correlated to sugar concentration. These scores depend on aroma release parameters only when aroma release and taste perception are likely to be less overlapped (i.e. after swallowing). It tends to prove that even when attempting to analyze the components of the flavor, panelists were not able to disentangle unitary percepts when those occurred simultaneously.

#### ***4.3. Strengths and limitations***

To our knowledge, this study was the first attempt to understand thanks to the measurement of *in vivo* aroma release, what panelists score when they are asked or not to assess every salient

descriptor. It was also the first study to show the contribution of temporal sequence of aroma release to multimodal perception. Because the global release of aroma compounds was not changed by the concentration of tastants, the previous use of a static method, in Arvisenet et al. (2016) suggested negligible physicochemical interactions. The use of dynamic *in vivo* release measurement showed that, whereas tastants did not affect global aroma release in terms of intensity, they rather changed aroma release temporality. Swallowing was shown to play key role in this temporality. One strength of this study was thus to follow the dynamic in-mouth release of aroma compounds from alcoholic beverages in real-time, via nosespace analysis. Thanks to this novel approach (Sémon, et al., 2018) it was possible to compare, in this category of products, the release of volatile compounds and the aroma perception.

Some potential limitations related to our experimental setting should be mentioned. Despite the small number of samples of our study, a clear pattern emerged for the effect of sugar on aroma perception. But it would be interesting to confirm the effect of acid on aroma perception with a larger range of concentrations. For the purpose of our study, it was necessary that the same panelists performed both sensory analyses and dynamic measurements of *in vivo* release of aroma compounds. While the number of panelists was usual for nosespace experiments (Arancibia, Castro, Jublot, Costell, & Bayarri, 2015; Heenan et al., 2012; Tyapkova et al., 2014), it may be considered to be low for sensory analysis. But the seven panelists who performed both sensory and nosespace analyses were selected among a larger group, on the basis of their accordance with the results of this group.

Dynamic sensory measurements are particularly interesting when it comes to the comparison with aroma release. Time-intensity (TI) was inapplicable in our study, because assessment of several attributes in the same session was required in the second session. Dynamic multi-attribute sensory methods do exist but none of them was well adapted to the products and

purpose of the present study. Temporal Dominance of Sensations (TDS) focuses on the dominant attributes rather than on the intensity of each individual attribute over time, and it is difficult to relate TDS results and dynamic aroma release (Deleris et al., 2011). Temporal Check-all-that-apply (T-CATA), based on the frequency of detection of attributes over time, does not either provides a score for each attribute perceived on the products (Castura, Antunez, Gimenez, & Ares, 2016; Pineau et al., 2009). Multi-attributes time intensity and progressive profiling provide discontinuous data collection and are not well adapted to the scoring of products with short consumption time span.

Of all the volatile compounds of the studied model wines, it seemed surprising that only ethyl octanoate release was correlated to the aroma perception. Many reasons can explain the high contribution of this compound to perceived aroma. First, ethyl octanoate was the least volatile compound among those that were followed by nosespace. The consequence of that was that its release was the most delayed, and thus, it was the least overlapped with taste percept. Secondly, ethyl octanoate had by far the highest OAV compared to the other compounds of the studied model wine (odor activity value, calculated with odor perception thresholds in water/ethanol, 90/10, w/w) (Arvisenet, et al., 2016; Guth, 1997). The other compounds in this study had very low OAVs compared to ethyl octanoate: between six and 160 times smaller (for isoamyl acetate and 3-methylbutan-1-ol, respectively). Nevertheless, this explanation should be considered with caution, since the perception of volatile compounds in a mixture does not depend only on their OAV, but also on Stevens' law exponents of each compound and on the composition of the mixture, due to odor-odor sensory interactions. Psychophysical functions were shown to vary with intra-modal interactions (Grosch, 2001). In addition, odor perception in a mixture is not the addition of the contributions of the different odorants: as an example the fruity odor could result from synergic or antagonistic effects, making it difficult



to identify the contribution of each compound to the perceived odor (Thomas-Danguin et al., 2014). Methodological development is still needed before being able to fully understand the contribution of each compound to perception.

## 5. Conclusion

We showed that despite their intensive training, panelists are still sensitive to dumping effect. Even when the test conditions allowed limiting the dumping effect, panelists were not able to completely dissociate aroma from the congruent taste perceived simultaneously. These results point out the limit of descriptive sensory tests: even for a well-trained panel, it seems difficult to evaluate independently every component of the flavor.

Our results suggest that trained panelists are able to distinguish between the concepts of retronasal aroma and taste, and that the post-swallowing decrease of taste resulted in more accurate aroma assessment (i.e. more correlated to aroma release). The high contribution of swallowing in perception was showed, but more work is needed to better understand the role of tastants on the aroma release over time and how panelists integrate changes of temporal aroma release.

## Acknowledgements

This work received financial support from the CSGA. The authors thank Damien Laurent, Thibaut Potiquet and Marine Vigneron for their help in carrying out the experiments.

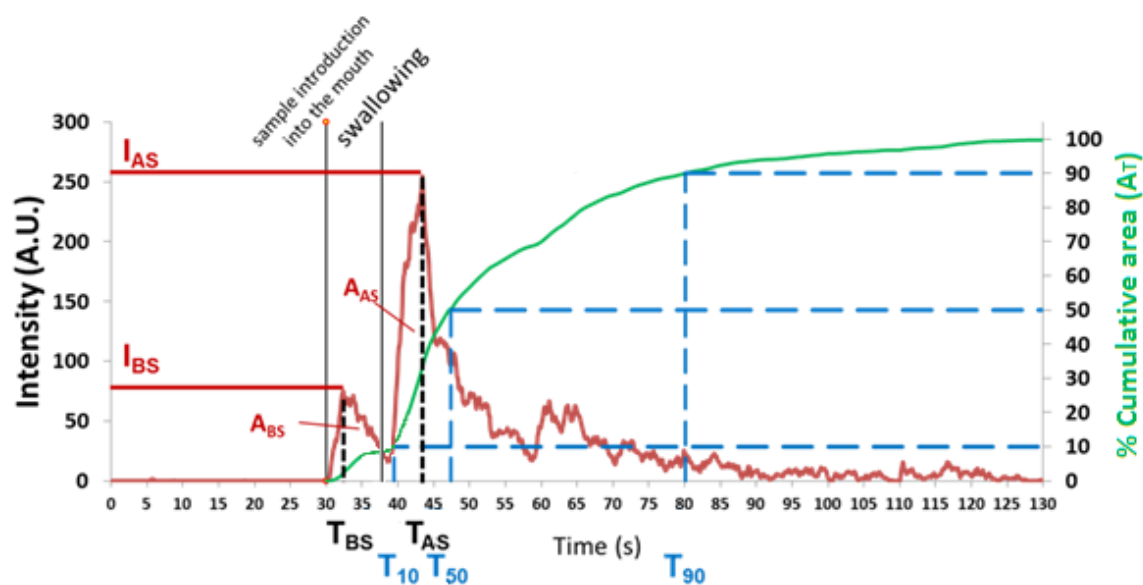
The authors are grateful to the reviewers for their constructive feedback, which highly contributed to improve this paper.

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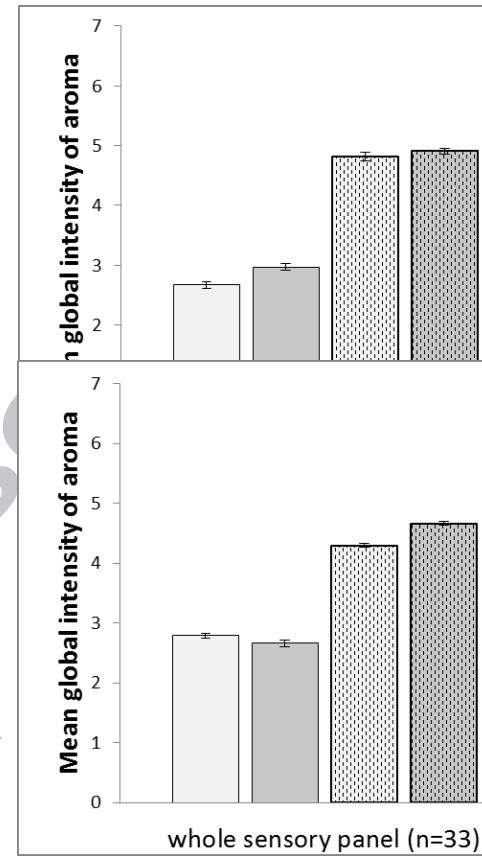
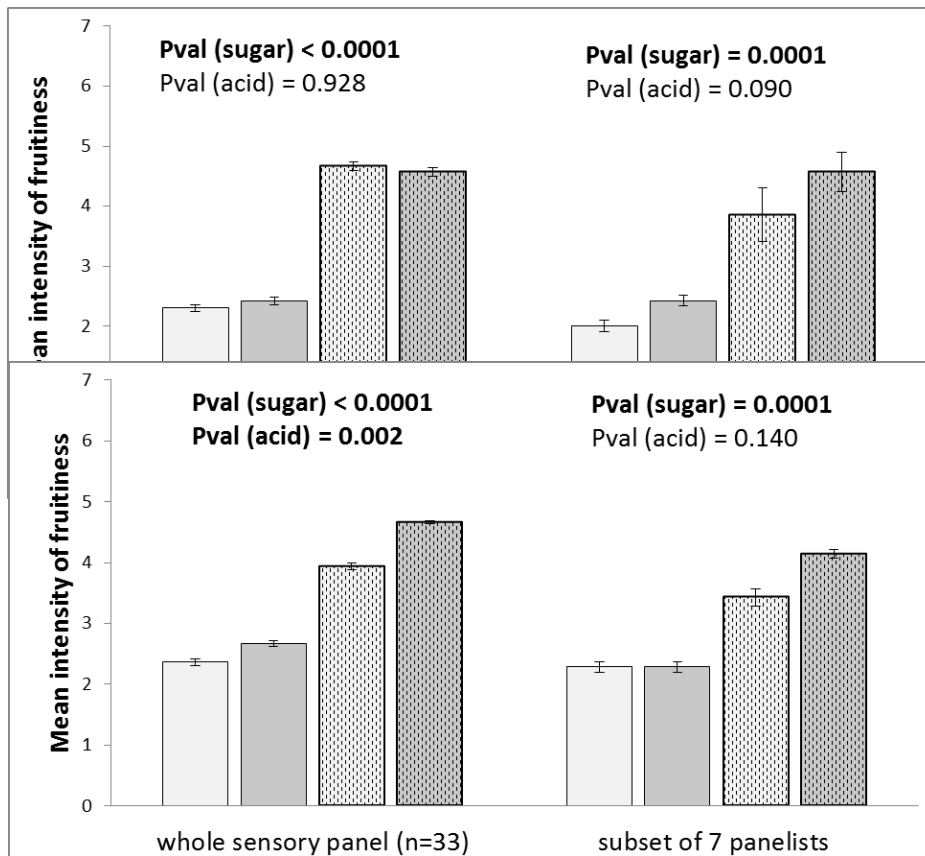
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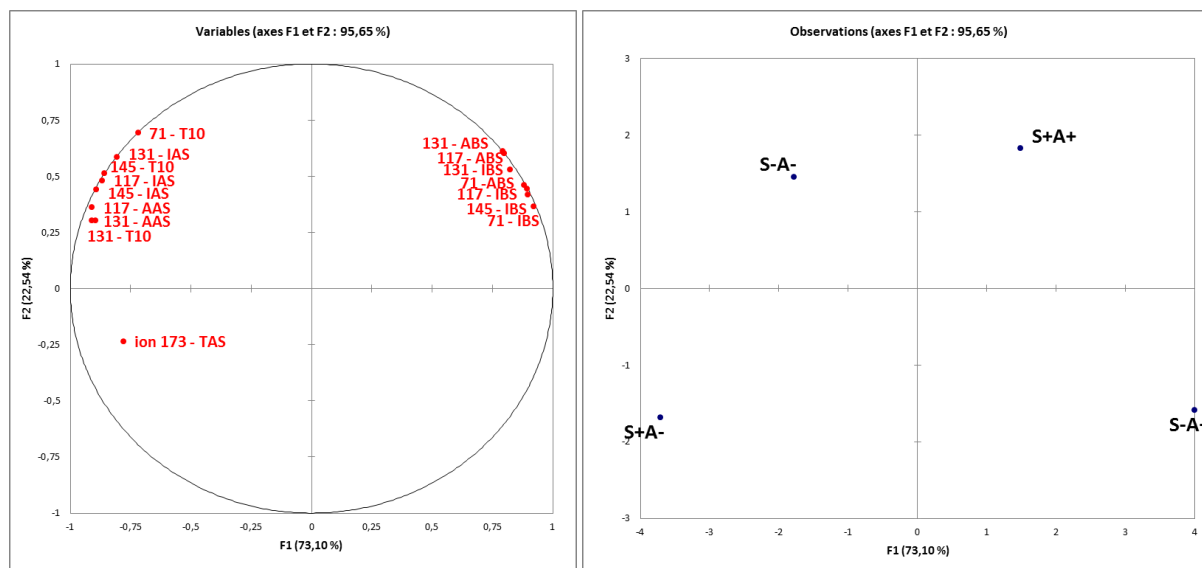
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**Figure 1.** Extraction of parameters from nosespace curves. Example of a smoothed *in vivo* release curve for the ion  $m/z$  71.086

Maximal intensity before swallowing ( $I_{BS}$ ) and after swallowing ( $I_{AS}$ ), time to reach maximal intensity before swallowing ( $T_{BS}$ ) and after swallowing ( $T_{AS}$ ), area under the curve before swallowing ( $A_{BS}$ ) and after swallowing ( $A_{AS}$ ), and time to reach 10% ( $T_{10}$ ), 50% ( $T_{50}$ ) and 90% ( $T_{90}$ ) of the cumulative total area ( $A_T$ ).





IBS: maximal intensity before swallowing; ABS: area under the curve before swallowing; TBS: time to reach maximal intensity before swallowing; T10: time to reach 10% of total cumulated area; IAS: maximal intensity after swallowing; AAS: area under the curve after swallowing; TAS: time to reach maximal intensity after swallowing

**Figure captions**

Figure 1. Extraction of parameters from nosespace curves. Example of a smoothed *in vivo* release curve for the ion  $m/z$  71.086

Figure 2. Mean retronasal aroma scores (and standard errors) given to model wines with different sugar and acid concentrations by the two panels (for model wine codes, see Table 1)

Figure 3. Factorial map (axes 1 and 2) of PCA for nosespace of model wines: (a) correlation plot showing the parameters of release curves correlated with tastants concentrations of model wines; (b) products plot.

*Parameters of nosespace curves IBS: maximal intensity before swallowing; ABS: area under the curve before swallowing; TBS: time to reach maximal intensity before swallowing; T10: time to reach 10% of total cumulated area; IAS: maximal intensity after swallowing; AAS: area under the curve after swallowing; TAS: time to reach maximal intensity after swallowing*



**Table 1. Non-volatile compound composition (g.L<sup>-1</sup>) and pH in the model wine solutions**

Compound (purity)	Model wine code			
	S-A-	S-A+	S+A-	S+A+
<b>Ethanol</b> (96%)	----- 80.0 -----			
<b>Glycerol</b> (87%)	----- 8.5 -----			
<b>Glucose</b> (100%)	1.7	1.7	26.7	26.7
<b>Fructose</b> (100%)	3.3	3.3	53.3	53.3
<b>Tartaric acid</b> (100%)	2.5	5	2.5	5
<b>Acetic acid</b> (99%)	----- 0.3 -----			
<b>Sulfur dioxide</b> (5%)	----- 0.6 -----			
<b>pH</b>	2.91	2.29	2.88	2.31

----- x ----- means that the concentration of the corresponding compound was the same, x, in all the solutions

**Table 2. Aroma compounds in the model wine solutions and their properties**

Compound	Formula	Molar mass	logP <sup>a</sup>	Odor description <sup>b</sup>	Concentration in model wine (mg.L <sup>-1</sup> )
Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	44.05	-0.17	Pungent, breathtaking	0.94
2-Methylpropan-1-ol	C <sub>4</sub> H <sub>10</sub> O	74.12	0.77	Sweet, sweaty-chemical, whiskey-like	16
3-Methylbutan-1-ol	C <sub>5</sub> H <sub>12</sub> O	88.15	1.26	Alcoholic, winey-brandy	95.2
Ethyl butanoate	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116.16	1.85	Ethereal, fruity odor, buttery, ripe fruit	0.42
2-Phenylethanol	C <sub>8</sub> H <sub>10</sub> O	122.16	1.57	Floral, rose, floral	24.2
Isoamyl acetate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	130.18	2.26	Sweet, fruity, banana, pear	2.54
Ethyl hexanoate	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144.21	2.83	Fruity, winey odor; apple, banana, pineapple	1.02
Linalool	C <sub>10</sub> H <sub>18</sub> O	154.25	3.38	Floral-woody odor with faint citrus note, sweet floral	0.36
<i>cis</i> -Rose oxide	C <sub>10</sub> H <sub>18</sub> O	154.25	3.58	Metallic, grassy-green, geranium	0.042
Ethyl octanoate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172.27	3.81	Fruity, winey, sweet odor, cognac-apricot	1.02
$\beta$ -Damascenone	C <sub>13</sub> H <sub>18</sub> O	190.28	4.21	Fruity-floral with apple-plum-raisin, tea, rose, tobacco	0.008
Ethyl decanoate <sup>d</sup>	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.32	4.79	Sweet, fatty, nut-like, winey-cognac odor	0.027

a: EpiSuite 4.1 software

b: Flavorbase (<http://www.leffingwell.com/flavbase.htm>)

c: Guth et al. (1997), except for ethyl decanoate: Ferreira, Lopez & Cacho (2000)

d: Compound present with  $\beta$ -damascenone

**Table 3. Volatile compounds detected by PTR-MS and m/z of their respective main protonated ion**

Compound	m/z	Fragment
3-Methylbutan-1-ol	71.086	(MH-H <sub>2</sub> O) <sup>+</sup>
Ethyl butanoate	117.091	MH <sup>+</sup>
Isoamyl acetate	131.107	MH <sup>+</sup>
Ethyl hexanoate	145.122	MH <sup>+</sup>
Ethyl octanoate	173.154	MH <sup>+</sup>

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Table 4. Pearson's correlation coefficients between sensory scores and nosespace parameters

Parameter occurrence	Parameter	Evolution of parameter with [tastant]	Correlation between nosespace parameter and retronasal aroma descriptors*			
			Global intensity <i>aroma scoring only</i>	Fruitiness intensity <i>aroma scoring only</i>	Global intensity <i>taste + aroma scoring</i>	Fruitiness intensity <i>taste + aroma scoring</i>
Before swallowing	71-I <sub>BS</sub>	↗ when [acid] ↗	<b>-0.389</b>	<b>-0.379</b>	-0.015	-0.166
	117-I <sub>BS</sub>	↗ when [acid] ↗	<b>-0.407</b>	-0.320	-0.189	-0.209
	131-I <sub>BS</sub>	↗ when [acid] ↗	<b>-0.392</b>	-0.328	-0.132	-0.145
	145-I <sub>BS</sub>	↗ when [acid] ↗	<b>-0.402</b>	-0.341	-0.207	-0.253
At T <sub>10</sub>	131-T <sub>10</sub>	↘ when [acid] ↗	-0.103	-0.136	<b>0.447</b>	0.216
	145-T <sub>10</sub>	↘ when [acid] ↗	-0.031	-0.046	<b>0.506</b>	0.233
After 1 <sup>st</sup> swallow	173-T <sub>AS</sub>	↗ when [sugar] ↗	0.253	0.364	<b>0.413</b>	<b>0.636</b>

\* Correlation coefficients in bold are significant at  $\alpha = 0.05$  (degree of freedom = 27)

## Highlights

Aroma was enhanced by sweetness in model wines

This enhancement differed whether panelists scored only aroma or taste and aroma

When taste was not scored, perceived aroma did not depend directly on aroma release

When taste was scored, aroma depended on ethyl octanoate release after swallowing

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