# Rules and Mechanisms of Perceptual Interaction of Odor Mixtures : Application to Icewine aroma

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# Règles et mécanismes des interactions perceptives dans les mélanges d'odeurs: application à l'arôme du vin de glace

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Composition du Jury :

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|---|---|---|
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**Titre:** Règles et mécanismes des interaction perceptives dans les mélanges d'odeurs: application à l'arôme du vin de glace

**Mots clés:** composés odorants, perception des arômes, intensité des odeurs, qualité des odeurs, effet induit par les mélanges d'odeurs

**Résumé:** Ce ne sont pas seulement des odorants clés dans un produit, mais également des interactions entre ces odorants différents qui façonnent l'arôme alimentaire global. Cependant, les interactions perceptives induites par des mélanges d'odeurs sont toujours un verrou majeur dans la compréhension et l'analyse des arômes des aliments. Dans cette thèse, le vin de glace a été utilisé comme objet expérimental, et des centaines de mélanges d'odeurs liés l'arôme de ce vin, ou à des aliments en général, ont été conçus et étudiés. L'objectif du travail de thèse était d'explorer les odorants clés des arômes alimentaires dont la perception est affectée par les effets de mélange et de tirer des lois générales qui supportent ces interactions perceptives olfactives. Quatre études principales ont été menées dans la thèse. La première étude a analysé les composés odorants et la perception de ces odorants générant l'arôme du vin de glace par une approche sensomic. La deuxième étude a évalué la contribution des composés odorants clés du vin de glace en considérant les interactions induites par les mélanges grâce à la technique Olfactoscan. La troisième étude a systématiquement exploré les interactions perceptives de plus de 150 mélanges d'odeurs liés aux odorants clés du vin de glace en utilisant un olfactomètre à dilution dynamique à 12 canaux contrôlé par ordinateur. La dernière étude a évalué 222 séries de mélanges binaires de composés odorants, touvés communément dans les aliments, afin d'explorer des lois générales des interactions olfactives et leur influence sur l'intensité et le caractère hédonique des odeurs.

**Title:** Rules and Mechanisms of Perceptual Interactions in Odor Mixtures: Application to Icewine Aroma

**Keywords:** aroma compounds, flavor perception, odor intensity, odor quality, odor mixture-induced effect

**Abstract :** It is not only key odorants in a product but also the interactions between different odorants that finally shape food aroma. However, perceptual interactions induced by odor mixtures are still a major lock in food flavor understanding and analysis. In this thesis, icewine was used as the experimental object, and hundreds of wine- or food-related odor mixtures were designed and investigated for the first time based on the identification and analysis of icewine' odorants. The aim of the thesis work was to explore the key odor elements that affected the perception of odor mixtures and the general laws behind olfactory perceptual interactions. There were four main studies conducted in the thesis. The first study analyzed the odorants and the odorants' aroma perception in icewine by Sensomic approach; The second study evaluated the contribution of odor-active compounds found in icewine considering mixture-induced interactions by Olfactoscan technique; The third study systematically explored the perceptual interactions of more than 150 odor mixtures which were related to the key odorants of icewine by using a 12-channels computer-controlled dynamic-dilution olfactometer. The last study evaluated 222 sets of binary odor mixtures in a large amount of food-related odor-active compounds to explore general laws of olfactory interactions and their influence on intensity and pleasantness.

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Yue MA

# Abstract

The diversity of food aroma is largely due to the combination of a limited number of odorants. Subtle changes in the concentration of odorants in an odor mixture can considerably change the mixture's odor perception, and particular the combination of several odorants also plays an important role in the overall perception of an aroma. Therefore, it is not only key odorants in a product but also the interactions between different odorants that finally shape food aroma. However, perceptual interactions induced by odor mixtures are still a major lock in food flavor understanding and analysis. This topic is of major relevance not only for the quality control and novel product development in the Fast Moving Consumer Goods industry, but also for basic research and technical development in the fields of environmental odor, artificial sense and intelligent sensors. In addition, understanding the mechanisms underpinning olfactory perceptual interactions is also one of the core issues in understanding the coding and signal processing of odor and aroma perception especially in humans. In this thesis, icewine was used as the experimental object, and hundreds of wine- or food-related odor mixtures were designed and investigated for the first time based on the identification and analysis of icewine' odorants. The aim of the thesis work was to explore the key odor elements that affected the perception of odor mixtures and the general laws behind olfactory perceptual interactions. The main studies of the thesis are as follows:

1. The first study analyzed the odorants and the odorants' aroma perception in icewine. By means of gas chromatography-olfactometry, namely aroma extract dilution analysis (AEDA) coupled with mass spectrometry (MS) on polar and nonpolar columns, a total of 59 odor-active aroma compounds in three ranks of Vidal icewines were identified, and 28 odorants (FD≥ 9) were further quantitated for aroma reconstitution and omission tests. *β*-Damascenone showed the highest FD value of 2187 in all icewines. 3-(methylthio)-1-propanal (methional) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) were observed for the first time as important odorants in Vidal icewine. Aroma recombination experiments revealed a good similarity containing the 28 important odorants. Omission tests corroborated the significant contribution of *β*-damascenone and the entire group of esters. Besides, furaneol and methional also had significant effects on icewine character, especially on apricot, caramel, and tropical fruit odor characteristics.

2. The second study evaluated the contribution of odor-active compounds found in icewine considering mixture-induced interactions. By comparing the impact of key odorants detected in icewine following a gas chromatography-olfactometry approach with an Olfactoscan-based methodology using a background odor of icewine, 69 odor zones were detected. The candidate odor-active compounds were identified by comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC-TOFMS). The results revealed that icewine odorants could exert masking or synergy on key odorants when they are considered in the complex wine aroma buffer. Several compounds can induce qualitative changes in the overall wine background odor. This study underlined the efficiency of Olfactoscan-like approaches to screen for the real impact of key odorants and to pinpoint

specific compounds that could be highly influential once embedded in the aroma buffer.

3. The third study systematically explored the perceptual interactions between key odor-active compounds. Based on the findings of the first two studies, more than 150 odor mixtures samples, related to the key odorants of icewine and wine aroma vectors, were designed to assess the influence of odor intensity, the number of odorants in the mixture, and combinations of odorants on the overall perception. The experiment was performed with a 12-channels computer-controlled dynamic-dilution olfactometer. The results show that (1) adding most of the impact odorants into icewine can affect the aroma of wine individually with respect to their odor quality (i.e. aroma descriptor); the higher the odor intensity the higher the impact; (2) the olfactory discrimination ability of human was more sensitive to compound addition compared to omission, especially for mixtures with less than three odorants, a result that strengthens the hypothesis of perceptual stability under certain restrictions; (3) the impact of odorants combination on the attributes of e.g. cheese, smoky, and caramel likely involved contrasted odor processing strategies such as configural and elemental perception. Besides, perceptual interactions between different odorants or between common wine aroma vectors were highlighted, which could be verified or assessed in the future by psychophysical and/or neurobiological techniques.

4. The last study explored the general laws of olfactory interactions and their influence on intensity and pleasantness. The study selected 72 representative odorants in food and beverage systems based on the differences in their structural parameters, and 222 sets of binary odor mixtures were designed among the 2556 possible combinations. By measuring the intensity and pleasantness of the binary odor mixtures and of their two unmixed components, general laws of perceptual interactions, as well as hypotheses on their putative influencing factors in a simple system, were established. For odor intensity, the results showed that the components' odor was perceived within the mixture in most cases, and their intensity remained the same as in the unmixed situation in 54.3% of cases. Masking was the second major effect (44.8%) and occurred more frequently when components' pleasantness was significantly different. Synergy occurred in a small number of cases (0.9%) and only for four compounds in the tested set. The overall odor intensity of the mixture was determined to be equal to the strongest component in most cases (73.9%), while partial addition was observed as the second most frequent effect (21.7%), especially when the components had equal intensity. For odor pleasantness, in most cases, the pleasantness of the binary mixtures was driven by the pleasantness and intensity of its components. Nevertheless, a significant pleasantness partial addition was observed in 6 binary mixtures consisting of 2 components with similar pleasantness ratings. A mathematical model involving the pleasantness of the components, as well as $\tau$-values reflecting components' odor intensity proportions was applied to predict mixture pleasantness. Using this model, the pleasantness of mixtures including two components with contrasting intensity and pleasantness could be efficiently predicted at the panel level ($R^2 > 0.80$, Root Mean Squared Error$< 0.67$). Overall, the results of this study should contribute to a better prediction of the outcome to expect when mixing key components of food odors.

Finally, the results obtained in the Thesis should be beneficial to the understanding of

interactions between odorants, to promote the quality control in the production of fast-moving consumer goods such as wine and to gain knowledge on the information processing mechanisms at play in the olfactory system.

**Keywords:** aroma compounds, flavor perception, odor intensity, odor quality, odor mixture-induced effect

# Description of abbreviations

| Abbreviation | Full name |
|---|---|
| AEDA | aroma extract dilution analysis |
| AON | anterior olfactory nucleus |
| ANOVA | analysis of variance |
| cAMP | cyclic adenosine monophosphate |
| CATA | Check-All-That-Apply |
| DA | descriptive sensory analysis |
| DF | detection frequency |
| EEG | electroencephalogram recordings |
| EOG | electro-olfactogram |
| ERP | event-related potentials |
| FD | flavor dilution factor |
| FID | flame ionization detector |
| fMRI | functional magnetic resonance imaging |
| GC × GC-TOFMS | comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry |
| GC-MS | gas chromatography-mass spectrometry |
| GC-O | gas chromatography-olfactometry |
| GC-PO | gas chromatograph-pedestal olfactometer |
| GPCR | G-protein coupled receptor |
| IP3 | inositol-1,4,5-trisphosphate |
| MTCs | mitral-tufted cells |
| NIF | nasal impact frequency |
| OAVs | odor activity values |
| OB | olfactory bulb |
| OC | olfactory cortex |
| ODP | olfactory detection port |
| OE | olfactory epithelium |
| OLFH | high level of Olfactoscan |
| OLFL | low level of Olfactoscan |
| ORs | olfactory receptors |
| OSNs | olfactory sensory neurons |
| OZs | odor zones |
| PC | piriform cortex |
| PCA | principal component analysis |
| PET | positron emission tomography |
| PLSR | partial least squares regression |
| PLS-DA | partial least squares discriminant analysis |
| PG | periglomerular interneurons |
| QDA™ | quantitative descriptive analysis |
| RATA | Rate-All-That-Apply |
| SPE | solid-phase extraction |

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using a paired t-test (*t.test* function). The significance of the difference for each aroma attribute is marked in the figure (*: $p$ <0.05).

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**Figure 10** Schematic representation of the GC-O and Olfactoscan analysis for icewine

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**Figure 12** Nasal impact frequency (NIF, %) comparisons between GC-O and Olfactoscan analysis. An NIF difference above 20% (4/19) was considered a threshold for a significant mixture-induced effect for a peak. **(a)** The NIF difference between GC-O analysis and Olfactoscan analysis within the aroma buffer of icewine at a high concentration. If the NIF for OLFH was significantly lower than the NIF for GCO, a masking effect (in purple color) occurred; if the NIF for OLFH was significantly higher than the NIF for GCO, synergy effect (in light blue color) occurred. **(b)** The NIF difference between GC-O analysis and Olfactoscan analysis within the aroma buffer of icewine at a low concentration.

**Figure 13** The NIF difference between GC-O analysis and Olfactoscan analysis at both high and low concentrations. **(a)** The peak of GC-O analysis and the effect occurring within the aroma buffer of icewine for each peak are marked. The effects including masking occurring at both concentrations (in red); synergy occurring at both concentrations (in dark blue); masking occurring at high concentration (in purple); synergy occurring at high concentration (in light blue); masking occurring at low concentration (in rose); synergy occurring at low concentration (in light green); and no significant effect occurring at either concentration (in black). **(b)** Peak of Olfactoscan analysis within the aroma buffer of icewine at high (deep orange) and low (light orange) concentrations. E.g.: For the NIF of peak 11, the aroma buffer of icewine at high level was marked in deep orange and the aroma buffer of icewine at low level was marked in light orange, the NIF at low level (47.4%) is higher than the NIF at high

$IM_{XO1HA}$), the attribute' intensity of the target component is increased, that is synergy (+) occurs; if $IM_{XA}$ < min ($IM_{XO1LA}$, $IM_{XO1HA}$), the attribute' intensity of the target component is reduced, that is masking (-) occurs; and if min ($IM_{XO1LA}$, $IM_{XO1HA}$)< $IM_{XA}$ < max ($IM_{XO1LA}$, $IM_{XO1HA}$), it was classified into a group namely not sure (n), where *independence* might occur. Attribute changes with *IM* values below 0.4 are also included in (n). In the figure:'++' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA}$ > max ($IM_{XO2LA}$, $IM_{XO2HA}$); '+' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)< $IM_{XA}$ < max ($IM_{XO2LA}$, $IM_{XO2HA}$); '+-' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA}$ < min ($IM_{XO2LA}$, $IM_{XO2HA}$); '-' represents $IM_{XA}$ < min ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)< $IM_{XA}$ < max ($IM_{XO2LA}$, $IM_{XO2HA}$); '--' represents $IM_{XA}$ < min ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA}$ < min ($IM_{XO2LA}$, $IM_{XO2HA}$); 'n' represents min ($IM_{XO1LA}$, $IM_{XO1HA}$)< $IM_{XA}$ < max ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)< $IM_{XA}$ < max ($IM_{XO2LA}$, $IM_{XO2HA}$).

**Figure 20** Schematic of psychophysical experiment data collection. The upper scale shown in the figure presented two ticks labeled as standard 1 and standard 2. This scale was only provided in an instruction sheet during the training session along with the standard samples. This instruction sheet was also provided at the beginning of the first two sessions to remind subjects with the intensity scale. However, for all the samples' evaluation, we only used the bottom scale without the two ticks.

**Figure 21** Intensity and pleasantness of 72 odorants calculated across all the subjects in all the trials

**Figure 22** Experimental observations of perceptual interactions within binary mixtures regarding odor intensity of components within the mixture. **Panel (a)** represents the frequency of mixture-induced effects on the intensity of components' odor within mixtures as a function of components' intensity and pleasantness: Group E included mixtures made with odorants that are not significantly different in their unmixed odor intensity and pleasantness; Group I included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness but significantly different in intensity; Group P included mixtures made with odorants that are not significantly different in their unmixed odor intensity but significantly different in pleasantness; and Group IP included mixtures for which a significant difference in both intensity and pleasantness. **Panel (b)** represents the frequency of mixture-induced effects on the intensity of components' odor within mixtures as a function of components' intensity and pleasantness: Group E+I (green color) included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness, and group P+IP (red color) included mixtures made with odorants that are significantly different in their unmixed odor pleasantness.

**Figure 23** Experimental observations of perceptual interactions within binary mixtures regarding overall mixture odor intensity. **Panel (a)** represents a plot of Sigma-Tau ($\sigma$-$\tau$) of the 222 binary mixtures classified into four different groups. **Panel (b)** represents the frequency of mixture-induced effects on the overall intensity of binary mixtures as a function of components' intensity and pleasantness: Group E included mixtures made with odorants that are not significantly different in their unmixed odor intensity and pleasantness; Group I included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness but are significantly different in intensity; Group P included mixtures made with

each small picture, where the code B6-R6L1L2L3L4L5L6 represents odorant L1 (3-methyl-1-butanol), L2 (ethyl 2-methyl butanoate), L3 (*β*-damascenone), L4 (3-methylbutanoic acid), L5 (guaiacol) and L6 (furaneol) mixed with R6 (icewine).

**Supplementary Figure 9** Analysis of the changes in odor attributes of mixed odor samples with different odorant combinations and the significance of differences. The column 'odorants' represents the odorants modified (added or omitted) in the sample. The descriptors marked in green cell were the dominant odors related to the different odorants (i.e. L1, alcoholic; L2, tropical fruit; L3, honey/ apricot; L4, cheese; L5, smoky; and L6, caramel). The column 'A' represents the percent change between the sample and the icewine odor sample; The column 'O' represents the percent change between the sample and the sample made of the icewine odor in which the 6 odorants were added (B6-R6L1L2L3L4L5L6). For example, for the sample L1L2L3L4L5L6, the value in column 'A' was calculated as following ($IM_{\text{B6-R6L1L2L3L4L5L6}}$-$IM_{\text{icewine}}$)/$IM_{\text{icewine}}$; the value in column 'O' was calculated as following ($IM_{\text{icewine}}$-$IM_{\text{B6-R6L1L2L3L4L5L6}}$)/$IM_{\text{B6-R6L1L2L3L4L5L6}}$. The significance of the difference between attributes is marked in each cell with different colors (yellow: $p$<0.05; light orange: $p$<0.01; dark orange: $p$<0.001); in column 'A', the difference was compared between $IM_{\text{sample}}$~ $IM_{\text{icewine}}$ and in column 'O', the difference was compared between $IM_{\text{sample}}$~ $IM_{\text{B6-R6L1L2L3L4L5L6}}$.

**Supplementary Figure 10** The influence of the different odorant combinations on the 13 odor attributes. The figure was drawn after collecting the odorant combinations which induced significant changes in the 13 attributes in Supplementary Figure 9. The column 'A' represents the influence in 'addition' test, the column 'O' represents the influence in 'Omission' test, and the column 'M' indicates whether the combination in 'A' and 'O' was the same (S) or complementary (C). In column 'A', the change was between $IM_{\text{sample}}$~ $IM_{\text{icewine}}$, and if $IM_{\text{sample}} > IM_{\text{icewine}}$, the font color of the label is red, If $IM_{\text{sample}} < IM_{\text{icewine}}$, the font color of the label is black; In 'O' column, the change was between $IM_{\text{sample}}$~ $IM_{\text{B6-R6L1L2L3L4L5L6}}$., and if $IM_{\text{sample}} > IM_{\text{B6-R6L1L2L3L4L5L6}}$, the font color of the label is red, if $IM_{\text{sample}} < IM_{\text{B6-R6L1L2L3L4L5L6}}$, the font color of the label is black. The difference between odor attributes could be found in Supplementary Figure 9, and the significance of the difference between odor attributes was marked in each cell with different colors (yellow: $p$<0.05; light orange: $p$<0.01; dark orange: $p$<0.001).

**Supplementary Figure 11** Subjects' overall performance and coherence checked by principal component analysis.

**Supplementary Figure 12** Subjects' overall performance and coherence checked by principal component analysis. **(a)** Results obtained from the original dataset. **(b)** Results obtained from the dataset without subject 47.

**Supplementary Figure 13** Repeatability of pleasantness. **(a)** Pleasantness of binary odor. **(b)** Pleasantness of odor A. **(c)** Pleasantness of odor B.

**Supplementary Figure 14** Distribution of intensity **(a)** and pleasantness **(b)** values for each odorant. Significant differences between the means of intensity or pleasantness compared to the mean value across odorants are indicated (ns: not significant, *: $p$< 0.05; **: $p$< 0.01; ***: $p$< 0.001; ****: $p$< 0.0001).

# List of Publication and Communication

## Publication

1. **Ma, Y.**, Guilbert, A., Béno, N., Tang, K, Xu, Y., & Thomas-Danguin, T. The effect of odor elements on the perception and perceptual interaction of icewine odor mixtures. (in progress)
2. **Ma, Y.**, Béno, N., Tang, K, Li, Y. Y., Simon, M., Xu, Y., & Thomas-Danguin, T. Assessing the contribution of odor-active compounds in icewine considering odor mixture-induced interactions through gas chromatography-olfactometry and Olfactoscan. (submitted)
3. **Ma, Y.**, Xu, Y., & Tang, K. (2021). Aroma of icewine: a review on how environmental, viticultural, and oenological factors affect the aroma of icewine. *J. Agric. Food Chem.*, 69(25), 6943–6957.
4. **Ma, Y.**, Tang, K., Xu, Y., & Thomas-Danguin, T. (2021). A dataset on odor intensity and odor pleasantness of 222 binary mixtures of 72 key food odorants rated by a sensory panel of 30 trained assessors. *Data in Brief*, 36, 107143.
5. **Ma, Y.**, Tang, K., Xu, Y., & Thomas-Danguin, T. (2021). Perceptual interactions among food odors: Major influences on odor intensity evidenced with a set of 222 binary mixtures of key odorants. *Food Chem.*, 353, 129483.
6. **Ma, Y.**, Tang, K., Thomas-Danguin, T., & Xu, Y. (2020). Pleasantness of binary odor mixtures: rules and prediction. *Chem. Senses*, 45(4), 303-311.
7. Tang, K., Tian, X., **Ma, Y.**, Sun, Y., Qi, X., Miu, C., & Xu, Y. (2020). Aroma characteristics of Cabernet Sauvignon wines from Loess Plateau in China by QDA®, Napping® and GC-O analysis. *Eur. Food Res. Technol.*, 246(4), 821-832.
8. Lyu, J., **Ma, Y.**, Xu, Y., Nie, Y., & Tang, K. (2019). Characterization of the key aroma compounds in Marselan wine by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission tests. *Molecules*, 24(16), 2978.
9. Tang, K., Xi, Y. R., **Ma, Y.**, Zhang, H. N., & Xu, Y. (2019). Chemical and sensory characterization of Cabernet sauvignon wines from the Chinese Loess Plateau region. *Molecules*, 24(6), 1122.
10. Huang, L., **Ma, Y.**, Tian, X., Li, J. M., Li, L. X., Tang, K., & Xu, Y. (2018). Chemosensory characteristics of regional Vidal icewines from China and Canada. *Food Chem.*, 261, 66-74.
11. **Ma, Y.**, Tang, K., Xu, Y., & Li, J.M. (2017). Characterization of the key aroma compounds in Chinese Vidal icewine by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission tests. *J. Agric. Food Chem.*, 65(2), 394-401.

## Communication

1. **Ma, Y.**, Tang, K., Xu, Y., Thomas-Danguin, T. Perceptual interactions in odor mixtures: focus on intensity with a set of 222 binary mixtures (Oral). *The 18th International Symposium on Olfaction and Taste (ISOT)*, Virtual Meeting, 3-7, August, 2020.
**2.** Tang, K., Wang, B., **Ma, Y.**, Xu, Y., Li, J.M. Effect of fermentation temperature on the flavor composition of Chinese icewine (Oral). *The 2nd International Flavour and Fragrance*

*Conference*, Wuxi (China), 28-31 May, 2018

3. **Ma, Y.**, Tang, K., Xu, Y., Li, J.M. Research on the aroma characteristics and impacts of the nonvolatile matrix composition on the aroma release of vidal icewine based on sensomics (Poster, flash oral). *The 15th Weurman Flavour Research Symposium*, Graz (Austria), 18-22 September, 2017

4. **Ma, Y.**, Béno, N., Tang, K., Simon, M., Xu, Y., Thomas-Danguin,T. The GC-PO technique used to evaluate the contribution of odor-active compounds in icewine (Poster). *The 16th Weurman Flavour Research Symposium*, Virtual Meeting, 4-6 May, 2021

5. **Ma, Y.,** Thomas-Danguin, T., Tang, K., Xu, Y. Prediction of hedonic judgments of binary odorant mixtures using their molecular structure features (Poster). *The 12th Wartburg Symposium on Flavour Chemistry & Biology*, Eisenach (Germany), 21-24 May, 2019.

# Introduction

From an evolutionary perspective, animals rely on smell because they need to capture much chemical information from the environment to complete some of the necessary tasks for their survival and reproduction, such as: finding prey, feeding, avoiding predators, identifying mates, and marking territory, navigating, identifying relatives and establish a social hierarchy. Compared with other animals, humans tend to rely on sight and hearing to collect information about the surrounding environment, but humans still retain the ability to detect and distinguish a large number of odorants and odors. This discrimination ability increases the complexity of our perception of odors and flavors and enables us to detect and identify many volatile molecules released from food and beverages.

The research on the odor of food and beverages has always been the core issue in flavor research. Human perception of food odor is based on the odorants present in the food and the signal processing of these odorants by the olfactory system. At present, a large number of studies related to the separation and identification of odorants in food have been carried out. These studies showed that there were no more than 40 key odorants in a food that ultimately determine its odor, and compared with other foods, the composition of key odorants in wine was more abundant (Dunkel et al., 2014). This means that compared to other foods, the interaction between wine odorants is more complex making wine one of the most challenging and representative food samples in odor mixture research. Determining the key odorants in wine and their contribution is the foundation of understanding wine aroma perception, and it is also critical for product quality control and flavor regulation. This type of research has been carried out for more than 30 years. This research is based on the use of gas chromatography to separate the complex odorants extracted from products, performing qualitative and quantitative analysis of single odorants when combined with olfactometry and mass spectrometry detection, and evaluating the impact of odorants on the overall odor perception of the product based on perceived intensity and odor activity value (OAVs). This method combined with recombination and omission experiments can ascertain the contribution of key odorants to the overall odor perception of the product under the non-volatile matrix conditions (Hofmann, Krautwurst, & Schieberle, 2018).

At present, the key odorants in most wines have been identified, but it is still impossible to predict the final aroma perception of wine based on the composition. Due to the complex perceptual interactions between different odorants, any change in the combination and intensity of odorants may affect the final aroma perception of wine. Therefore, analyzing the perceptual interactions laws and the mechanisms of odor mixtures perception is the most critical step in achieving product aroma prediction. However, due to the complexity of odor mixture perception and the lack of systematic research and support from large sample data, some key issues in the perceptual interaction are still inconclusive. These academic issues include: how do different odor characteristics (intensity, composition number, odor combinations, pleasantness, etc.) affect perception and perceptual interactions in odor mixtures either in simple systems or in real food samples, and what are the rules of odor

interactions.

Due to the variability of perceptual interactions and the diversity of influencing factors, the analysis of the perception mechanism of odor mixtures has always been challenging and has attracted attention in the research field of olfaction. Moreover, it is also one of the core issues in understanding the coding and signal processing of ecological odor perception in both humans and animals. In addition, research on laws and mechanisms of the olfactory interaction is directly related to quality control and novel product development in Fast Moving Consumer Goods industry such as wine, food, flavors and fragrances and the topic is also involved in basic research and technical development in the fields of environmental odor, artificial sensing and intelligent sensors.

This rationale funded the main objective of this thesis, which was to get a better understanding of rules and mechanisms of perceptual interactions in odor mixtures. Icewine was used as an experimental object, and hundreds of wine- or food-related odor mixtures were designed and investigated for the first time based on the identification and analysis of icewine' odorants. The thesis work aimed to explore the key odor elements that affected the perception of odor mixtures and the general laws behind olfactory perceptual interactions. The thesis manuscript contains seven chapters:

The literature review presented in Chapter 1 gives a brief description of the olfactory system and odor perception. Then, odor mixtures perception is highlighted through examples of interactions between odorants at the perceptual level. Research progress in perceptual interactions between odorants observed in foods and beverages and methodologies developed in these researches are also briefly summarized.

A complementary review of the literature (Chapter 2) aims to provide comprehensive information related to our research object, the odor of a special wine, namely icewine, which is made from late-harvested and frozen grapes by the unconventional production process. The uniqueness of icewine's aroma, the mechanism of odor generation in icewine and the effects of natural and human factors in the vineyard and winery on the aroma composition are systematically presented. The current research status and future research needs for icewine aroma are also put forward in this chapter. Some of these researches needs have been further considered in this thesis work.

Chapters 3 to 6 describe the experimental work of the thesis, which is graphically summarized in Figure 1. The first experimental study (Chapter 3) analyzed the odorants and the odorants' perception in icewine. In this study, the key aroma compounds of Chinese Vidal icewine were characterized by means of gas chromatography–olfactometry (GC-O) coupled with mass spectrometry (MS) on polar and non-polar columns. Aroma recombination experiments and omission tests were used to verify and rank the aroma contribution for investigating the key aroma compounds.

The second experimental study (Chapter 4) is among the very first attempts to evaluate the contribution of odor-active compounds considering the mixture-induced perceptual effect on a complex aroma (here icewine). In this study, we used the advanced Olfactoscan setup, which allowed us to consider the impact of a single odorant, identified by GC × GC-TOFMS, on the global wine aroma delivered as a background odor during GC-O analysis. To analyze the data,

a new semiautomatic method was developed to allow the identification of odor zones in a similar way both in GC-O and Olfactoscan approaches based on the detection frequency (DF) method.

The third experimental study (Chapter 5) systematically explored the perceptual interactions between selections of key odor-active compounds. Based on the findings of the first two studies, more than 150 odor mixtures samples related to the key odorants of icewine and wine aroma vectors were designed to assess the influence of odor intensity, the number of odorants in the mixture, and combinations of odorants on the overall perception. The experiment was performed with a 12-channels computer-controlled dynamic-dilution olfactometer.

The last and fourth experimental study (Chapter 6) selected 72 representative odorants in food and beverage systems based on the differences in their structural parameters, and 222 sets of binary odor mixtures were designed among the 2556 possible combinations. By measuring the intensity and pleasantness of the binary odor mixtures and of their two unmixed components, general laws of perceptual interactions, as well as hypotheses on their putative influencing factors in a simple system were established.

The manuscript ended with a general discussion and conclusion (Chapter 7) about the four experimental studies, then the innovation of this work and the perspectives for further research were addressed.

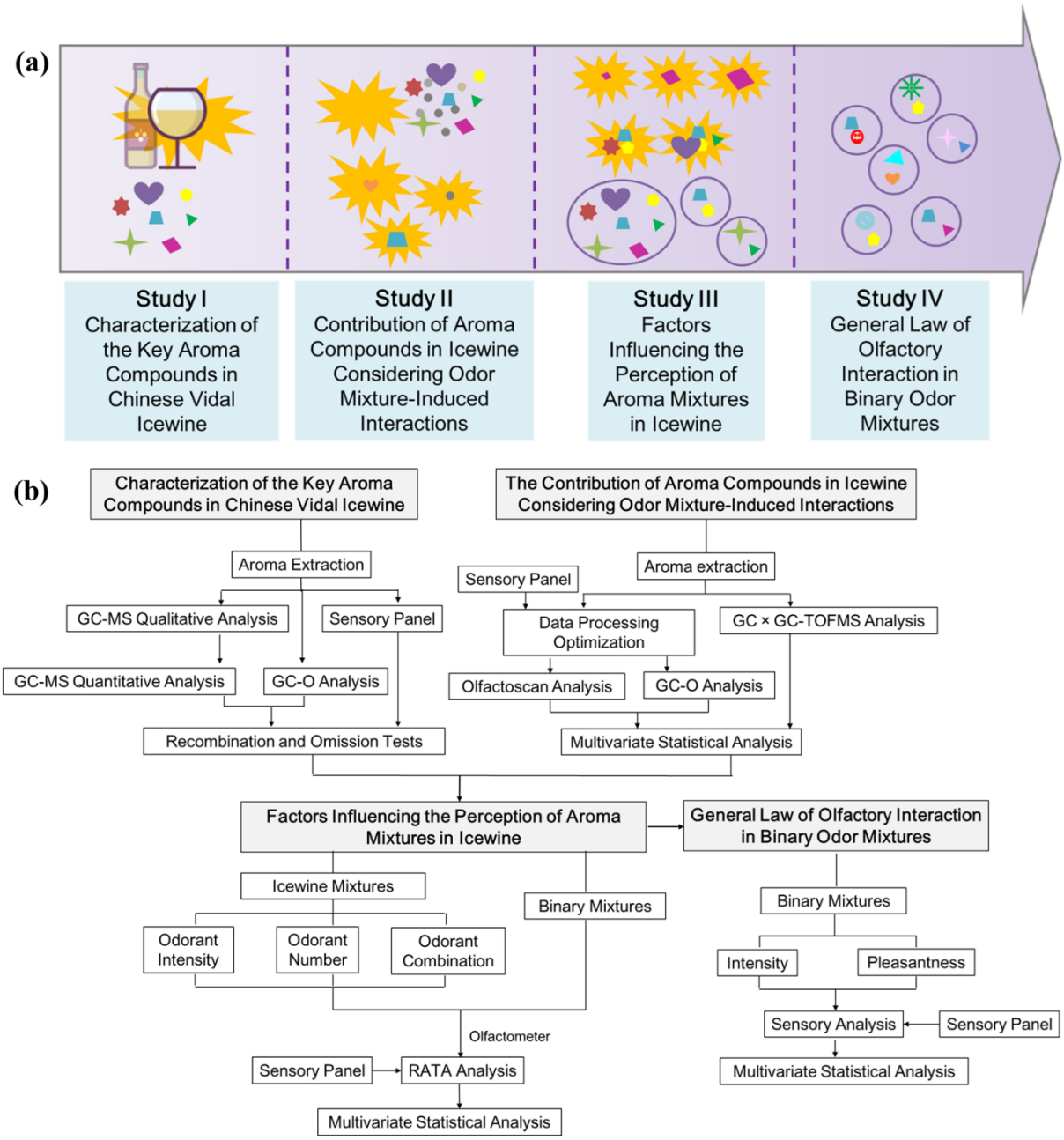


**Figure 1** Research content and technology schematic diagram. **(a)** Experimental content illustration. **(b)** Experimental technique flow chart.

# Chapter I Review of Literature on Odor Perception

## 1.1 Olfactory System

The perception of odor relies on the olfactory system to capture, detect and recognize odorants. The olfactory system is not a unified anatomical structure, but a collection of subsystems with different anatomical structures, different receptor libraries, different transduction mechanisms, and different central projections. All these subsystems are combined through their functions to detect chemical substances in the environment and convert them into corresponding neural signals (Trimmer & Mainland, 2017).

The olfactory system includes three main levels (Figure 2), namely, the olfactory epithelium (OE, peripheral level), the olfactory bulb (OB, the first central relay), and the olfactory cortex (OC, cortex level). As a very ancient system in terms of phylogeny, the olfactory system has developed various adaptive strategies to face the odor world. It can perform elemental coding on odors based on physiological needs or motivational events, and can also perform configural processing (or synthetic processing), or both (P. Duchamp-Viret, Lacroix, Kuszewski, & Baly, 2016; Thomas-Danguin et al., 2014).

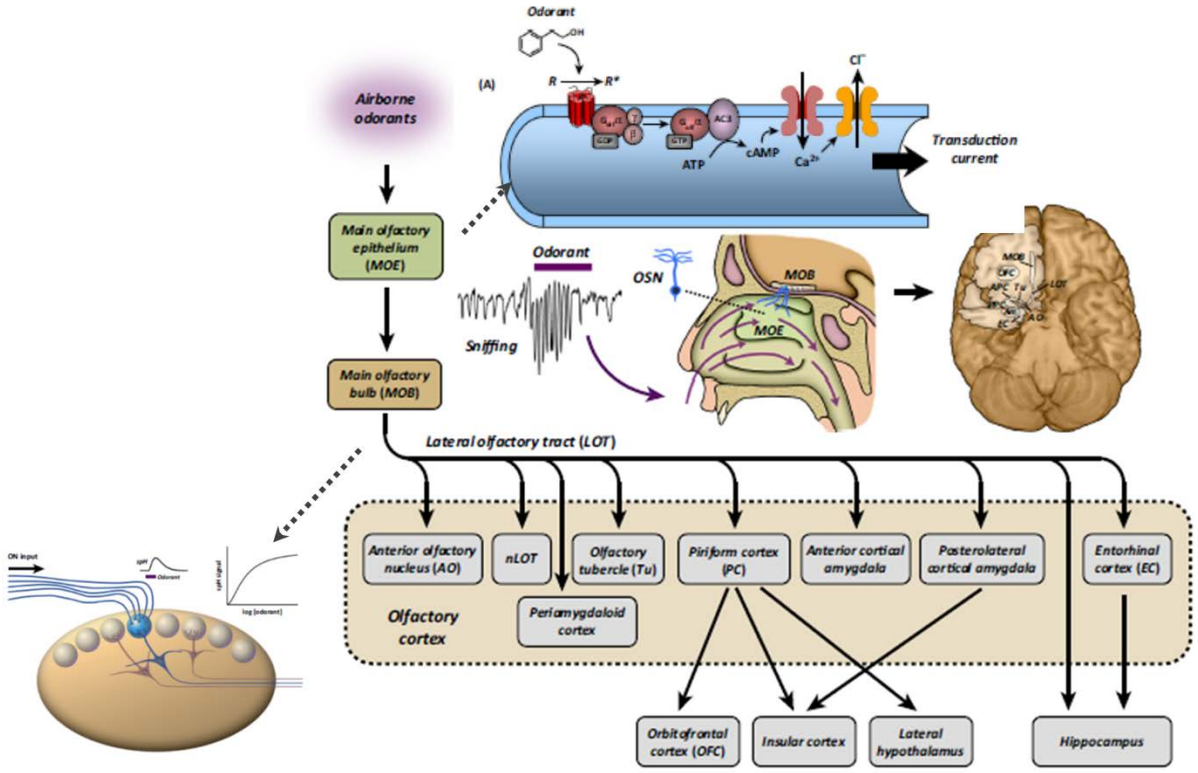


**Figure 2** Illustration of the olfactory system (Mainland et al., 2014)

### 1.1.1 Olfactory Epithelium

The processing of olfactory molecular information in the OE stage depends on the interaction between odor-active volatile molecules (called odorants) and specific olfactory receptors expressed by olfactory sensory neurons (OSNs). In the OE, there are three main steps in the processing of olfactory molecular information, namely binding, signaling and coding. The OE area is composed of supporting cells, basal cells, olfactory glands, and

olfactory cells or olfactory sensory neurons (OSNs). The OE contains nearly 50 million OSNs, and each OSN has 4-50 olfactory cilia at the end (de Souza & Antunes, 2007). Olfactory receptors (ORs) are located on the olfactory cilia. The top part of the nasal cavity is covered by a mucus layer specific to the olfactory system and different from the respiratory mucus. When specific odorants are captured by the olfactory mucus, they pass through the mucus layer and bind to specific ORs. An OR is a G-protein coupled receptor (GPCR), which consists of seven membrane-spanning helices connected by extracellular loops and intracellular loops to form a socket-like structure. Inside the socket, chemical molecules can bind to the proteins (Buck & Axel, 1991). There are about 400 types of ORs in humans, which are divided into two categories based on sequence homology: type I and type II. Class I receptors are the only type of ORs that exist in fish, so they are thought to have evolved earlier and are more sensitive to water-soluble ligands (Trimmer & Mainland, 2017). Soon after the discovery of ORs, the researchers also found that there is only one OR subtype in each OSN (Buck & Axel, 1991; Ngai et al., 1993), that is, each OSN and its corresponding OR can be regarded as a single unit to ensure that the OE and OB have the same precise connection in the cyclic update of the OSN. A single OSN-OR unit can interact with a large number of odorants according to its affinity with molecules, thereby constructing a combination code for odor perception (P Duchamp-Viret, Chaput, & Duchamp, 1999). It is worth mentioning that slight alterations in an odorant structure, or a change in its concentration result in changes in the combination of receptors that recognize the odorant (Malnic, Hirono, Sato, & Buck, 1999). At present, there are two main theories about the recognition of odorants by OSN-OR units. One is the docking theory based on the shape and weak bond interaction between the odorant and the receptor. The other less accepted theory is the vibration theory considering that the OR can recognize molecules through its vibration frequency (Horsfield, Haase, & Turin, 2017). Since each OSN-OR unit can interact with a large group of odorants, and most olfactory stimuli are a mixture of odorants, some odorants that constitute the signal have the opportunity to act simultaneously with the same OSN. This possibility accounts for the interaction between odorants that can occur at the OR level (Rospars, Lansky, Chaput, & Duchamp-Viret, 2008).

Odorants are usually volatile, hard-to-react, and hydrophobic compounds, so that they can pass through the mucus layer and bind to olfactory receptors more easily. When an odorant is combined with its compatible OR, it initiates the intracellular signal transduction mechanism, thereby converting chemical stimuli into electrical signals (Ronnett & Moon, 2002). This combination of the OR and its chemical ligand induces a conformational change in the OR, which leads to the activation of olfactory-specific G protein. The activation stimulates adenyl cyclase and increases the concentration of the second messenger, cyclic adenosine monophosphate (cAMP), which causes the cyclic nucleotide-gated channel to open. When the cyclic nucleotide gated channel opens, sodium ions and calcium ions enter into the cell. Then calcium ions act as the third messenger to promote the flow of chloride ions, and finally form the activation and triggering of olfactory neurons (P. Duchamp-Viret et al., 2016). The OSN response is a graded receptor potential. Once the trigger threshold is reached, action potentials will be triggered (P. Duchamp-Viret et al., 2016). These action potentials will drive along the

axons of the OSN to reach the OB. In the olfactory signal transduction, cAMP is an indispensable signal to produce olfactory response, but in addition to the main cAMP, olfactory response also involves inositol-1,4,5-triphosphate (IP3) and cyclic guanyl cyclase related intracellular signal transduction (P. Duchamp-Viret et al., 2016).

### 1.1.2 Olfactory Bulb

After the olfactory signal is integrated by the OSN, it is transmitted to the olfactory bulb (OB). In the OB, the peripheral representation of odorants may be modified by the organization of the projections from the OE to the OB (Giraudet, Berthommier, & Chaput, 2002), by intrabulbar inhibitory circuits involving periglomerular and granular cells (Yokoi, Mori, & Nakanishi, 1995) and by centrifugal controls exerted by more central structures (Shipley & Ennis, 1996). In the OB, the OSN axons generate synapses on discrete and dense subspherical nerve fiber structures, called glomeruli. The transduction of olfactory signals follows the rule of one OR one OSN, and OSNs expressing the same OR converge on the same olfactory glomerulus, that is, each olfactory glomerulus only accepts one type of OR information. This rule ensures that olfactory signals can be stably transducted during the OSN renewing. At this stage, the OB filters and converts olfactory information into the olfactory cortices. An olfactory glomerulus and its main output neurons, namely mitral-tufted cells (MTCs), which are connected to it, receive the information of the olfactory peripheral system from thousands of OSNs expressing the same OR. This quantitative convergence can amplify weak signals, and reduce irrelevant noise , so as to enhance the signal-to-noise ratio of the signal, to achieve normalization and contrast enhancement before transmission (Persaud, 2013). In addition to MTCs, periglomerular interneurons (PG), mainly GABAergic neurons, can receive OSN signals and form simple inhibitory and reciprocal synapses with MTC, thereby regulating the concentration range of what MTCs can encode (P. Duchamp-Viret et al., 2016). Since the OSN gathered in the OB strictly follows the one OR one OSN rule, this suggests that distinguishing different olfactory stimuli should rely on the coding of OB, which is targeted and activated by the OSNs. Studies have found that the OB of rats can be divided into areas with different functions that can be activated by different types of odorants, and it is believed that the olfactory bulb areas with similar receptive domains are very close in space and form a large functional domain to detect a class of odor molecules, and this functional domain constitutes the spatial coding within the OB (Uchida, Takahashi, Tanifuji, & Mori, 2000). However, this hypothesis was subsequently questioned by Soucy et al (2009). They found that the relevance of the response of different olfactory glomeruli to certain odors had nothing to do with the space in which they were located, and the response of adjacent olfactory glomeruli to odors was not particularly similar (Soucy, Albeanu, Fantana, Murthy, & Meister, 2009). Alternatively, other study suggested that odor code would be based on fine temporal tuning of spiking activity within cell assemblies (Wehr & Laurent, 1996). These synchronously discharged assemblies use gamma oscillations as a clock to transfer sensory information throughout the burst sequence, and the brain would assign specific odor object to the temporal pattern which was successfully assembled by the MTCs. Besides, it is believed that the OB odor encoding could be affected by attention, experience and memory, as well as

the physiological relevance of stimuli (P. Duchamp-Viret et al., 2016).

### 1.1.3 Olfactory Cortex

The olfactory information projection from the OB is connected to many areas in the brain through MTCs. All brain areas that receive direct input from MTCs axons are defined as the primary olfactory cortex (OC), of which the piriform cortex (PC) is the largest sub-area, suggesting that the PC may be the area where odor perception is formed. It seems that the anterior part of the PC (aPC) and the posterior part of the PC (pPC) likely have distinct roles in odor encoding (Thomas-Danguin et al., 2014): encoding of odorant (chemical) identity may occur in the aPC while encoding of odor (perceptual) similarity or odor quality may occur in the pPC (Kadohisa & Wilson, 2006; Yoshida & Mori, 2007). The projection of olfactory information on the PC does not follow any obvious topographical rules (Wilson & Stevenson, 2003b). Indeed, a single odor activates distributed neuron subgroups throughout the cortex without spatial preference, that is, the MTCs projection pattern on PC neurons diverges in space but converges in quantity. The lack of spatial association between the OB and the PC means that the activation pattern of cortical neuron collections is based on the representations of different odors (Stettler & Axel, 2009). Different odor-active molecules will trigger the scattered and spatially different (but partially overlapping) activity patterns of PC neurons. PC neurons affected by multiple MTCs will only be activated when related MTCs are stimulated synchronously. This mechanism increases the distinction between structurally similar odorants. In fact, although two similar odorants generate very close spatial patterns in OB glomeruli, they will activate different and multiplexed PC units (P. Duchamp-Viret et al., 2016). PC has rapid neuroplasticity in olfactory signal processing. A 10 s or 50 s odor stimulus will establish a synthetic representation of the odor mixture in PC neurons and distinguish it from a single odor component. Therefore, experience, learning and memory affect the coding procedures and outcomes. This processing characteristic of PC may be based on the result of natural selection, because mammals in nature most commonly deal with tasks related to overall mixture recognition, rather than the analysis of different odor components. Implicit memory and learning will be extremely facilitated in the PC odor processing to analyze the location, time and background of odor perception (Wilson & Stevenson, 2003b). In addition, the PC may balance stability and discrimination in the processing of olfactory signals to ensure the recognition (stability) of specific perceptions in the entire ‘complete process’ when certain elements were missing or added to a mixture system, while still ensuring the distinction and selection of variability (discrimination) in the ‘separation process’. In the olfactory cortex, olfactory information is also combined with information from other sensory organs and compared with previous experience to give the final odor perception (P. Duchamp-Viret et al., 2016).

## 1.2 Dimensions of Odors

The main features of odor percepts include odor intensity, odor quality, and odor pleasantness.

### 1.2.1 Odor Intensity

Intensity is a perceptual variable, which is a basic feature for all senses; it can facilitate

comparisons of different objects within a single modality as well as across modalities. In olfaction, intensity is a common feature of all odors (Engen, 1964), and the perceptual organization of intensity is conserved across the mammalian species (e.g. rats and humans). It has been shown that neuronal activity in the piriform cortex, entorhinal cortex (Rolls, Kringelbach, & de Araujo, 2003), and amygdala (Anderson et al., 2003) correlate with intensity perception, but how neural activity at specific stages in olfactory processing contributes to this perceptual variable is unclear (Sirotin, Shusterman, & Rinberg, 2015). Perceived intensity for a given odor is a function of at least two variables: the odorant(s) concentration and the sampling duration (Sirotin et al., 2015). The perceived intensity of an odorant is a monotonic, sigmoidal function of the logarithm of odorant concentration. A comparison of models found that the Hill equation is the most suitable model for a wide variety of odorants (Chastrette, Thomas-Danguin, & Rallet, 1998)(Equation 1), where $I_{max}$ is maximal odor intensity, $C$ the odorant concentration, $K$ the concentration at the inflection point on the log axis, and $n$ the Hill exponent. Given that the Hill exponent varies between odors, intensity is not an intrinsic property of an odorant (Mainland et al., 2014).

$$I = I_{max} C^n / (K^n + C^n) \quad \text{(Equation 1)}$$

### 1.2.2 Odor Pleasantness

It has been suggested that odor pleasantness (hedonic dimension or valence) is an important feature (Block, 2018; Rolls et al., 2003; Wise, Olsson, & Cain, 2000). In particular, when a wide range of odors are assessed at a similar odor intensity, the hedonic dimension was found to be the most salient (Zarzo, 2008). The pleasantness of an odor not only affects judgment but also causes changes in individual physiological parameters. Exposure to odors with different pleasantness levels can modify heart rate, skin conductance, and skin temperature (He, Boesveldt, de Graaf, & de Wijk, 2014). Odor pleasantness is determined by many factors, including molecular structure (Khan et al., 2007), odor quality (Kermen et al., 2011) and odor intensity (Doty, 1975); but also individual features, such as genetic (Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007) and cognitive factors, aging (Konstantinidis, Hummel, & Larsson, 2006), culture (Ayabe-Kanamura et al., 1998; Seo et al., 2011) and physiological status (Rouby, Pouliot, & Bensafi, 2009); and temporary environmental factors, such as visual stimuli (Hummel et al., 2017).

### 1.2.3 Odor Quality

Odor quality mainly refers to the description or the identity of an odor, namely of its source, such as apple, rose, nut, etc. Odor quality is also one of the dimensions of odor perception, and it is the most important perceptual dimension for humans to recognize odors and name them. The range of odor quality that humans can perceive is usually considered as the odor space. Bushdid et al. (2014) speculated on the range of the perception space of human smell and concluded that humans can distinguish 1 trillion molecules (Bushdid, Magnasco, Vosshall, & Keller, 2014). However, after a revision by Gerkin et al. (2015), it is believed that the amount of odors that humans can distinguish is still to be determined (Gerkin & Castro, 2015). The odor quality is mainly related to the physical, chemical and structural parameters of the

odorant or mixtures, but the recognition and naming of odors by humans is related to learning and memory (Wilson & Stevenson, 2003b). Early studies explored the odor characteristics of newly synthesized molecules by changing the structural characteristics of certain types of molecules. Various studies found that the structural parameters of compounds such as chain length, degree of unsaturation, double bond position, functional group position and molecular stereochemical factors can affect odor quality of the odorants (Jelen & Gracka, 2016). The research based on quantitative structure activity relationship (QSAR) modeling in the past five years has consolidated the relationship between the structural parameters of odorants and odor quality (Keller et al., 2017; Keller & Vosshall, 2016; H. Li, Panwar, Omenn, & Guan, 2017).

## 1.3 Odor Mixtures

### 1.3.1 Perception of Odor Mixtures

At present, the key odorants in lots of foods have been identified (Dunkel et al., 2014; Hofmann et al., 2018), but it is still impossible to predict the final odor perception of foods based on the composition of odorants. The odor perception of food is based on the perception of odor mixtures, and when it comes to the perception of an odor mixture, due to the mechanism of binding and encoding of chemical signals by the peripheral and central olfactory system and the configural processing (or synthetic processing) (Livermore & Laing, 1998) or elemental processing (Berglund & Engen, 1993), the odor mixtures could be perceived as homogeneous or heterogeneous and different odor mixture-induced effect, such as synergy effect, masking effect and blending effect could occurred (Figure 3) (Thomas-Danguin et al., 2014). Thus, the perception of an odor mixture is not a linear combination of the elemental perception of the single odorants (Thomas-Danguin, Barba, Salles, & Guichard, 2017).

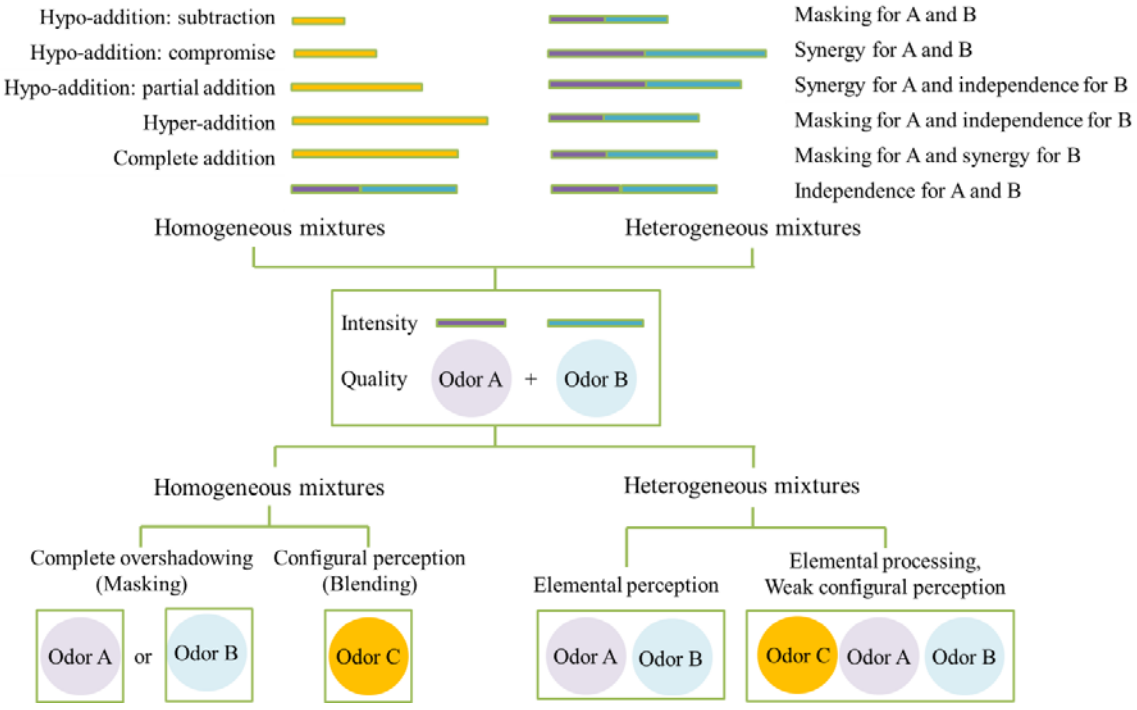


**Figure 3** Illustration of aroma mixture perception, adapted from (Thomas-Danguin et al., 2014)

Odor mixture interactions can be characterized with regard to the odor quality and intensity (Berglund, Berglund, & Lindvall, 1976). In a binary odor mixture, the perception can be heterogeneous when the two odors of the two mixed components are perceived as separate odors once in the mixture. Conversely, the perception for a binary mixture can be homogeneous when only a single odor is perceived from the mixture, which is possible if one of the components is completely overshadowed (Kay, Crk, & Thorngate, 2005) or when odor blending occurs (Le Berre, Thomas-Danguin, et al., 2008). Regarding the odor intensity, previous research (Berglund et al., 1976; V. Ferreira, 2012a; Thomas-Danguin et al., 2014) have defined that perceptual interactions for a heterogeneous perception include synergy, independence, or masking (partial overshadowing), while those for homogeneous perception include hyper-addition, complete addition, or hypo-addition, and hypo-addition can be further divided into partial addition, compromise, or subtraction based on whether the mixture intensity is higher or lower than the intensities of single components (Figure 3 and Figure 4). These perceptual interactions are still a major lock in food flavor understanding and analysis since there are millions of possibilities for combining different food odorants at various concentration levels. Thus, revealing the influencing factors and regular patterns of these interactions is crucial to understanding odor mixture perception, which has major relevance for food flavor perception and its underlying chemistry.

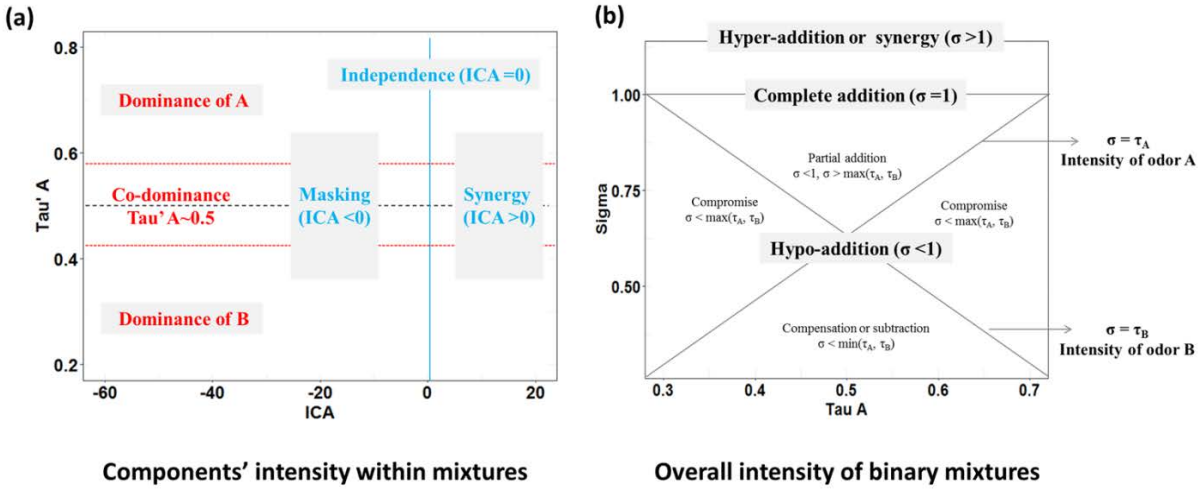


**Figure 4** Schematic representations of the effects that can be theoretically observed as the result of perceptual interactions in odor mixtures. **In panel (a)**, the different terms refer to the type of perceptual interactions within binary mixtures regarding component's odor intensity. *IC* stands for percent of mixture-induced odor intensity change (Equation 2) for the odor of a given individual odorant perceived within a binary mixture with another odorant. Tau' ($\tau'$) represents the relative intensity of one component, which is chosen as a reference, within the mixture (Cain et al., 1995) (Equation 5). **In panel (b)**, the different terms refer to the type of perceptual interactions within binary mixtures regarding overall mixture odor intensity. Sigma ($\sigma$) accounts for the deviation from complete addition and is calculated as the intensity of the mixture divided by the sum of the (unmixed) intensities of the two components(Patte & Laffort, 1979) (Equation 3). Tau ($\tau$) reflects the perceptual proportion (i.e., in terms of intensity) of one component, which is chosen as a reference. This proportion is calculated as the intensity of the reference divided by the sum of the (unmixed) intensities of the two components (Patte & Laffort, 1979) (Equation 4).

$$IC_A = \frac{I_{Amix} - I_A}{I_A} \times 100 \text{ or } IC_B = \frac{I_{Bmix} - I_B}{I_B} \times 100 \quad \text{(Equation 2)}$$

$$\sigma = \frac{I_{AB}}{I_A + I_B} \quad \text{(Equation 3)}$$

$$\tau_A = \frac{I_A}{I_A + I_B} \text{ or } \tau_B = \frac{I_B}{I_A + I_B} \quad \text{(Equation 4)}$$

$$\tau'_A = \frac{I_{Amix}}{I_{Amix} + I_{Bmix}} \text{ or } \tau'_B = \frac{I_{Bmix}}{I_{Amix} + I_{Bmix}} \quad \text{(Equation 5)}$$

In the context of a mixture system, the perceptual properties of an odorant may be different from its original perception, that is, the mixed aroma will tend to produce new odors after mixing. In addition, perceptual interaction may also cause many odorants to lose their original odor qualities in the mixture system (Laing, 1991). Studies have shown that in a mixture containing up to 8 odorants, humans couldn't recognize more than 3 or 4 odorants. In a complex mixture where more than two odorants are mixed, the total intensity of the odor didn't increase with the increasing number of constituents (Laing & Francis, 1989; Livermore & Laing, 1998). However, it should be noted that the odor qualities of the odor compounds we cannot perceive do not mean that they have no contribution to the perception of odor mixture. In addition to changes in odor quality and intensity, the interaction between odorants could also affect pleasantness, but few research studies have been carried out on this topic.

Perceptual interactions in mixtures of odorants could arise from several biochemical or neurobiological interactions from the periphery to the brain (Mainland et al., 2014; Thomas-Danguin et al., 2014). Several factors can significantly influence different stages of olfactory information processing within the olfactory system and include odorants' ratio (Coureaud, Gibaud, Le Berre, Schaal, & Thomas-Danguin, 2011; Laing, Panhuber, Willcox, & Pittman, 1984; Olsson, 1994, 1998; Romagny, Coureaud, & Thomas-Danguin, 2018), odorants' chemical similarity (Linster et al., 2001), exponents of Stevens' law (Laffort, Etcheto, Patte, & Marfaing, 1989), polarity (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b), odor intensity (Laing et al., 1984), odor quality (Barkat, Le Berre, Coureaud, Sicard, & Thomas-Danguin, 2012; Kay et al., 2005), odor familiarity (Rabin, Cain, & Cain, 1989; Sinding et al., 2015), and odor pleasantness (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b).

### 1.3.2 Interactions Occurred at Different Stages of the Olfactory System

The perception of odor mixtures is based on the results of various qualitative and quantitative interactions that may occur at different stages of the olfactory system (V. Ferreira, 2012b; Thomas-Danguin et al., 2014).

Most single odorants contain multiple chemical features that can be detected by different ORs, and individual ORs generally exhibit broad tuning properties showing the ability to interact with many odorants, leading to the building of a combinatorial coding model in which odors are uniquely represented in the periphery by the specific combination of activated OSNs (Brann & Datta, 2020). Single odorant in odor mixtures can act as agonists or antagonists of ORs. Agonists are molecules that are able to bind to the main receptor site and activate the receptor, while antagonists are molecules that are able to bind to the main receptor site but are unable to activate it. Different odorants have different affinities to bind to a receptor and

different efficacy to activate the receptor. However, it seems that the OR would be not simply activated or inactivated, but would adopt many conformations; each of them leading to a highly specific ligand-receptor interaction, and each conformation acting in turn with a specific intracellular signaling complex (P. Duchamp-Viret et al., 2016). Competition interactions and noncompetition interactions can occur at the periphery. By investigating the electrophysiological responses of rat ORNs *in vivo* to odorant agonists and their binary mixtures, it showed that a competitive interaction related model accounted for all concentration-response curves obtained with single odorants and for about half of those obtained with binary mixtures and the other half suggested a noncompetitive interaction (Rospars et al., 2008). Competitive effects result from two molecules, specifically, two agonist odorants or one agonist and one antagonist, bind to the same receptor binding site and the most refined and/or concentrated odorant would bind ORs at the expense of the least one. Noncompetitive effects involve allosteric phenomena which may result from various mechanisms, including agonist binding to another site, which modifies the receptor properties at the main binding site, leading to the changes in the affinity and/or efficacy of ORs (P. Duchamp-Viret et al., 2016; Rospars et al., 2008).

Depending on the odorants included in a mixture and their concentration ratios, different interactions have been observed in electrophysiological studies by comparing the responses of OSNs to binary mixtures and their components (Chaput et al., 2012; P. Duchamp-Viret, Duchamp, & Chaput, 2003; Kang & Caprio, 1997; Steullet & Derby, 1997). Compromise or the subtraction levels of hypo-addition were observed when the response intensity of OSNs to the mixture was lower than the response to the most efficacious component, and conversely, partial addition or hyper-addition was observed when the response intensity of OSNs to a mixture was higher than that induced by the most efficacious component. A given type of interaction was mostly observed over the whole concentration range, but a shift to another interaction type as a function of odorant concentration was observed in some cases (Thomas-Danguin et al., 2014). A study of the binary mixture of citral and octanal showed that synergy mainly occurs on the lowest concentration range of odorants, thus boosting the detection power of single OSNs, for either the mixture regarding the single compounds, or one of the compounds, depending on if the coding mode is elemental or synthetic for the considered mixture (P. Duchamp-Viret et al., 2016).

In addition to the interactions observed at the periphery (ORs, OSNs), central processing also takes part in forming the mixture perception. Studies conducted on patterns of afferent glomerular activity (representing OB input) and the MTC odor responses (representing OB output activity) of binary mixture interactions showed significant interactions in MTC responses rather than their input activity patterns, indicating that the OB contributes to the processing of odor mixtures (Tabor, Yaksi, Weislogel, & Friedrich, 2004). Single MTC responses could encode both elementary information about mixtures' chemical composition, or synthetic olfactory objects (P. Duchamp-Viret et al., 2016). When MCT responses to mixtures were very similar to responses to one of the components, it should appear as mainly devoted to the elemental mixture coding. By contrast, when temporal response patterns to mixtures were clearly distinct from those to single components, such emergence of a specific

response pattern for mixture would rather favor synthetic mixture coding (P. Duchamp-Viret et al., 2016; Wilson & Stevenson, 2003a). By comparing the single-unit responses of MTC to pure odors and to their binary mixtures, it was shown that in most cases, the mixture pattern was closely similar to one of the component patterns, and this dominance of a component over the other one was related to the responsiveness of the cell to the individual components of the mixture, to the molecular nature of the stimulus, and to the coarse shape of individual response patterns (Giraudet et al., 2002), but it is difficult to predict which of the components in a mixture will dominate. It has been shown that MTC responses to binary mixtures of food extracts were dominated less by one component but acquired some novel properties, suggesting that mixture interactions become more complex with increasing overlap of afferent component representations (Tabor et al., 2004). A study which assessed MTCs responses to a set of 348 odorants, presented both individually and in mixtures at behaviorally relevant concentrations, showed that cells typically responded to effective compounds presented both individually and in mixtures, although firing rates evoked by mixtures typically showed partial suppression (Davison & Katz, 2007). The research also showed that MTC activation by odorants was markedly selective and multiple odorants activating a single neuron commonly shared clear structural similarity, but MTC tuning also frequently extended beyond obviously defined chemical categories (Davison & Katz, 2007).

Beyond MTC, the olfactory information is processed in superior areas of the brain, such as the anterior olfactory nucleus (AON) and the PC. The PC also has a major role in mixture perception, but the processing of olfactory information in the PC and the OB is quite different. By examining and comparing responses of AON neurons and OB mitral cells to a panel of structurally diverse odorants presented either as mixtures or as individual components using intracellular and extracellular recordings, it has been shown that a majority of individual AON neurons could be synaptically activated by several mixtures of structurally dissimilar components and by several dissimilar components in an effective mixture, while the majority of mitral cells were activated by only one or two components in a single mixture. The result of the study further suggested that individual AON neurons synaptically integrate several functionally distinct mitral cell inputs (Lei, Mooney, & Katz, 2006). Compared to OB, the PC was able to rapidly discriminate a mixture from its components, in other words, to minimize cross-habituation to components after habituation to the mixture (Kadohisa & Wilson, 2006; Wilson, 2003). However, in the case of omission of individual components from a mixture, the response of the PC is less sensitive than MTCs. It was shown that the PC failed to decorrelate the 10 component mixture from that missing a single component, while ensembles of mitral/tufted cells decorrelated all the various mixture morphs and the standard 10 component mixture (Barnes, Hofacer, Zaman, Rennaker, & Wilson, 2008; Wilson, 2009). However, as more components were missing from the mixture, or novel contaminants added, the PC decorrelated the mixtures even more strongly than the MTCs (Barnes et al., 2008). All these results show that the PC can remain acute for discriminating the perception variability throughout ‘separation’ processing, while it appears capable of ensuring the perception stability throughout ‘completion’ processing (P. Duchamp-Viret et al., 2016). Interestingly, new data suggest that the balance between pattern separation and completion is highly

malleable based on task demands and occurs in concert with changes in perceptual performance (Chapuis & Wilson, 2012).

## 1.4 Research Progress Related to Olfactory Perceptual Interactions

### 1.4.1 Perceptual Interactions between Odorants Observed in Foods and Beverages

Perceptual interactions between odorants belonging to different categories have been described. The results especially obtained in wine, showed that some esters (Lytra, Tempere, de Revel, & Barbe, 2012; Lytra, Tempere, Le Floch, de Revel, & Barbe, 2013), norisoprenoids, dimethyl sulfide (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007) and some volatile fatty acids (San-Juan, Ferreira, Cacho, & Escudero, 2011) enhanced fruity aroma, while fractions containing diacetyl, acetoin, acetic acid, $\gamma$-butyrolactone (Lytra et al., 2012), 4-ethylphenol, acetic acid, phenylacetaldehyde, methional (San-Juan et al., 2011), and mixture of higher alcohols had a masking effect on fruity aroma (Cameleyre, Lytra, Tempere, & Barbe, 2015). Ethyl decanoate and methyl salicylate at subthreshold concentrations were also likely to contribute to overall aroma (Niu et al., 2019) and various concentrations of ethyl isobutanoate and ethyl isovalerate gave additive or synergistic odor effects for mixtures (Niu, Zhu, & Xiao, 2020). Aldehydes such as benzaldehyde, furfural, and vanillin could induce synergistic effects in a *Huangjiu* aroma reconstitution (Yu et al., 2020). Acetic and butyric acids at subthreshold concentrations could enhance the rated intensity of moderately intense sensations (Miyazawa, Gallagher, Preti, & Wise, 2008a). Combinations of some $\gamma$-lactones might act additively or synergistically to contribute to the 'apricot' aroma of white wine (Siebert, Barker, et al., 2018). Binary mixtures of monophenols could induce different perpetual interactions ranging from partly additive to strong synergistic for almost all combinations of monophenols (Sterckx, Missiaen, Saison, & Delvaux, 2011). Ethylphenols had a masking effect on wine fruity notes even at subliminal concentrations (Tempere et al., 2016).

All these results highlighted the complexity of perceptual interactions between odorants, and also the variety of approaches that have been used: several studies used detectability functions approach (Lytra et al., 2013; Miyazawa, Gallagher, Preti, & Wise, 2008b; Niu et al., 2019; Yu et al., 2020), OAV approach (Niu, Zhu, et al., 2020) or $\sigma$-$\tau$ approach (Cameleyre et al., 2015; Niu et al., 2019). However, very few studies have set up a systematic work to assess the contribution of different odorants combinations to the global odor of mixtures, or the aroma profile of real products. In addition, most of the conclusions obtained in reconstitution and addition or omission experiments where conducted in more or less complex model solutions. However, the partition coefficient between the liquid phase and the gas phase is largely influenced by the matrix constituents, which adds additional variable with respect to the perceptual aspect under study. Therefore, a systematic approach based on the use of an olfactometer to produce the mixed odor stimuli can help to focus on the specific law and mechanisms at play in olfactory perceptual interactions. Other approaches based on the GC-O procedure have been developed. For instance online recombination techniques can be used such as Olfactoscan, which relies on the combination of a dynamic dilution olfactometer and GC-O device, allows to study the odor-odor interaction between target odorants and a

background odor (Béno, Loison, Villière, Le Fur, & Thomas-Danguin, 2015; Burseg & de Jong, 2009).

### 1.4.2 Methodologies Involved in Research Related to Odor Mixture Interactions

Odor-odor interactions can occur at the level of the peripheral olfactory system (Singh, Murphy, Balasubramanian, & Mainland, 2019) and/or at the level of the central olfactory system (Thomas-Danguin, Barba, Salles, & Guichard, 2016; Thomas-Danguin et al., 2014). To explore neurophysiological interactions, cellular explorations and neuroimaging techniques could be useful (Cerf-Ducastel & Murphy, 2004; Chaput et al., 2012; El Mountassir, Belloir, Briand, Thomas-Danguin, & Le Bon, 2016). Cellular approaches such as calcium imaging of ORs activation, electrophysiological recordings of OSNs, and analyses of olfactory mucosa electro-olfactogram (EOG) responses are advantageous to measure peripheral responses and changes induced by odor mixtures (Chaput et al., 2012; El Mountassir et al., 2016). Neuroimaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), electroencephalogram recordings (EEG) or other event-related potentials (ERP) recording methods are especially adapted to study brain activity. These methods have been used to explore odor-odor interaction (Ishii et al., 2008) and have also been used in wine research to discover how the brains of sommeliers represent flavor (Castriota-Scanderbeg et al., 2005; Pazart, Comte, Magnin, Millot, & Moulin, 2014).

Most of the time, olfactory perceptual interactions are studied using sensory analysis methods, possibly combined with other approaches. Sometimes the purpose of the study is only to confirm the impact of the mixture and not to describe the extent of changes. For instance, threshold tests or reconstruction and omission tests, aim to determine if a difference exists between two or more samples. The selection of the appropriate difference test is often determined by the nature of the potential difference (e.g., known or unknown), amount of sample available, number of samples, testing conditions, and specific test objectives (Drake, 2007). Standard discrimination methods such as 2-alternative forced-choice method (2-AFC, D. M. Green & Swets, 1966), 3-alternative forced-choice method (3-AFC, D. M. Green & Swets, 1966), duo–trio method (Dawson & Harris, 1951), triangular (triangle) method (Dawson & Harris, 1951) are the most widely used tests. In applications involving the detection of small differences with high confidence, the 2-AFC and 3-AFC methods were proven to be superior to the duo-trio and triangular methods (Ennis, 1990), but they should be used in situations where the nature of the difference tested is known.

Numerous sensory analysis methods have been developed to characterize profiles, differences, and pleasantness of foods and wines, and some of these methods can also be used to study the olfactory perceptual interaction from a psychophysical point of view. Descriptive analysis (DA) involves the discrimination and description of both the qualitative and quantitative sensory features of a product by a small number of panelists (from 8 to 15) who provide intensity ratings for a set of selected attributes at a certain time or some periods over time (Murray, Delahunty, & Baxter, 2001; Valentin, Chollet, Lelièvre, & Abdi, 2012). In wine research, DA has been used to the most salient sensory properties of a sample (Ferrer-Gallego,

Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; J. A. Green, Parr, Breitmeyer, Valentin, & Sherlock, 2011; Sokolowsky, Rosenberger, & Fischer, 2015; Tang, Xi, Ma, Zhang, & Xu, 2019), and how one attribute can affect other attributes (Arvisenet et al., 2019; De-La-Fuente-Blanco, Fernández-Zurbano, Valentin, Ferreira, & Sáenz-Navajas, 2017), or to link sensory properties with instrumental measurements (J. A. Green et al., 2011; Tang et al., 2019).

Several different conventional methods of descriptive analysis, such as the Flavour Profile method (Cairncross & Sjöström, 1997), the Spectrum™ method (Meilgaard, Civille, & Carr, 2007), Quantitative Flavour Profiling (Stampanoni, 1994), Free-choice Profiling (A. A. Williams & Langron, 1984), and especially Quantitative Descriptive Analysis ™ (Stone, Sidel, & Bloomquist, 2008) have obvious advantages to provide detailed and specific information of aroma characteristics and intensity, and can be used to build relationships between sensory data and instrumental measurements. These methods can be extremely useful in studying the olfactory perceptual interaction when significant changes happen in target attributes. Although these conventional methods can provide good quality data, the cost for accuracy and reliability is extensive training, money and effort. To address the needs in some cases, when a short project lack of training time or funding, rapid descriptive methods were alternative plans, and they provide products discrimination that is generally comparable to conventional methods (Eva Campo, Ballester, Langlois, Dacremont, & Valentin, 2010; dos Santos Navarro et al., 2013). These methods can be classified as 1). verbal-based methods, such as flash profile (Dairou & Sieffermann, 2002), Check-All-That-Apply (CATA) (Adams, Williams, Lancaster, & Foley, 2007) and Rate-All-That-Apply (RATA) (Ares et al., 2014); 2), similarity-based methods, such as free sorting task (Lawless, 1989), projective mapping (Risvik, McEwan, Colwill, Rogers, & Lyon, 1994), napping® (Pagès, 2005); and 3), reference-based methods, such as polarized sensory positioning (Teillet, Schlich, Urbano, Cordelle, & Guichard, 2010) and Pivot© profile (Thuillier, Valentin, Marchal, & Dacremont, 2015; Valentin et al., 2012).

The most advanced basic studies exploring odor mixture interactions used sensory methods that provide odor intensity and seldom considered odor quality or blending effect. In terms of the approach related to odor intensity of binary mixtures, the $\sigma$-$\tau$ approach (Patte & Laffort, 1979) has been the most commonly used. In this approach, the overall perceived intensity of a mixture is compared to the intensities of each individual component, and the perceptual interactions are defined based on the $\sigma$-$\tau$ plot (Figure 4b). To assess the perceptual interactions at subthreshold levels in a binary mixture, two approaches, namely the dose addition (Cometto-Muniz, Cain, & Abraham, 2003) and the response addition (Cometto-Muniz, Cain, & Abraham, 2005) based on the detectability functions were widely used. A specific explanation for the two approaches can be found in the review by Ferreira (V. Ferreira, 2012a). For instance, the response addition approach has been used in a study to evaluate sensory interactions among red wine fruity esters in a model solution (Lytra et al., 2013). The 3-AFC discrimination test was used to measure the detection threshold which was defined as the concentration at which the probability of detection was 50%. The detectability function used in this study was a psychometric function and fitted a sigmoid curve and the detection

probability was corrected using the chance level. Once the detection probabilities of the two components in a binary mixture were determined, the probability of detecting the mixture p(AB) is defined as follows: $p(AB) = p(A) + p(B) - p(A)p(B)$, where p(A) represents the probability of detecting component A and p(B) that of detecting component B. Then, mixture interaction patterns were compared based on the simple additive response model. If the panel's detection performance for the mixture was below the sum of probabilities, some degree of suppression had occurred relative to statistical independence. A performance above the sum of probabilities indicated that some form of mutual enhancement or synergy had occurred. Moreover, if detection performance matched the sum of probabilities, no mixture interaction had occurred (Lytra et al., 2013).

Another approach to assess the perceptual interactions related to threshold level is the OAV approach (V. Ferreira, 2012a). This approach has been applied to evaluate sensory interactions among esters in alcoholic beverages (Niu, Zhu, et al., 2020). In this approach, the sum of individual OAV of the components ($OAV_i$) was divided by the OAV of the mixture ($OAV_{mix}$). The resulting parameter, called X ($X=\Sigma OAV_i/OAV_{mix}$) was equal to 1 in case of odor additivity; if $X> 1$, it represented suppression; if $X< 1$, it represented higher degree of cooperation or synergy. GC-O analysis is kind of special case in the combination of instrumental sampling and sensory analysis.

# Chapter II Review of Literature on Aroma of Icewine

**Related to the publication**

Ma, Y., Xu, Y., & Tang, K. (2021). Aroma of icewine: a review on how environmental, viticultural, and oenological factors affect the aroma of icewine. *J. Agric. Food Chem.*, 69(25), 6943–6957.

## Abstract

The aroma of a wine is mostly driven by not only the factors in the vineyard, such as the grape variety and harvest time, but also the fermentation process. Icewine is a unique, intensely sweet wine made from late harvested grapes that have frozen naturally on the vine. Different from normal table wines, the grapes used in icewine naturally undergo a dehydration process and freeze-thaw cycles and the must for icewine making has to be pressed from frozen grapes. This pressing process leaves water behind as ice crystals and allows the grape juice to be concentrated with more sugars, acids, and other dissolved solids, resulting in a slower-than-normal fermentation. These special procedures can lead to a unique aroma characteristic of icewine. This review delves into recent advances in chemical compounds related to icewine aroma characteristics and addresses how changes in these aroma characteristics and composition are influenced by environmental, viticultural, and oenological factors in the vineyard and winery. Deficiencies in previous studies and future trends related to the flavor science of icewine were also briefly addressed.

**Key words:** sweet wines, frozen grapes, odor, viticulture, oenology

## 2.1 Introduction

Icewine (*Eiswein* in Germany) started to be produced more than 200 years ago in Germany. It is an intensely sweet wine made from grapes that must be frozen on the vine at or below -7°C [European Union (EU) regulations, International Organization of Vine and Wine, 2003] or -8°C (Canada, Vintners Quality Alliance 1999) during the harvest and pressing processes. True icewine is made only in regions where a cool climate can be guaranteed, so that grapes are able to be frozen on the vine and keep frozen during harvest and pressing procedures. There are limited areas that meet this requirement, such as Mosel, Rheinhessen, Pfalz and Rheingau in Germany, Burgenland in Austria, Ontario and British Columbia in Canada. These three countries are the most traditional producers. The precious nature of icewine is reflected not only by its limited production regions but also in its yield. Because the harvest time of icewine grapes is long past the normal harvest season, the remaining grapes experience dehydration and animal predation and are at risk of rotting. As a consequence, the yields of icewine grapes are only 15–20% that of table wine, even under ideal climate conditions (Pickering, 2006). The relatively low yield makes for a relatively high price for a bottle of icewine, with $ 40–80 for a 375 ml bottle of wine.

For wine products like icewine, flavor is not the only but is the most important aspect in defining the quality of the product. It is believed that flavor perception is the result of a complex pattern of chemical and physical interactions that trigger the response of the brain to gustatory, trigeminal and olfactory stimuli (Bisson, Waterhouse, Ebeler, Walker, & Lapsley, 2002), and normally the aroma plays a very important role in terms of unique flavor perceptions such as fruity, floral, and herbal attributes. Most of the sweet wines that undergo a dehydration process on-vine or off-vine or that are fortified with alcohol tend to present distinguished aromas that are clearly very different from those of normal table wines (Reboredo-Rodríguez, González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015). Compared to sweet wines made by adding alcohol such as Port, Sherry, and Madeira wines, naturally sweet wines, such as botrytized wines and icewines, are likely to receive higher rankings in some attributes such as honey and dried fruit (González-Álvarez, Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013). Lexicons that include honey, caramel, apricot, tropical fruit, raisin, nutty, and floral have been used to describe the aroma of icewines (Cliff, Yuksel, Girard, & King, 2002; Huang et al., 2018; Nurgel, Pickering, & Inglis, 2004).

The variety of aroma attributes primarily depends upon the composition of volatile compounds, especially the odor-active compounds in wine, and the multitude of interactions between all of the chemical components. These odor-active compounds in wines can be traced to the direct contribution of compounds from grapes, oak and fermentation microbes (Dunlevy, Kalua, Keyzers, & Boss, 2009), and they can be influenced or altered by various grape processing, winemaking, and/or storage processes (Parker, Capone, Francis, & Herderich, 2017; Schreier & Jennings, 1979). The compounds that arise from grape sources are primarily determined in the vineyard through the interplay between the vine genotypes, vineyard management practices and natural environment (Lund & Bohlmann, 2006), while the

compounds that arise from oak and fermentation microbes are affected by oenological strategies. In addition, some of the compounds that arise from grapes can also be modified by the fermentation process (released from glycosides such as terpenes and thiols). To generate the typical aroma of some sweet wines such as icewine, the factors in the vineyard, such as the grape variety and harvest time, are more influential than other factors (Mencarelli & Tonutti, 2013). Considerable progress has been made over the past decade in understanding the evolution of the aroma composition during the grape-growing and winemaking processes (Baiano, Scrocco, Sepielli, & Del Nobile, 2016; Gambetta, Bastian, Cozzolino, & Jeffery, 2014; Parker et al., 2017; Polášková, Herszage, & Ebeler, 2008; Robinson et al., 2014a, 2014b; Roland, Schneider, Razungles, & Cavelier, 2011; Schreier & Jennings, 1979; Sumby, Grbin, & Jiranek, 2010). This knowledge has been highly beneficial to winemakers in their quest to produce high-quality wines. However, the aroma composition in special wines and its influencing factors which closely related to the production requirements of the wines have been less frequently addressed.

Thus, this review begins with the special process of icewine production, followed by the characteristics of the aroma profile and the composition of icewine and then delves into the impact of different factors that affect these compounds during grape growing and winemaking. Deficiencies in previous studies related to the flavor science of icewine were also briefly addressed in the part of perspectives.

## 2.2 Uniqueness of Icewine and Its Aromas

Icewine is made with late-ripening grapes that have frozen naturally on the vine. The icewine making process and some factors that are important in the quality control of icewine are shown in Figure 5, and the basic parameters of quality control for icewine production in different countries are shown in Table 1.

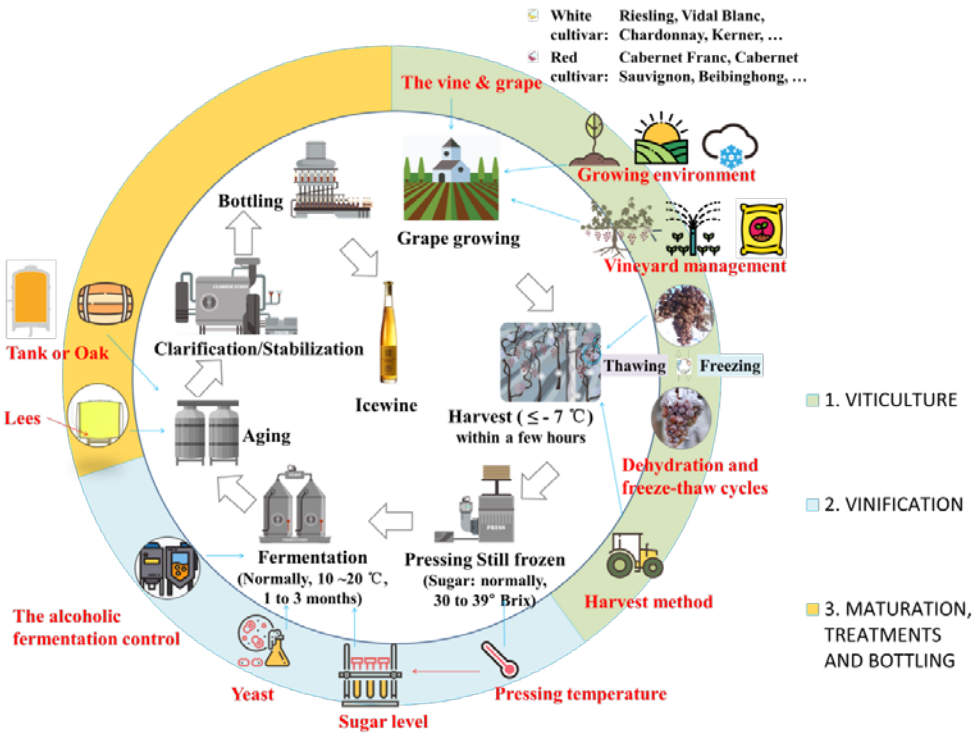


**Figure 5** Icewine making process and some factors those are important in icewine's quality control.

**Table 1** Basic Parameters of Quality Control for Icewine Production in Different Countries

| country | cultivar | harvest temperature (◦C) | soluble solid (◦Bx) | volatile acidity (g/L) | titratable acidity (g/L) | ethanol (% v/v) | residual sugar (g/L) | total SO2 (mg/L) | free SO2 (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| OIV (OIV, 2003) | – | ≤ –7 | ≥ 25.3 | ≤ 2.1 | – | ≥ 5.5 | – | – | – |
| Canada (Bowen, 2010; Soleas & Pickering, 2007) | Riesling, Sauvignon Blanc, Kerner, Vidal Blanc, Chardonnay, Gewürztraminer (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) Cabernet Sauvignon, Cabernet Franc (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) | ≤ –7* | ≥ 35.0* | ≤ 2.1* | ≥ 6.5* | 7–14.9* | ≥ 125* (Ontario) ≥ 100* (British Columbia) | 130-400 | 10-50 |
| Germany (Bowen, 2010; Wang et al., 2018) | Riesling, Chardonnay, Kerner, Silvaner (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) Pinot Noir, Merlot, Dornfelder, St. Laurent, Trollinger (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) | ≤ –7* | ≥ 29.6* | ≤ 2.1* | > 10 | > 5.5* | ≥ 100 | ≤ 400 | – |
| Austria (Bowen, 2010; Wang et al., 2018) | Grüner Veltliner, Kerner, Neuburger (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) Lemberger, Zweigelt (Bowen, 2010; Soleas & Pickering, 2007; | ≤ –7* | ≥ 29.6* | ≤ 2.1* | > 10 | > 5.5* | ≥ 100 or ≥ 250 | – | – |

| country | cultivar | harvest temperature (◦C) | soluble solid (◦Bx) | volatile acidity (g/L) | titratable acidity (g/L) | ethanol (% v/v) | residual sugar (g/L) | total SO2 (mg/L) | free SO2 (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| China (Wang et al., 2018) | Wang et al., 2018) GWelschriesling, Vidal Blanc (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) Cabernet Sauvignon, Beibinghong, Merlot (Bowen, 2010; Soleas & Pickering, 2007; Wang et al., 2018) | ≤ –8* | 33.0–38.0 | 0.8–1.5 | 8.0–10.0 | 11.0–12.0 | 140–180 | 32-588 | 0-58 |

Note: The references for the parameters of icewine making from each country were given in the ‘country’ column, and the value marked by ‘*’ was required by regional regulations for icewine production.

Like other grapes, the icewine grape growing involves two sigmoidal periods called formation phase and ripening phase. The formation phase is characterized by rapid cell division and growth, embryo development, and the accumulation of malate and other organic acids in the vacuoles, while the ripening phase is characterized by sugar accumulation, softening, and changes in color, followed by an increase in pH and accumulation of polyphenols and flavor compounds (Zamboni et al., 2010). The grapes normally ripen in October, but they are left on the vines under a cloak of protective netting until December or January when the first deep freeze of winter comes. Between the end of the ripening and the harvest, dehydration occurs in the grapes and the dehydration concentrates the sugars and acids in the juice and intensifies the flavor. The grapes for making icewine have to be harvested in their naturally frozen state and pressed while still frozen under a higher-than-normal pressure. Under the pressing, the water in the juice remains frozen as ice crystals, therefore only a smaller amount of juice with more concentrated sugar and flavor compounds is obtained. After pressing, the concentrated juice is separated from seeds, skins, and stems as it drains out from the press and then is fermented by selected yeast. Normally, the sugar concentration of regular grape juice for making table wines is 16–26°Bx (approx. 16–26% w/v), while icewine juice must reach a minimum of 25.3°Bx (EU regulations, International Organization of Vine and Wine, 2003) or 35°Bx (Canada, Vintners Quality Alliance 1999) and sometimes it can be as high as 50°Bx. Therefore, fermentations of highly concentrated icewine juice are often sluggish. Yeast fermenting the icewine juice at 40% (w/v) sugars begins to consume sugar immediately and continues for 17 to 21 days depending on the type of yeast to reach the desired ethanol level (approximately 11.0% v/v), and after which, no further sugar is consumed over the next 21 days for icewine fermentation (Erasmus, 2005; Pigeau, 2006). Most icewines usually have high levels of volatile acidity (Kontkanen, Inglis, Pickering, & Reynolds, 2004) and they do not undergo malolactic fermentation to maintain their acidity and freshness. After fermentation, some icewines are aged on lees in a barrel or steel tank, and some icewines are aged in oak barrels for a variable time, but normally oak aging of icewine is not a common practice.

Icewine owes much of its uniqueness to a high level of acidity which gives balance to the concentration of sugar in the grapes and it is also characteristically higher in special aromas. Through collections and calculations of the frequency of aroma descriptors used in icewine descriptive analysis, caramel, honey, apricot, dried fruit, floral, raisin, tropical fruit were shown to be the major aroma characteristics used to describe icewines (Table 2). These typical aroma characteristics can be influenced by the grape varieties, technologies used in the cellar (Nurgel et al., 2004) and production regions (Cliff et al., 2002; Huang et al., 2018; Nurgel et al., 2004). There were more than 200 volatile compounds identified in more than 130 icewines (Setkova, Risticevic, & Pawliszyn, 2007). However, not all of these compounds contribute to the aroma characteristics of icewine. It is widely accepted that the typical aroma is caused only by a small portion of the volatile compounds, which can be screened from among the huge body of volatiles extracted from wine samples and can be localized by gas chromatography-olfactometry (GC-O) though human ‘sniffing’ detection (Dunkel et al., 2014; Hofmann et al., 2018). By collecting the primary odor-active compounds detected by GC-O

analysis and comparing the odor-active compound pattern between icewine and other type of wines, it showed that the odorant pattern of icewines was not similar to the odorant patterns of other type of wines, although it was closer to the odorant patterns of botrytis wines and sparking or white wines (Figure 6).

A total of 80 odor-active compounds have been detected by GC-O in icewine, and *β*-phenethyl alcohol, ethyl hexanoate, *β*-damascenone, ethyl 3-methylbutanoate, *β*-phenylethyl acetate, 1-hexanol, *γ*-nonalactone, 1-octen-3-ol, ethyl benzeneacetate, and *cis*-rose oxide were detected as impact odorants in all three icewines from different grape varieties (Bowen & Reynolds, 2012; Lan et al., 2019; Ma et al., 2017). Interestingly, different researches showed that the key compounds in icewine largely overlapped with those in dry wines (Bowen & Reynolds, 2012; Lan et al., 2019), which suggested that the difference of sensory attributes between dry wines and icewines was ascribed to complex interactions (such as blending, synergy or masking effect) among aroma compounds, with huge variations in their concentration. Compared with dry red and white wines, icewines contained higher concentrations of most aroma compounds, especially for *β*-damascenone, 1-octen-3-ol, and some terpenes, lactones and furanones (Bowen & Reynolds, 2012; Qian et al., 2020). Partial least squares-discriminate analysis showed that *γ*-hexa-, *γ*-octa-, *γ*-nona-, *γ*-deca-, *δ*-hexa-, and *δ*-decalactone, as well as 5,6-dihydro-6-pentyl-2H-pyran-2-one, sotolon and homofuraneol contributed greatly to the discrimination between icewines and dry wines (Qian et al., 2020).

The contribution of aroma compounds ranked by the result of GC-O analysis is based on their threshold in air and not in the respective wine matrix, but this bias can be corrected by the use of odor activity values (OAVs) which was defined as the ratio of the concentration of an odorant in the food and its odor threshold in an appropriate matrix (Dunkel et al., 2014). In this approach, odorants with OAV above 1 are normally selected for further investigation. Through odor recombination, addition and omission studies, more refined contribution of odorants can be determined (V. Ferreira, 2010; Frank, Wollmann, Schieberle, & Hofmann, 2011; Grosch, 1993; Nicolotti, Mall, & Schieberle, 2019). Based on their contribution in wines, the detected odor-active compounds can be divided into 1) genuine impact compounds or key odorants, 2) major contributors, 3) net contributors, 4) secondary or subtle contributors, and 5) aroma enhancers and aroma depressors (V. Ferreira, 2010). All the odorants with OAV above 1 are considered as main contributors (key odorants, major contributors and net contributors). key odorants are normally present above their recognition threshold, and they provide the distinct aroma characteristic to the wine (V. Ferreira, 2010). In icewines, *β*-damascenone was shown to be a key odorant that not only transmits its honey-like aroma to the icewines but also influences the entire aroma typicality (Ma et al., 2017). The major contributor and net contributor affect the aroma profile or the intensity of a primary generic descriptor in a given wine; however, it might provide an aroma characteristic that is not the same as the specific descriptor (V. Ferreira, 2010). Esters could be a major contributor to icewines (Lan et al., 2019; Ma et al., 2017), since it affected the aroma profile or the intensity of a primary generic descriptor like ‘fresh fruity’ (Lan et al., 2019). Furaneol® presents a caramel aroma and methional presents a cooked potato aroma, and they were verified as net contributors to icewine for their significant effects on the apricot, caramel, and tropical fruit

characteristics (Ma et al., 2017). Some odorants such as 1-octen-3-one, *cis*-rose oxide, ethyl hexanoate, 2,3-butanedione, ethyl 2-methylbutanoate, ethyl isobutanoate, 1-octen-3-ol, *γ*-octa-, *γ*-nona-, and *γ*-decalactone, as well as furaneol and homofuraneol could also be key odorants, major contributors or net contributors since they had a relatively higher OAVs (Bowen & Reynolds, 2012; Lan et al., 2019; Ma et al., 2017; Qian et al., 2020), which calculated from the odor threshold data and quantification. However, the roles they play in icewine have not been verified separately by omission test.

**Table 2** Major Aroma Characteristics Used to Describe Icewines in Sensory Evaluation

| country | cultivars [a] | methods | descriptors [b] | ref. |
|---|---|---|---|---|
| Canada (British Columbia) | Riesling (11), Vidal (1), Kerner-Riesling (1) | 10-cm unstructured line scale; 25 trained subjects | dried/cooked fruit, raisin/dried fruit, honey/caramel, nutty/oily, apple/pear, tropical fruit/pineapple, peach/apricot | (Cliff et al., 2002) |
| Canada (Ontario) | Vidal (9) | 10-cm unstructured line scale; 25 trained subjects | raisin/dried fruit, dried/cooked fruit, honey/caramel, tropical fruit/pineapple, peach/apricot, apple/pear, nutty/oily | (Cliff et al., 2002) |
| German | Riesling (3) | 10-cm unstructured line scale; 25 trained subjects | nutty/oily, dried/cooked fruit, honey/caramel, raisin/dried fruit, tropical fruit/pineapple, peach/apricot, apple/pear | (Cliff et al., 2002) |
| Canada | Chardonnay (2), Erhenfelser (1), E-V-R (1), Kerner (1), Pinot Blanc (2), Riesling (5), Vidal(5), Oak-aged Vidal (3) | 15-cm scales, 9 well trained subjects | oxidized, caramel, honey, oak, citrus, floral, pineapple, raisin, walnut, apricot, apple | (Nurgel et al., 2004) |
| Canada | Riesling (1) | 10-cm line scale with anchors at 0 and 10 cm, 12 well trained subjects | fresh fruit, earthy/mushroom, dried fruit, volatile acidity, oxidation, fusel oils | (Clary, Gamache, Cliff, Fellman, & Edwards, 2006) |
| China | Vidal (1) | 7-point scale, 12 well trained subjects | apricot, honey, tropical fruit, caramel, rose, raisin | (Ma, Tang, Xu, & Li, 2017) |
| Ukraine | Marselan (1) | grade scale with anchors at 0 to 7, 10 trained subjects | fruit, walnut, honey, floral, citrus, spicy, caramel | (V. Ostapenko, O. Tkachenko, & E. Iukuridze, 2017) |
| German | Riesling (1) | choosing up to five descriptors from the list, 29 trained subjects | citrus fruits, muscat, lemon (more related descriptors) | (Avizcuri-Inac, González-Hernández, Rosáenz-Oroz, Martínez-Ruiz, & Vaquero-Fernández, 2018) |
| Spain (La Rioja) | Tempranillo-Grenache (1), Tempranillo (1) | choosing up to five descriptors from the list, | woody, prune, dry fig, dry fruits (more related descriptors) | (Avizcuri-Inac et al., 2018) |

| country | cultivars [a] | methods | descriptors [b] | ref. |
|---|---|---|---|---|
| China | Vidal (4) | 29 trained subjects 10-cm line scale, 8 trained subjects | nut, honey, caramel, apricot, tropical fruit, rose | (Huang et al., 2018) |
| Canada | Vidal (4) | 10-cm line scale, 8 trained subjects | apricot, honey, nut, tropical fruit, caramel, rose | (Huang et al., 2018) |
| China | Beibinghong (2) | 11-point scale, 12 trained subjects | dried fruit, honey/sweet, floral, caramel, smoky, apricot/peach, fresh fruity, herbaceous | (Lan et al., 2019) |

Note: [a] The cultivars and its sample numbers that were evaluated in sensory evaluation. [b] Descriptor was order by its intensity obtained in sensory evaluation from high to low.

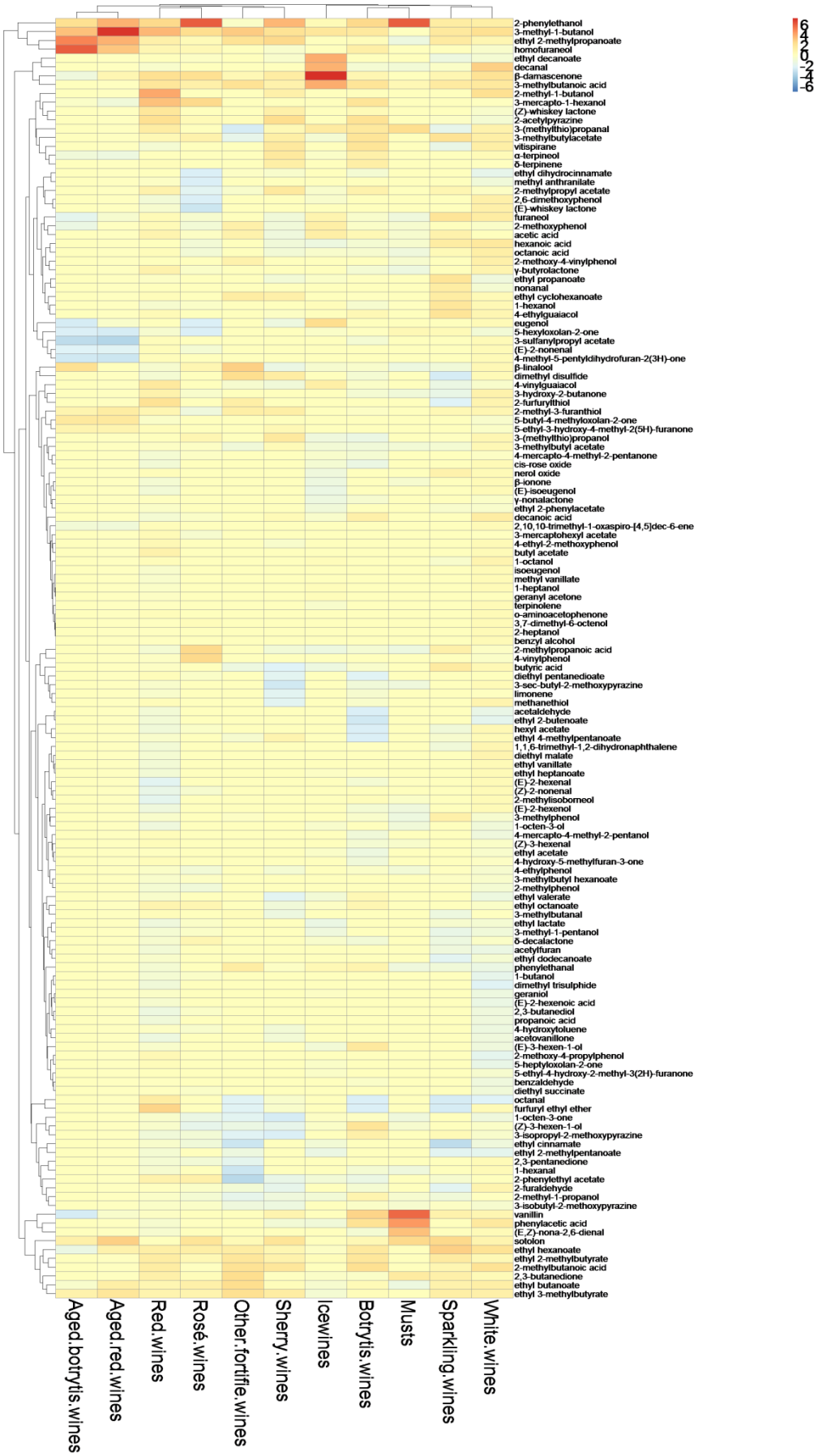


**Figure 6** Hierarchical cluster analyses and heat map visualization of different wine types (row) and major odorants detected by GC-O analysis (column). A total of 39 peer reviewed publications in the year from 1999 to 2019 were considered into our database after assessing the quality of the data (The references were

provided as Supplementary Table 1). The database included 131 samples and 309 odorants which were detected by GC-O analysis in these samples. These 131 samples were classified into 13 categories only considering wine categories to see the differences of odorants patterns of icewines from other wine types. Because different GC-O analysis method was used in these studies and the scale for each sample was different, the GC-O values of all the samples were first convert to standardized scores (Z-scores) for each odorants and then the mean value of each odorant in every category were calculated. The final data of the mean value for each odorant in every category was treated by means of a cluster analysis, and visualized as the heatmap. Statistical analysis was performed with *R* software (version 3.5.3). Distance measure used in clustering rows (wine samples) is Pearson correlation using the *pheatmap* functions from the *pheatmap* package (Kolde & Kolde, 2015). To highlight more important compounds, only the odorants which occurred in more than 8 wine samples were included in the heatmap.

## 2.3 Origin of Icewine Aromas

It is widely accepted that the odorants in wines can be divided into three groups, namely, the odorants originating from grapes (primary aromas); the odorants generated or modified during yeast fermentation and any other secondary microbial fermentations(secondary aromas); and the odorants produced or changed during aging/storage (tertiary aromas) (Schreier & Jennings, 1979). The classes of some odor-active compounds in icewines are shown in Figure 7.

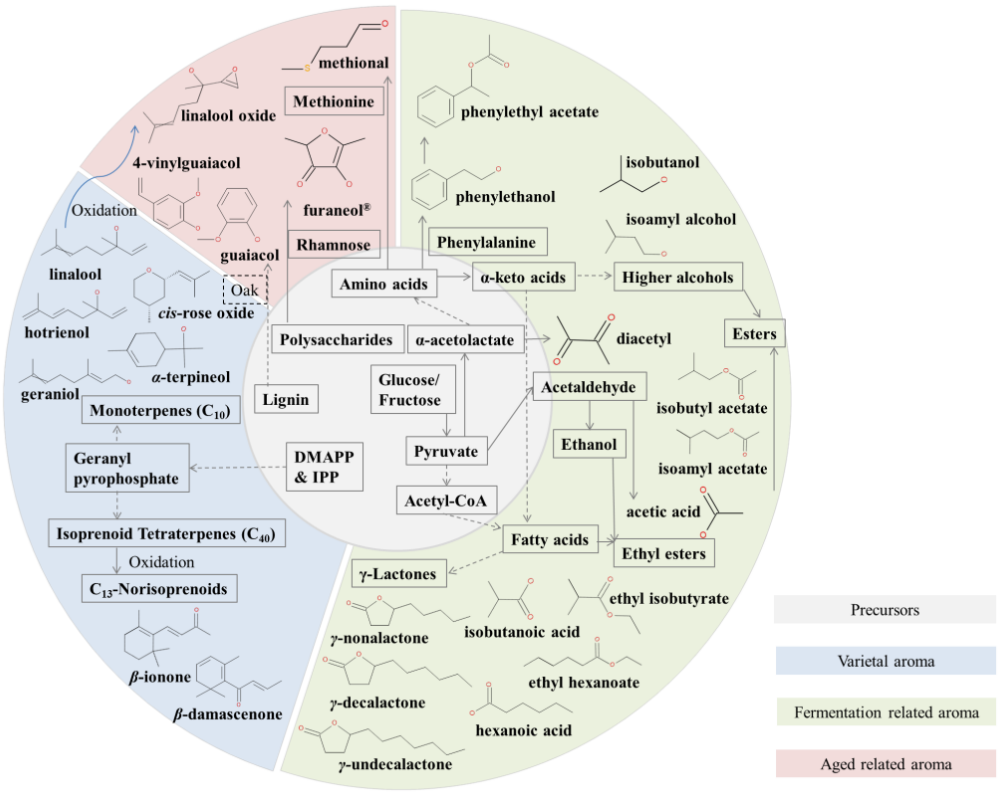


**Figure 7** Formation of selected odor-active compounds in icewines

The odorants that originated from the grapes are more related to the metabolites from grapes, and they are mostly released from the hydrolysis of glycosides or conjugate precursors or the breakdown of lipids during the grape crushing or winemaking processes (Roubelakis-Angelakis, 2009). These compounds include monoterpenes, norisoprenoids, methoxypyrazines, some phenylpropanoids, aliphatics, $C_6$ aldehydes and volatile sulfur compounds. The most important grape-derived compounds detected as potential odorants in

icewines are monoterpenoids, which are the $C_{10}$ representatives of the terpenoid family, and $C_{13}$-norisoprenoids. Among them, the most important monoterpenes in icewine are *cis*-rose oxide, geraniol, and linalool, and the most important $C_{13}$-norisoprenoid is *β*-damascenone (Bowen & Reynolds, 2012; Lan et al., 2019; Ma et al., 2017). Monoterpenoids diverge from higher isoprenoid biosynthesis at the level of geranyl pyrophosphate (Croteau, 1987), while $C_{13}$-norisoprenoids are formed as biodegradation products of carotenoids (Black, Parker, Siebert, Capone, & Francis, 2015).

Although some of the odor-active compounds in wines are primary aromas which are variety dependent and highly influenced by the grapes' growing conditions, most of the odor-active compounds, especially esters, are produced during fermentation at relatively well-defined proportions based on the wine type (V. Ferreira, 2010). This conclusion also applies to icewines. More than half of the odor-active compounds in icewines are derived from the yeast metabolism of sugar, fatty acids, amino acids or other precursors. These precursors do not contribute directly to the wine aroma but may change the yeast metabolism and associated yeast-derived or yeast-altered aroma compounds (Dunlevy et al., 2009; Robinson et al., 2014a). The yeast-derived compounds are highly affected by grape constituents, esterase activity, and fermentation parameters such as the temperature, $SO_2$ and oxygen levels or even juice clarification (Roubelakis-Angelakis, 2009). Among these odorants, aliphatic organic acids such as 2-methylpropanoic acid, hexanoic acid, octanoic acid, and decanoic acid are produced as byproducts of fatty acid metabolism. Higher alcohols such as isobutanol, 3-methyl-1-butanol and *β*-phenylethanol are produced as a byproduct of amino acid metabolism. However, several $C_6$ alcohols are probably formed enzymatically by grapes after mechanical damage (Waterhouse, Sacks, & Jeffery, 2016). Most esters are the metabolic byproducts of yeast action, accumulating to the maximum extent in wines after cell division has slowed or essentially ceased (Jackson, 2014; Lee, Rathbone, Asimont, Adden, & Ebeler, 2004). Fatty acid availability is the primary factor influencing the synthesis of ethyl esters, whereas the production of acetates depends on the activity of alcohol acetyltransferases (Rollero et al., 2015). The major esters reported in wine, the enzymes responsible for their hydrolysis and synthesis and their formation during primary and malolactic fermentation have been summarized well before (Sumby et al., 2010). In addition to the esters in icewines that are generated from enzymatic esterification during the fermentation process, these compounds can also be generated from chemical esterification during long-term aging (Bowen, 2010). Some *γ*-lactones that provide the peach or coconut-like aromas in icewines are produced by the cyclization of the corresponding hydroxycarboxylic acids (Perestrelo, Barros, Câmara, & Rocha, 2011). It seems that dessert wines have higher concentrations of longer-chain lactones than red wines and white wines (Cooke, Capone, van Leeuwen, Elsey, & Sefton, 2009; Huang et al., 2018), but explanations for these effects are unclear. However, these lactones are present as enantiomers with a racemic distribution in wine, and these enantiomers are slightly different in terms of their sensory properties (Cooke et al., 2009).

Aging-derived aroma compounds largely depend on the aging/storage conditions. If wines are stored or fermented in oak barrels, the aroma compounds can be extracted during the fermentation and storage of wine and vary depending on the origin, seasoning, and heating of

the wood (Robinson et al., 2014a). Some compounds detected in icewine such as guaiacol, eugenol and syringol might arise from the thermal degradation of lignin, and then extracted during oak storage (Waterhouse et al., 2016). However, oak aging of icewine is not a necessary practice and some icewines which have not spent any time in oak are also possible to show an oak related aroma. Apart from the extraction-related compounds created during storage, some important odor-active compounds detected in icewine have been considered to be related to the aging or oxidation processes, such as methional and phenylacetaldehyde (Escudero, Hernández-Orte, Cacho, & Ferreira, 2000; Soares da Costa et al., 2004). However, the origins of these aldehydes are not always clear and they do not always arise from the oxidation of the related alcohol (Waterhouse et al., 2016). One possibility is that they are generated when berries are harvested following freeze and thaw cycles, which is analogous to an oxidative process (Boulton, Singleton, Bisson, & Kunkee, 1996).

## 2.4 Factors Affecting the Primary Aromas of Icewine

### 2.4.1 Grape-growing Environment

The development of wine aroma compounds or aroma precursors in grapes is profoundly affected by the grape-growing environment, such as the topography of a prospective site, the local soil and the microclimate, which vary for a vineyard depending on its size and natural slope. Most high-quality icewines are produced in a region with a lake. The lake effect protection can prevent early frost and vine-destroying deep freezing and provide for the development of more complex and intense grape flavors during ripening than the region without the lake effect protection (Ostapenko, 2016). Most soil types are suitable, but the sites that are susceptible to water stress will not be as productive due to restrictions in yield (Bowen, 2010). The climate and microclimate exert a profound influence on the vine phenology and grape composition and then affect the vinification conditions and wine microbiology, ultimately affecting the chemistry and sensory aspects of the wines (De Orduna, 2010). Compared with other microclimate factors, the growing season length and temperatures are the factors that exert the greatest influence on the wine quality because they affect the ability of the grapes to ripen with optimal levels of sugar, acids, and odorants. For icewine grapes, the vineyard must be located at a site that can reach temperatures below –8°C after the traditional harvest period to sufficiently freeze the berry (Bowen, 2010), but the temperatures cannot be too low, or the yield will be decreased and there will be increased pressing difficulties with fermentation. The optimal temperature is between –10°C and –12°C at harvest to provide optimum sugar levels and good aroma or flavor in the grapes (Turvey, Weersink, & Celia Chiang, 2006).

### 2.4.2 Grape Varieties

Compared to table wine varieties, a cultivar destined for icewine should resist disease and be cold or winter hardy and be late-ripening, and its grape berries should have a thick skin to maintain the integrity of the berry under harsh weather and limit invasion by saprophytic or parasitic microbes (Jackson, 2014). Furthermore, because of the extreme sweetness and high alcohol content of icewine, it is necessary to balance the taste and aroma to improve the flavor,

so the grapes should come from an aromatic variety with high natural acidity.

Riesling is an ideal and the most expanding cultivar for icewine production and has been selected by 14 producing countries (Ostapenko, 2016). This variety is a classic dessert wine varietal that is fairly disease resistant and grows well in many cool climate regions which are suitable for icewine production. It does not rot while hanging on the vine past normal harvest and products high sugars but retain its acidity at full maturity (Bowen, 2010). Another common grape used is Vidal Blanc, which is a white French-American hybrid used to produce icewine of excellent quality, owing to its cold hardiness (it is more cold-hardy than *Vitis vinifera* varieties), bunch rot resistance and especially its versatile and desirable wine style (Dami, 2014). Vidal Blanc is high-acidity and yields a wine of Riesling-like character under optimal conditions (Jackson, 2014). Icewines made from these two grape varieties possess apricot, pineapple, honey, caramel, raisin, oak, *etc.* aromas (Cliff et al., 2002; Nurgel et al., 2004), and they share many odor-active compounds such as *β*-damascenone, cis-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate, ethyl 3-methylbutanoate (Bowen & Reynolds, 2012).

The majority of icewine from North America and China is produced using Vidal Blanc, while European countries prefer Riesling (Ostapenko, 2016). In other countries, white varieties such as Welschriesling, Sauvignon Blanc, Grüner Veltliner, Pinot Gris, Pinot Blanc, Chardonnay, Gewürztraminer, Kerner, and Traminer are also used for icewine production (Pickering, 2006; Wang et al., 2018). Initially, icewine was produced only from white grapes because of their predominance in planting areas in cool regions, since red grape varieties need more sunlight and heat to ensure the physiological ripeness of the tannins in their skins (Ostapenko, 2016). Recently, research into using red cultivars for icewine has attracted increasing interest, and winemakers are testing red varieties to expand their range. Cabernet franc is the third-most frequently used cultivar after Vidal Blanc and Riesling for icewine production (Bowen, 2010). Other red varieties such as Cabernet Sauvignon, Lemberger, Pinot Noir, Zweigelt, Merlot, and Beibinghong are used for icewine production in different countries (Wang et al., 2018). However, it is not possible to produce icewine with a strong red color because the icewine has to be pressed immediately after harvest in order to press frozen grapes and a skin contact for color extractions is required for at least few days. Besides, extracting color from the juice without extracting the astringent compounds is a challenge posed by red icewine production (Bowen, 2010).

The aroma difference between different countries and different grape varieties has been studied. However, the aroma profiles were largely obtained from white varieties such as Riesling and Vidal Blanc, and aroma of icewines from other white varieties and red varieties has not been widely studied to date. It showed that icewine made from different grape varieties can be different in some aroma characteristics such as raisin, honey or caramel, and some odor active compounds could be only detected in individual varieties, such as ethyl valerate, 1-heptanol for Vidal Blanc, and ethyl cinnamate, *β*-ionone for Riesling (Bowen & Reynolds, 2012; Nurgel et al., 2004). For the same grape varieties, icewines made by different countries could be distinguished by their aroma characteristics. Vidal icewines from China were characterized by nutty and honey aromas, while Vidal icewines from Canada expressed

caramel, fruity and rose or floral aromas (Huang et al., 2018). Canadian Riesling icewines had higher fruity and floral aromas than German icewines, which had more nutty or oily characters (Cliff et al., 2002). Considering the grape variety as a random effect, the regional effect was still significant between Canadian icewines. Ontario icewines processed higher apricot, raisin, honey and oak aromas, while British Columbia icewines had higher intensities of pineapple and oxidized aromas (Nurgel et al., 2004).

### 2.4.3 Dehydration and Freeze-thaw Cycles in Icewine

In the time between the end of the growing season and the harvest, some special phenomena, such as grape dehydration accompanied by the occurrence of freeze-thaw cycles, occur in icewine grapes. Grape dehydration includes off-vine and on-vine dehydration and icewine allows only on-vine dehydration and freezing except when the grape/cluster falls on the vine naturally and are captured in the net. Normally, wines undergoing dehydration presented the aroma properties of dry fruits, caramelized, floral, phenolic, burned and raisins (Allamy, Darriet, & Pons, 2018; Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013). For grapes produced from on-vine dehydration, it showed that they presented an advantage in terms of the development of a honey or syrup aroma, with a high correlation with $\beta$-ionone and geranial (K. Chen, Wen, Ma, Wen, & Li, 2019). The dehydration process leads to water loss, aroma composition changes, and increases in the soluble solids, total acidity, total polyphenols, etc. (Panceri, Gomes, De Gois, Borges, & Bordignon-Luiz, 2013), resulting in a higher level of water stress in grapes, and it can further involve the activation of specific osmotic and oxidative stress response genes (Zamboni et al., 2010). The modification of the gene expression not only is involved in hormone and sugar metabolism as well as defense mechanisms (Rizzini, Bonghi, & Tonutti, 2009) but also regulates some genes associated with the accumulation of volatile compounds in various cultivars (Bonghi et al., 2012; Chkaiban et al., 2007; Cirilli et al., 2012; Costantini, Bellincontro, De Santis, Botondi, & Mencarelli, 2006; Rizzini et al., 2009).

In addition to grape dehydration, freeze-thaw cycles also occur in icewine grapes. It is believed that the freeze-thaw cycles affect both primary and secondary metabolism in grape berries and generate the distinct flavor profile of icewine, which is quite different from that of other sweet wines and from wines made from fresh grapes (Bowen, 2010). This special procedure cannot be duplicated by freezing alone or by other nondestructive dehydration. In regions where the climatic conditions do not permit this process, microwave vacuum dehydration, cryoextraction and reverse osmosis have been used to mimic the production of concentrated juices for making icewine-like wines. However, they seem to lack the flavor changes that develop during long over-ripening in the vineyard (Jackson, 2014; V Ostapenko, O Tkachenko, & E Iukuridze, 2017). It showed that the astringency and bitterness tastants such as vanillic acid, caffeic acid, (-)-epigallocatechin gallate, gentisic acid and syringic acid can be used to distinguish artificial icewine from real icewine (Tian et al., 2009), but there is no research reported on distinguishing artificial icewine from real icewine by aroma compounds.

Berries harvested after freeze and thaw cycles are subject to processes analogous to wine

aging, which is an oxidative process (Boulton et al., 1996). The aging process can increase the monoterpene oxides, dimethyl sulfide, 3-(methylthio)-1-propanol, diethyl sulfide, and diethyl disulfide but decrease the monoterpene alcohols, 2-mercaptoethanol and ethyl mercaptan (Bowen, 2011; Fedrizzi, Magno, Badocco, Nicolini, & Versini, 2007). A striking alteration in the volatile profile occurred at sub-zero temperatures, particularly for the free fractions such as the $C_6$ alcohols, higher alcohols and oxidative terpene derivatives, which implied that the freeze and thaw cycles had significant effects on some aroma compounds in grapes (Lan et al., 2016), but the trend details are still a mystery. During the freezing phase, the formation of ice crystals causes the perforation of the berry's cellular components, permitting reactions among the components from different parts of the grape and changes in the pH and oxidation-reduction potential. In addition, osmotic pressure caused by freezing may affect the reactions to follow when the temperature warms up. During the thawing process, the ice crystals will melt and the blending components may trigger a series of complex reactions, such as biotransformation, oxidation, the Maillard reaction (Boulton et al., 1996; Lan et al., 2016). Over the full freezing and thawing cycles, sugars, acids, and flavor compounds are concentrated by the separation of part of the frozen water in the form of pure ice crystals, which directly modifies the composition of the must and the final flavor (Tsakiris, Koutinas, Psarianos, Kourkoutas, & Bekatorou, 2010). The freezing and thawing process accompanied by juice concentration created caftaric acid oxidized by polyphenol oxidases (Bowen, 2011), and the release of catechins imbues icewine with a golden color (Jackson, 2014; Kilmartin, Reynolds, Pagay, Nurgel, & Johnson, 2007). This analogy may help to explain why the color and flavor characteristics, such as caramel and raisin, which appear more often in aged wines, are also detected in icewine.

### 2.4.4 Harvest

The most often explored and influential viticulture practice factor that influences icewine flavor is the harvest time, which largely determines the primary characteristics of icewine via complex but still unknown biochemical and chemical reactions. Late harvest and normal Gewürztraminer wines had higher fruity and sweet aroma compound series, whereas Gewürztraminer icewines had higher terpenic, floral, chemical, pungent and ripe fruit aroma compound series (Lukić, Radeka, Grozaj, Staver, & Peršurić, 2016). Riesling wines harvested in mid-December had the highest fresh fruit descriptor intensities, whereas wines harvested in mid-January were highest in dried fruit and nutty descriptors (Bowen, Reynolds, & Lesschaeve, 2016). For Vidal, the concentrations of most of the phenolic acids and flavan-3-ol in musts increased with the harvest time delay (Tian et al., 2009) and had higher aroma/flavor intensities (Bowen et al., 2016). The most odor-active compounds associated with the harvest date in the Vidal and Riesling wines are *β*-damascenone, *cis*-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate, and 4-vinylguaiacol (Bowen & Reynolds, 2015a). In addition to the direct changes in odor-active compounds in late harvest wines, some aroma precursors are known to form under postharvest conditions through grape metabolism (Allen et al., 2011) and are related to the harvesting time (Kobayashi et al., 2012).

Delaying the harvest date also increases the soluble sugars and pH while decreasing the

titratable acidity (Dami, 2014). The changes in the sugars in the fruit may cause osmotic changes that induce other pathways responsible for producing flavor compounds, but whether pathways leading to aroma accumulation coordinately or independently result from some relative stimulus that induces sugar production is still unknown. For icewine, the harvest temperature determines the °Bx value of the icewine must, it showed that temperatures ranging from –9°C to –11°C give a soluble solid concentration in the must between 38 and 40°Bx (Bowen, 2010; Ziraldo & Kaiser, 2007). Icewine grapes are usually harvested from mid-December to late January to achieve a balance between the flavor profile and yield (Bowen & Reynolds, 2015a).

Harvesting methods may also have an effect on the icewine flavor. Hand picking ensures the grapes are not damaged or rotten, which is very important and significant for the production of a great icewine with a fruity aroma and good acidity. Based on the research results on other types of wine, machine harvesting produces cleaner juices but can increase the sulfur aroma compounds such as 3-mercaptohexanol and 3-mercaptohexyl acetate (Allen et al., 2011). Since the temperature for icewine harvest is between –10°C and –12°C, hand picking in this weather is arduous and can last all night long. Most medium-sized wineries hand-pick the grapes and larger wineries use machine harvesting.

### 2.4.5 Other Vineyard Management

In addition to the date and time of harvest, other viticulture practices such as training and pruning, vine propagation and grafting, irrigation and fertilization may influence the aroma of icewine and their precursors by affecting the primary and secondary metabolic processes of grapes. However, only limited work has focused on the relationship between the parameters from these viticulture practices and the composition of icewine. The relationship between the crop level and characteristics of icewine and its grapes has been investigated (Bowen & Reynolds, 2015a; Bowen et al., 2016; Bowen, Reynolds, & Pickering, 2006; Ennahli, Dami, & Scurlock, 2009). A high crop level decreased the total soluble solids and pH of Vidal Blanc grapes during three of five years (Dami, Ennahli, & Scurlock, 2013), while thinning treatments lead to a higher fruity, honey, sherry and nut aroma/flavor intensities in Vidal Blanc and Riesling icewines (Bowen et al., 2016). Bowen et al. studied the effect of three crop treatments, namely fully-cropped, cluster thin at fruit set to one (basal) cluster per shoot and cluster thin at veraison, on icewine chemical and aroma compound profile, and it showed that most of the aroma compounds were highest in thin clusters at veraison and lowest in the cluster per shoot (Bowen & Reynolds, 2015b).

### 2.4.6 Healthy Grape-growing Condition

The potential for botrytis infection in icewine grapes is very high and would result in changes in the chemical and sensory profiles of the wine (Bowen, 2011). Botrytis infection increases the biosynthesis of terpenes and fatty acid aroma precursors and alters the berry metabolism by inducing biotic and abiotic stress responses (Blanco-Ulate et al., 2015). Although botrytis infection can significantly modify the important aroma components of some wines (Fedrizzi et al., 2011), it is a defect for grapes intended for icewine, because the botrytis characters might detract from the purity of the fruit aromas found in icewines that are

derived from healthy grapes (Kaiser, 2006). Compounds including 3-sulfanylpentan-1-ol, 3-sulfanylheptan-1-ol, 2-methyl-3-sulfanylbutan-1-ol (Sarrazin, Shinkaruk, et al., 2007), 1-octen-3-ol, phenylacetaldehyde, N-(3-methylbutyl) acetamide, γ-nonalactone, 4-carbethoxy-γ-butyrolactone and furaneol® were positively linked to botrytis-infected grapes (Tosi et al., 2012).

## 2.5 Factors Affecting the Secondary Aromas of Icewine

### 2.5.1 Pressing

In vinification practice, the first step is pressing. The major types of pressing are the vertical, horizontal, and pneumatic forms. Vertical pressing is used more in icewine production due to its advantage in pressing many small fruits and in pressing frozen grapes (Jackson, 2014). The pressing practice primarily influences the concentration of the pressed icewine juice, which primarily relies on the temperature at pressing. Pressing at a warmer temperature will speed up the process but lead to a more dilute juice, while pressing at a colder temperature results in decreasing yields, difficulty associated with fermenting a high soluble solids must, and poses a risk of serious equipment damage. Pressing temperatures of –8°C and –14°C would result in icewine musts with 35°Bx and 55°Bx, respectively (Bowen, 2010), and considering the pros and cons mentioned above, the temperature for icewine pressing narrows from –10°C to –7°C (–8°C in Canada) (Butzke, 2010). The pressing temperature also determines the choice of pressing pressure. Generally, the lower the temperature, the higher the pressing pressure is required. It showed that wines from the lighter press fraction had higher tropical fruity aromas while wines from the higher press fraction had higher rose aromas (Ma et al., 2017); however, little has been known about the dynamics of aroma extraction and the changes during pressing until the present.

### 2.5.2 Yeast and Fermentations

For making icewine, the yeast used in icewine fermentation gives a different initial response because the yeasts are under high osmolarity and anaerobic conditions. Hyperosmotic stress on yeast will upregulate the genes involved in the synthesis of acetic acid and succinic acid and genes related to the glycolytic and pentose phosphate pathways while downregulating the genes involved in the *de novo* biosynthesis of purines, pyrimidines, histidine and lysine (Erasmus, 2005). Icewine fermentation generates and accumulates more glycerol than table wine and produces much more acetic acid (Wang et al., 2018), which further leads to a relatively higher ethyl acetate content in icewine. A high acetic acid content might result from the $NAD^+$-dependent aldehyde dehydrogenase encoded by *ALD3* (Heit, Martin, Yang, & Inglis, 2018; Pigeau, 2006; Yang, Heit, & Inglis, 2017), and the high glycerol needed to balance the external osmotic pressure is related to glycerol-3-phosphate dehydrogenases *GPD1* (Pigeau, 2006). The acetic acid and ethyl acetate that are present above their sensory detection thresholds will impart vinegar and nail polish odors, respectively, to the wine, and they can be considered undesirable faults at high concentrations (Martin, 2008).

The yeasts involved in icewine fermentation have a smaller cell size, less biomass and a

lower peak concentration than those involved in table wine fermentation (Bowen, 2010). To optimize icewine fermentation, factors related to the yeast must be assessed carefully, including but not limited to the yeast strain selection, the amount of added yeast and the addition of yeast micronutrients.

The yeast destined for icewine fermentation should have the ability to ferment under high-sugar conditions, be alcohol-tolerant and produce relatively lower volatile acidity. The optimal fermentation temperature, hydrogen supply, and assimilable nitrogen and oxygen required by yeast should also be considered (Bowen, 2010). Considering the factors above, V1116 (*Saccharomyces cerevisiae*), ST (*Saccharomyces cerevisiae*), N96 (*Saccharomyces cerevisiae*), EC1118 (*Saccharomyces bayanus*), VL1 (*S. cerevisiae*), and AWRI 1572 (a hybrid between *S. cerevisiae* and *S. bayanus*) were optimal yeast strains for producing icewine (Crandles, Reynolds, Khairallah, & Bowen, 2015; Erasmus, Cliff, & van Vuuren, 2004; Yang, 2011). A study conducted on botrytized musts showed that a mixed culture of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* was the best combination for improving the analytical profile of sweet wine, particularly volatile acidity and acetaldehyde production (Bely, Stoeckle, Masneuf-Pomarède, & Dubourdieu, 2008). The yeast species not only differ in their fermentation properties in high-sugar grape juice but also produce distinguished odorant profiles. Cabernet Franc icewine musts fermented by V1116 had the highest decanal, while those fermented by EC1118 had the highest concentrations of furfural, hexanoic acid, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), *β*-damascenone and some alcohols and esters, and those fermented by VL1 had the highest linalool, *cis*-rose oxide, ethyl octanoate and ethyl decanoate (Synos, Reynolds, & Bowen, 2015). The hybrids generated by *Saccharomyces cerevisiae* and *Saccharomyces bayanus* produced much lower concentrations of acetic acid relative to the industrial wine yeast parent, and they provided wines with novel aroma and flavor profiles (Bellon, Yang, Day, Inglis, & Chambers, 2015).

Indigenous yeasts found on the bloom of grape skins and in winery environments might have a significant effect on the wine aroma (Synos et al., 2015). The strains identified in icewine included *Saccharomyces* and some non-*Saccharomyces* yeasts, which primarily belonged to the genera *Metschnikowia*, *Hanseniaspora*, *Torulaspora*, *Candida*, and *Debaryomyces* (Hong, Li, & Chen, 2019). Strains with better tolerance for sugar, ethanol, and acid and have satisfactory multi-enzyme activities, such as *Candida railenensis* HC08 (Hong et al., 2019), can be used to design mixed fermentations to produce a characteristic icewine. Icewine obtained by spontaneous fermentation was unique in its production of geranyl acetone and ethyl benzoate and contained higher 1-hexanol, phenylethanol, nerol, *β*-phenyl acetate, and *γ*-nonalactone in Cabernet Franc, Riesling and Vidal icewines (Crandles et al., 2015; Synos et al., 2015). The co-fermentation of *S. cerevisiae* with different indigenous yeasts could generate a distinct aroma quality. Compared with *S. cerevisiae* (SC45) monoculture, a mixed fermentation of *M. pulcherrima CVE-MP20* with *S. cerevisiae* (SC45) produced higher contents of acetate esters, *β*-damascenone and lower $C_6$ alcohols, while a mixed fermentation of *H. vineae CVE-HV11* with *S. cerevisiae* (SC45) generated the highest amounts of $C_6$ alcohols, higher alcohols, acetate esters, *cis*-rose oxide, *β*-damascenone, and phenylacetaldehyde (Zhang, Shen, Duan, & Yan, 2018). *Saccharomyces cerevisiae*,

*Metschnikowia spp.*, *Hanseniaspora uvarum*, *Lactococcus lactis*, *Leuconostoc spp.*, *Aureobasidium pullulans* and *Rhodotorula glutinis* were notable indigenous yeasts that colonized grapes under icewine-harvesting conditions (Alessandria et al., 2013; Bučková et al., 2018; J. Li, Hu, Huang, & Xu, 2018), and *Metschnikowia* and *Saccharomyces* were predominant at the end of spontaneous fermentations (J. Li et al., 2018). These strains exhibited interesting esterase and *β*-glucosidase properties, which are important for aroma formation in wine and could be used in the future to modulate icewine characteristics (Bučková et al., 2018).

When yeast strains are chosen, using a sufficient inoculum level combined with stepwise acclimatized inoculations results in a sufficient viable cell concentration and yeast biomass to ferment more sugar (Kontkanen et al., 2004). To reach a target ethanol concentration of 10% v/v (79 g/L) in icewine, a yeast inoculum level higher than 0.2 g/L is needed (Kontkanen et al., 2004). In a mixed fermentation of *S. cerevisiae* with different indigenous yeasts, sequential inoculation could achieve higher aroma diversity and produce a higher intensity of fruity, flowery, and sweet attributes in icewine than simultaneous inoculation (Zhang et al., 2018). The addition of a yeast micronutrient, GO-FERM®, can modify the final wine quality by increasing the intensities of honey, orange or pineapple aromas (Kontkanen, Pickering, Reynolds, & Inglis, 2005).

## 2.6 Factors Affecting the Tertiary Aromas of Icewine

Some icewines are aged on lees in a barrel or steel tank, and some icewines are aged in oak barrels for a variable time, but oak aging of icewine is not a common practice, and there is no published research showed that the oak aging can improve the sensory acceptance of icewine. The process of aging on lees can help to improve the aromatic complexity and soften the acidity, making a better-balanced wine, and the simultaneous use of lees and oak aging together with agitation may result in a smooth buttery wine with toffee characteristics (Pérez-Serradilla & De Castro, 2008; Wang et al., 2018). However, the quantitative data for the related odorant contribution is unknown until now. Although aging is an important process for the aroma evolution of icewine, residual sugars increasingly constitute a spoilage risk at higher concentrations, which is particularly marked in sweet wines with low acid and alcohol levels (Jackson, 2014). Considering that the high sugar contents of icewines makes them easy to contaminate by spoilage bacteria during the aging process, special procedures might be required to prevent undesirable yeast and bacterial growth. To prevent microbe contamination and oxidation, higher $SO_2$ combined with lysozyme is often used during icewine processing. A study on the effects of $SO_2$, lysozyme and oligomeric proanthocyanidin (OPC) on the volatile compounds over a 1-year aging process showed that adding 180 mg/L to 200 mg/L of $SO_2$ contributes to the synthesis of a fruity and floral bouquet during aging, and the addition of lysozyme and oligomeric proanthocyanidin can promote the evolution of certain esters, such as isoamyl acetate, ethyl lactate and dibutyl phthalate (K. Chen, Han, Li, & Sheng, 2017).

## 2.7 Conclusions and Perspectives

The aroma depends on the odor-active compounds and a multitude of interactions between all the chemical components, and it plays a major role in the overall flavor perception of icewine. Clarifying the impact odorants would facilitate aroma control by targeting the key steps that affect the aroma characteristics in icewine at a molecular level. Most but not all odorants have been found to be responsible for the aroma characteristics of Vidal and Riesling icewines. It showed that compounds like $\beta$-damascenone, 1-octen-3-ol, some terpenes, lactones and furanones contributed greatly to the discrimination between icewines and dry wines, and some of them have been confirmed as key odorants or major contributors by recombination and omission studies in icewines. For the aroma characteristic that has been well reproduced by combining the impact odorants in icewine, the findings might reduce the difficulties involved in not only optimizing viticulture and enological practices but also exploring other scientific question, such as tracking target odorant's generation or analyzing the diversity of odor interactions in icewines. For the aroma characteristic that has not been well reproduced, it clarifies the urgency of using modern analytical technologies to increase our knowledge of new key odorants; to understand the perceptual and physicochemical interactions between odorants and other icewine components; or to explore the contribution of the odorants with sub-threshold, low dilution factors, or low OAVs to the global aroma profile of icewine, since the actual impact of these minor odorants could be strengthened either by interactions with non-volatile compounds of the wine matrix or by perceptual interactions with other odor-active compounds in the aromatic buffer (Atanasova, Thomas-Danguin, Langlois, et al., 2005; V. Ferreira et al., 2016; Lytra et al., 2012; Robinson et al., 2009; D. Ryan, Prenzler, Saliba, & Scollary, 2008; Villamor, Evans, Mattinson, & Ross, 2013; Villamor & Ross, 2013).

Like other wine products, the odor-active compounds in wine normally originate or form from the grapes and then altered by other various processes employed during production or aging. It showed that some special processes involved in ice grape-growing, such as freeze-thaw cycles, or the winemaking process affect the metabolism of grape berries or yeasts differently and generate quite different chemical compositions, resulting in distinct aroma profiles in icewines. To understand the evolution of the icewine aroma composition during the grape-growing and winemaking processes, environmental, viticultural and oenological factors in the vineyard and winery that highly influence the quality of icewine need to be taken into consideration. The effect of different factors on the aroma of icewine which has been studied up to date is summarized in Table 3. However, to maintain the highest icewine quality, many of factors remain unknown with respect to icewine making practices. In the future, more research about the effects of fermentation control and post-fermentation on the aroma profile and the composition in icewine is needed. Among all the factors that affect the quality of icewines, the grape-growing environment plays a key role in determining whether icewines can be produced successfully, since authoritative icewine is made only in regions where a cool climate and frosts can be guaranteed. With the influences predicted by climate change over the coming decades, icewine regions throughout the world may face gradual alterations in their growing and harvesting seasons and the majority of traditional

icewine making regions may be difficult to reach enough low temperature for grape freezing. All of these changes will raise problems in wine making as well as in flavor compound accumulation, because the warmer climate will short the period of grape ripening and the grapes will be more susceptible to rot before they are harvested in winter. In response to these potential challenges, studies focusing on the factors related to the growing environment should be posed as future trends. Diverse cold-hardy cultivars have been used for icewine production in many European countries, but the most common cultivars and frequently studied cultivars are Riesling and Vidal Blanc. Studying the aroma characteristics of icewine made from other varieties could be also useful to understand the viticulture and the oenological aspects for improving the flavor quality of icewines and to understand the regulatory mechanisms involved in viticulture and oenological factors. Since most of the key odorants in icewines are also derived from the fermentation process as primarily generated from yeast metabolism, understanding the role of the yeast species in odorant formation and their properties and changes in the diversity of the yeast population during icewine fermentation would be helpful for selecting indigenous yeast strains to manufacture wines with territorially unique characteristics.

The increased awareness of wine flavor obliges the wine researcher not only to understand the generation of icewine compounds but also to understand which compounds has major relevance with icewine's sensory profile and consumer's preference. However, research related to this topic has been rarely explored. In previous studies, researchers often used well-trained subjects to determine an orthonasal aroma profile of icewine. However, it is not the orthonasal aroma but the retronasal aroma perception that mainly determines a consumer's preference. Considering this, it would be interesting to shift the focus of studying the orthonasal perception to studying the retronasal perception by using not only professional assessor but also consumers. In terms of retronasal aroma perception, it is influenced by some other complicated issues primarily corresponding to the aroma dynamic released in the mouth or the diversity of cross-model interactions that occur between the aroma, taste and other sensory perceptions (Thomas-Danguin, Sinding, Tournier, & Saint-Eve, 2016). Some studies have shown that adding sucrose significantly enhanced the intensity of some aromas like cherry, citrus and vanilla (Fujimaru & Lim, 2013; B. G. Green, Nachtigal, Hammond, & Lim, 2012). Since icewine owes much of its uniqueness to a high level of sugar, acidity and special aromas, researches related to perception interplay between taste and aroma should also be considered for future research. Furthermore, the diversity of cross-model interactions can result in temporal dimension changes, such as the length of the 'finish' and the time of appearance for specific aromas (Baker & Ross, 2014; Goodstein, Bohlscheid, Evans, & Ross, 2014). In these studies, that temporal dimension is a central feature, and methods such as quantitative descriptive analysis or other forms of profiling analysis using a category or other scales is not suitable since these methods implicitly regard the sensory properties under investigation as static phenomena, resulting in a loss of critical information (Dijksterhuis & Piggott, 2000). In that case, dynamic methods should be preferred for future research.

**Table 3** Effects of Environmental, Viticultural and Oenological Factors in the Vineyard and Winery on the Aroma of Icewines

| Factors | Affected aroma or aroma compounds in grape or wines | Ref. |
|---|---|---|
| **Growing environment** | | |
| This factor could refer to the research in other wines and grape varieties (regional and site climate or weather (Mendez-Costabel et al., 2014; Šuklje et al., 2019), geographical features (Alessandrini et al., 2017), soil (Scacco et al., 2010)) | | |
| **The vine** | | |
| Species and varieties | *Vidal*: *β*-damascenone, 1-octen-3-ol, ethyl hexanoate, *cis*-rose oxide.<br>*Riesling*: *β*-damascenone, ethyl octanoate, ethyl hexanoate, 1-octen-3-ol, and ethyl isobutanoate. | (Bowen & Reynolds, 2012) |
| Rootstocks | Unknown | |
| **Viticulture** | | |
| Canopy management | *Thinned treatments*: peach, apple/pear, dried fruit and caramelized aromas, tropical fruit, and tangerine flavors. | (Bowen et al., 2016) |
| Soil treatment | Unknown | |
| Harvesting | *Harvest date*: fresh fruit, dried fruit and nutty descriptors, esters, higher alcohols, monoterpenol, oxides, *cis*-rose oxide, nerol oxide, *γ*-nonalactone, *β*-damascenone, linalool, 1-octen-3-ol, 4-vinylguaiacol, citronellol, acetaldehyde.<br>*Machine harvesting*: unknown | (Bowen & Reynolds, 2015a; Bowen et al., 2016; Lukić et al., 2016) |
| **Vinification** | | |
| Crushing and pressing | *Pressing*: tropical fruity, rose aroma. | (Ma et al., 2017) |
| Yeast selection | *EC1118, V1116, VL1*: furfural, hexanoic acid, TDN, *β*-damascenone, linalool, *cis*-rose oxide, some alcohols, esters. | (Synos et al., 2015) |
| | *Spontaneous fermentation* (*W5B3, R3A10)*: ethyl hexanoate, ethyl octanoate, *β*-damascenone, 1-octen-3-ol, ethyl 2-methylbutanoate, and 3-methyl-1-butanol. | (Feng et al., 2020) |
| | *Spontaneous fermentation*: 1-hexanol, phenylethanol, nerol, *β*-phenyl acetate, *γ*-nonalactone. | (Crandles et al., 2015; Synos et al., 2015) |

| Factors | Affected aroma or aroma compounds in grape or wines | Ref. |
|---|---|---|
| | *Saccharomyces:* ethyl acetate and isoamylol; *Hanseniaspora and Candidawere:* 1-hexanol; *Lactobacillus and Streptococcus:* 2,3-butanediol, ethanol, ethyl acetate, isoamylol and isoamyl acetate. | (Y. Chen et al., 2020) |
| | *Mixed fermentation*: acetate esters, *β*-damascenone, $C_6$ alcohols, higher alcohols, *cis*-rose oxide, and phenylacetaldehyde. | (Zhang et al., 2018) |
| Fermentation control | *Sequential inoculation in mixed fermentation*: fruity, flowery, and sweet attributes<br>*Enzyme*: unknown<br>*Temperature*: unknown | (Zhang et al., 2018) |
| Sulfur dioxide | *The addition of lysozyme and oligomeric proanthocyanidin*: isoamyl acetate, ethyl lactate and dibutyl phthalatet. | (K. Chen et al., 2017) |
| Post-fermentation | *Clarification:* could decrease volatile compounds such as rose oxide, isoamyl acetate, linalool, ethyl hexanoate, *β*-damascenone, especially treated by membrane filtration. | (T. Z. Ma et al., 2020) |
| | *Oak aged*: raisin, honey, oak, caramel aroma. | (Nurgel et al., 2004) |

# Chapter III Characterization of the Key Aroma Compounds in Chinese Vidal Icewine

**Related to the publication**

Ma, Y., Tang, K., Xu, Y., & Li, J. M. (2017). Characterization of the key aroma compounds in Chinese Vidal icewine by gas chromatography–olfactometry, quantitative measurements, aroma recombination, and omission tests. *J. Agric. Food Chem.*, 65(2), 394-401.

## Abstract

The key aroma compounds of Chinese Vidal icewine were characterized by means of gas chromatography-olfactometry (GC-O) coupled with mass spectrometry (MS) on polar and nonpolar columns, and their flavor dilutionfactors (FD) were determined by aroma extract dilution analysis (AEDA). A total of 59 odor-active aroma compounds in three ranks of Vidal icewines were identified, and 28 odorants (FD≥ 9) were further quantitated for aroma reconstitution and omission tests. *β*-Damascenone showed the highest FD value of 2187 in all icewines. Methional and furaneol were first observed as important odorants in Vidal icewine. Aroma recombination experiments revealed a good similarity containing the 28 important aromas. Omission tests corroborated the significant contribution of *β*-damascenone and the entire group of esters. Besides, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) and 3-(methylthio)-1-propanal (methional) also had significant effects on icewine character, especially on apricot, caramel, and tropical fruit characteristics.

**Key words:** Vidal icewine, gas chromatography−olfactometry (GC-O), odor active compounds, aroma recombination and omission

## 3.1 Introduction

Icewine is produced from grapes that have frozen naturally on vine under the temperature of –8°C or below. The frozen grapes on vine will be air-dried, shrunk and then picked and pressed in a continuous process while they are still frozen. The frozen grapes on vine will finally result in a wine concentrated with sugars, acids, pigments and flavor compounds. These compounds are vital components composing the specific flavor of icewine. Icewine production in China has developed rapidly in recent years, and China has become an important icewine production country. Chinese icewine is commonly made from Vidal Blanc (Vidal). It is a French hybrid variety, and due to its relatively thick skins of berries and the cold-resistance of vines, it becomes the typical cultivar used for icewine making (Cliff et al., 2002; Nurgel et al., 2004).

Aroma is one of the important factors that determine the characters of wine. It can also influence perceived wine quality and consumer acceptance (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014). In recent years, studies on icewine aroma have shown aroma attributes of icewine differ substantially from different regions of origin. Canadian icewines had the higher fruity and floral aromas while German icewines had higher nutty or oily character (Cliff et al., 2002). Even from the same country, Canada, those Riesling icewines from Ontario and British Columbia could be simply distinguished by principal components analysis (PCA) based on their volatile compounds (Setkova et al., 2007). There are more than 200 volatile compounds which have been identified in more than 130 of Canadian and Czech icewines, and the concentrations of these compounds would be changed during the harvest time. For Vidal icewine, some volatile compounds such as ethyl isobutanoate, ethyl 3-methylbutanoate, 1-hexanol, 1-octen-3-ol, *cis*-rose oxide, *β*-damascenone had higher context in the latest harvest date (Bowen & Reynolds, 2015b). Most Vidal volatile compounds also differed with crop level (Bowen & Reynolds, 2015a). All of these researches have focused on the identification and quantitation of volatile compounds. However, it widely accepted that not all of volatiles present in foods contributed to the specific smell of the food (Dunkel et al., 2014), so identifying the key odorants and clarifying the contribution of the key odorants to the overall aroma are important to icewine quality control.

The search for the key odorants of foods gave the birth of the ‘sensomics’ approach, and in this approach, performing a reconstitution study is an important step to confirm the identification and quantitation experiments and, therefore, to verify that all important compounds have been detected (Schieberle, 1995). Recombination experiments and omission tests have been successfully used to verify and rank the aroma contribution for investigating the key aroma compounds of alcoholic beverages (Table 4). Before the aroma reconstitution and omission test, first, gas chromatography-olfactometry (GC-O) is commonly used to determine which odor-active compounds in a chromatographic run contribute to the wine aroma. Using GC-O and odor activity values (OAVs) analysis, *β*-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate were found as the highest odor activity compounds for both Canadian Riesling and Vidal icewines (Bowen & Reynolds, 2012).

However, up to now, no comprehensive study employing the sensomics approach has been performed on icewines.

**Table 4** Examples of Sensomics Approach Used in Alcoholic Beverages' Aroma Researches

| year | alcohol type | sensory profile | components with larger contribution | reference |
|---|---|---|---|---|
| 2016 | Zhima aroma-type baijiu | ethanolic , roasted, malty, floral, fruity, etc. | ethyl hexanoate, 3-methylbutanal, ethyl pentanoate, methional, ethyl hexanoate, etc. | (Zheng et al., 2016) |
| 2016 | commercial rum | ethanolic, malty, butter-like, clove-like, vanilla-like, fruity | *cis*-whiskey lactone, vanillin, decanoic acid, and 2- and 3-methylbutanol, ethyl butanoate, 1,1-diethoxyethane, etc. | (Franitza, Granvogl, & Schieberle, 2016) |
| 2015 | commercial Amontillado sherry wine | ethanolic, honey, floral, fruity, cooked apple, etc. | 2-phenylethanol, ethyl methylpropanoate, ethyl (2S,3S)-2-hydroxy-3-methylpentanoate, 1,1-diethoxyethane, 2- and 3-methylbutanals, methylpropanal, etc. | (Marcq & Schieberle, 2015) |
| 2015 | Chixiang aroma-type baijiu | fatty, grassy, sweet, ethanolic, floral, etc. | (E)-2-nonenal, (E)-2-octenal, 2-phenylethanol, etc. | (Fan, Fan, & Xu, 2015) |
| 2014 | Shiraz wine (Australia) | chocolate, fruity, oak, pepper, green, nail polish remover | ethyl propanoate, dimethyl sulfide (DMS), 2- and 3-methylbutanoic acid, rotundone, etc. | (Mayr et al., 2014) |
| 2014 | light aroma type baijiu | fruity, floral, ethanolic, mushroom, coconut, grassy, etc. | *β*-damascenone, ethyl acetate, ethyl lactate, geosmin, acetic acid, 2-methylpropanoic acid, etc. | (Gao, Fan, & Xu, 2014) |
| 2012 | Sauvignon Blanc wine ( New Zealand) | passionfruit, apple, citrus, grassy, cats pee, etc. | *β*-damascenone, 3-mercaptohexanol, 3-mercaptohexyl acetate, etc. | (Benkwitz et al., 2012) |
| 2011 | Dornfelder red wine | fruity, flowery, clove-like, smoky, vanilla-like, malty, etc. | (S)-2 and 3-methyl-1-butanol , 2-phenylethanol , 3-hydroxy-4,5-dimethylfuran-2(5H)-one , (S)-ethyl 2-methylbutanoate, (S)-2- and 3-methylbutanoic acid, etc. | (Frank et al., 2011) |
| 2002 | Grenache rose´ wine | fruity, caramel, citric, flowery, etc. | 3-mercapto-1-hexanol, furaneol, homofuraneol, ethyl esters, isoamyl acetate, *β*-damascenone, etc | (V. Ferreira, Ortin, Escudero, Lopez, & Cacho, 2002) |

The objectives of this study include: 1) Identify the key aroma compounds in Chinese Vidal

icewine. 2) Determine the contribution of different aroma compounds to the profile of Chinese icewine by GC-O, quantitative measurements, aroma recombination and omission tests.

## 3.2 Materials and Methods

### 3.2.1 Chemicals

Ethanol absolute (≥99.8%, HPLC grade), dichloromethane (≥ 99.8%, HPLC grade), methanol (≥99.9%, HPLC grade), and analytical standards, with at least 97% purity were purchased from Sigma-Aldrich China Co. (St. Louis, Missouri, USA). These analytical standards were ethyl acetate, ethyl isobutanoate, ethyl butanoate, ethyl 2-methylbutanoate, isoamyl acetate, ethyl isovalerate, ethyl valerate, ethyl hexanoate, ethyl octanoate, 3-methyl-1-butanol, 1-hexanol, 1-heptanol, 1-octen-3-ol, *cis*-rose oxide, geraniol, phenethyl acetate, phenylethyl alcohol, guaiacol, vinyl guaiacol, *β*-linalool, *β*-damascenone, 3-(methylthio)-1-propanal (methional), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), *γ*-decalactone, *γ*-undecalactone, 1-octen-3-one, 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol), 2,3-butanedione, and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). *L*-menthol (internal standard, IS1), octyl propionate (IS2) and *p*-fluorobenzaldehyde (IS3) were purchased from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Ultrapure water made with Milli-Q purification system (Millipore, Bedford, MA) was boiled for 5 min before used. Analytical-grade anhydrous D-fructose, tartaric acid, sodium sulfate, sodium chloride, sodium carbonate, and sulfuric acid were purchased from China National Pharmaceutical Group Corp. (Shanghai, China). Dichloromethane was freshly distilled before used.

### 3.2.2 Icewine Samples

Three ranks experimental icewines, based on the level of pressing, Black label, Blue label and Yellow label, were made from Vidal grapes harvested in 2010 from ChangYu Winery in Huanren-on-the-Huanlong Lake, Liaoning province (North-east China). The Black label icewine received the lightest pressing, followed by the Blue label, with the Yellow label reserved for wines from the highest press fraction. Grapes were harvested, destemmed, crushed and pressed at –8ºC to –9ºC, and then, the grape juice was transferred to a stainless-steel container and mixed after adding 60–80 mg/L $SO_2$ and 30 mg/L of pectinase HC (Lallemand, France). Alcoholic fermentation was carried out at 10–12°C for 40–60 d with 200 mg/L of dried active yeast K1 (LALVIN, Canada). Malolactic fermentation was not induced. Stabilization, fining and filtration were involved before bottling, and were commercialized after 12-month aging time. All samples were stored horizontally at 18ºC in the dark prior to analysis. Three bottles were provided for each sample and were analyzed in duplicate.

### 3.2.3 Aroma Extraction Methods

Solid-phase extraction (SPE) method was used to extract volatile compounds. The column (LiChrolut EN, Merck; 0.5 g of phase) was first rinsed with 6 mL of dichloromethane, then 6

mL of methanol and 6 mL of a water-ethanol mixture (11%, ethanol by volume). 50 mL of sample was passed through the column at a flow rate of 1 mL/min. Sugars, pigment and other low-molecular-weight polar compounds were eliminated with 20 mL of ultrapure water. Finally, the sorbent was eluted with 10 mL of dichloromethane. Using nitrogen stream, the organic phase was concentrated to a final volume of 250 $\mu$L for GC-O and GC-MS analysis.

### 3.2.4 Gas Chromatography-Olfactometry and Gas Chromatography Mass Spectrometric Analysis

The instruments used were an Agilent 6890 gas chromatograph equipped with an Agilent 5975 mass-selective detector (MSD) and a sniffing port (ODP 2, Gerstel, Germany). The analytical columns were a DB-FFAP column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent, Torrance, CA) and HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses, Agilent, Torrance, CA). The front inlet was programmed in splitless mode for SPE (1 uL injected), and the oven temperature was initially held at 50°C for 2 min, then raised to 230°C at 6°C/min and held for 15 min. The carrier gas was helium at constant flow rate of 2 mL/min. The effluent supplemented with Helium was split to the olfactory port installed back of the GC detector. The sniffing time was 45 min for each analysis and the capillary, which was connected with the sniffing port, was kept at 250°C. The data acquisition [electron impact (EI) at 70 eV] was in scan mode, 35–500 Da for compound identification.

GC-O analysis was conducted by a panel of four well-trained assessors (two females and two males) from Laboratory of Brewing Microbiology and Applied Enzymology at Jiangnan University. The assessors first analyzed the extracts on both DB-FFAP column and HP-5MS column and record the retention time and descriptors of the odor peak for each compounds. After discussing, checking the aroma descriptor with the chemical standards and remembering the aroma characteristic, aroma extract dilution analysis (AEDA) was used for searching important odorants.

### 3.2.5 Aroma Extract Dilution Analysis

For AEDA, the concentrated fraction was diluted stepwise (1:3) with dichloromethane. Each dilution was submitted to GC-O analysis under the same GC conditions described above until no odorant could be detected. The flavor dilution (FD) factor of each compound represented the maximum dilution in which the odorant could be perceived. Analysis was repeated in duplicate by each assessor. Only the odorants detected among more than two assessors were recorded.

### 3.2.6 Aroma Identification and Quantitation

Aroma compounds identification was achieved by comparison of their odors, NIST 05 a.L database (Agilent Technologies Inc., Santa Clara, CA, USA), and their retention indices (RI) on both columns with those of pure standards. RI of the odorants were calculated from the retention times of *n*-alkanes ($C_5$–$C_{30}$), according to a modified Kovats method (Cates & Meloan, 1963).

Three methods were involved in aroma quantitation (Table 5). Standard curve concentrations and compounds were quantified on a DB-FFAP column, based on the ratio of

the peak area of the compound relative to the peak area of the internal standard to determine the concentration of the analytes. Standard curve concentrations and compounds were quantified in icewine model solution. The formula was referred from icewine model solution (Bowen & Reynolds, 2012) and prepared based on the true concentration in Chinese icewine (12.2 g/L total acid, tartaric acid was used; 159.0 g/L residual sugar, fructose was used; and 11.0% ethanol by volume, with a pH of 3.4).

Methional, guaiacol, furaneol, homofuraneol and *γ*-decalactone were enriched by SPE methods and quantified by GC-MS. *L*-menthol (314 mg/L) was used as internal standards. Selective ion monitoring (SIM) mass spectrometry was used to quantitate some aroma compounds, *m/z* 104 for methional, *m/z* 128 for furaneol and *m/z* 142 for homofuraneol. The ion monitored of *L*-menthol in the SIM run was *m/z* 138.

*Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry.* A 50/30 μm DVB/CAR/PDMS fiber (Supelco, Inc.,Bellefonte, PA) was used for aroma extraction. Except for methional, guaiacol, furaneol, homofuraneol and *γ*-decalactone, other compounds were enriched by headspace solid-phase microextraction (SPME) method and quantified by GC-MS. *L*-menthol (314 mg/L) and octyl propionate (181 mg/L) were used as internal standards. 8 mL of sample was placed into a 20 mL glass vial with a silicon septum, then added to 10 μL internal standard and saturated with 3 g sodium chloride, and was equilibrated at 60°C for 15 min and extracted for 30 min under stirring at the same temperature. After extraction, the fiber was inserted into the injection port.

*Headspace Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry after Derivatization.* 2, 3-butanedione and 1-octen-3-one were quantified after derivatization with PFBHA. First, 8 mL of sample was placed into a 20 mL glass vial and saturated with 3 g sodium chloride, then added to 10 μL of *p*-fluorobenzaldehyde (1.24 mg/L), which was an internal standard. Finally, 120 μL of PFBHA (50 g/L in water) was added. Then, it was equilibrated at 65°C for 10 min and extracted for 45 min under stirring at the same temperature, then transferred the fiber to the injector for desorption at 250°C for 300 s.

The front inlet was programmed in splitless mode, and the oven temperature was initially held at 50°C for 2 min, then raised to 100°C at a rate of 6°C/min and held for 0.1 min, then to 160°C at a rate of 2°C/min and held for 0.1 min, and finally at 5°C/min to 230°C and held for 10 min. The carrier gas was helium at constant flow rate of 1 mL/min.

The mass spectrometer was operated in electron ionization mode at 70 eV with SIM.

The ion monitored for *p*-fluorobenzaldehyde after derivatization was m/z 319. Monitored ions of 2, 3-butanedione and 1-octen-3-one after derivatization were 279 and 140 respectively.

**Table 5** Chemical Standards, Quantitative Ions, and Calibrated Intervals of Quantitative Analysis

| compounds | CAS registry No. | methods[a] | IS[b] | quantitative ion *(m/z)* | standard curve | | | calibrated interval （μg/L） | LOD （μg/L） | recovery (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | slope | intercept | $R^2$ | | | |
| ethyl acetate | 141-78-6 | SPME | IS2 | 61 | 0.022 | 0.211 | 0.9954 | 12.7–3248.0 | 73.8 | 125.7 |
| ethyl isobutanoate | 97-62-1 | SPME | IS2 | 116 | 0.010 | -0.003 | 0.9980 | 0.6–568.0 | 4.8 | 121.4 |
| 2,3-butanedione | 431-03-8 | SPMED | IS3 | 279 | 0.037 | 0.005 | 0.9961 | 48.8–3120.0 | 0.3 | 118.5 |
| ethyl butanoate | 105-54-4 | SPME | IS2 | 71 | 0.065 | -0.006 | 0.9961 | 11.8–12065.6 | 9.8 | 103.9 |
| ethyl 2-methylbutanoate | 7452-79-1 | SPME | IS2 | 57 | 0.101 | 0.001 | 0.9970 | 1.0–1018.6 | 1.9 | 107.4 |
| ethyl isovalerate | 108-64-5 | SPME | IS2 | 88 | 0.128 | 0.000 | 0.9980 | 1.0–1039.5 | 1.1 | 97.2 |
| isoamyl acetate | 123-92-2 | SPME | IS2 | 43 | 0.262 | 0.148 | 0.9970 | 18.3–18744.0 | 21.1 | 109.2 |
| ethyl valerate | 539-82-2 | SPME | IS2 | 85 | 0.129 | 0.002 | 0.9940 | 0.4–420.0 | 3.5 | 101.7 |
| 3-methyl-1-butanol | 123-51-3 | SPME | IS1 | 70 | 0.006 | –0.004 | 0.9980 | 228.6–234090.0 | 228.6 | 108.9 |
| ethyl hexanoate | 123-66-0 | SPME | IS2 | 88 | 0.640 | –0.097 | 0.9990 | 18.8–19285.2 | 20.2 | 95.6 |
| 1-octen-3-one | 4312-99-6 | SPMED | IS3 | 140 | 0.004 | 0.152 | 0.9982 | 17.9–2296.1 | 28.3 | 125.7 |
| 1-hexanol | 111-27-3 | SPME | IS1 | 56 | 0.049 | –0.011 | 0.9990 | 19.9–20326.7 | 27.1 | 95.5 |
| *cis*-rose oxide | 16409-43-1 | SPME | IS1 | 139 | 2.094 | 0.038 | 0.9930 | 0.6–631.0 | 1.9 | 103.4 |
| ethyl octanoate | 106-32-1 | SPME | IS2 | 88 | 4.682 | –4.391 | 0.9960 | 17.9–18340.0 | 19.2 | 105.5 |
| 1-octen-3-ol | 3391-86-4 | SPME | IS1 | 72 | 0.027 | 0.003 | 1.0000 | 1.6–1686.5 | 18.0 | 88.0 |
| 1-heptanol | 111-70-6 | SPME | IS1 | 56 | 0.064 | 0.002 | 0.9991 | 0.3–288.4 | 2.4 | 91.2 |
| 3-(methylthio)-1-propanal (methional) | 3268-49-3 | $SPE_{SIM}$ | IS1 | 104 | 48.534 | 0.007 | 0.9997 | 12.1–776.0 | 2.6 | 85.8 |
| *β*-linalool | 78-70-6 | SPME | IS1 | 71 | 0.225 | 0.105 | 0.9996 | 7.6–7829.0 | 8.9 | 98.7 |
| phenethyl acetate | 103-45-7 | SPME | IS2 | 104 | 0.941 | –0.121 | 0.9990 | 1.7–1713.7 | 1.5 | 97.2 |
| *β*-damascenone | 23696-85-7 | SPME | IS1 | 121 | 0.028 | –0.020 | 0.9940 | 2.8–2835.2 | 5.9 | 86.2 |
| guaiacol | 90-05-1 | SPE | IS1 | 124 | 0.811 | 0.016 | 0.9992 | 2.7–688.0 | 4.6 | 105.5 |
| geraniol | 106-24-1 | SPME | IS1 | 69 | 0.420 | –0.043 | 0.9960 | 1.1–1164.5 | 1.1 | 83.2 |
| phenylethyl alcohol | 60-12-8 | SPME | IS1 | 91 | 0.010 | 15.257 | 0.9980 | 771.7–790240.0 | 964.7 | 90.5 |

| compounds | CAS registry No. | methods[a] | IS[b] | quantitative ion *(m/z)* | standard curve | | | calibrated interval （μg/L） | LOD （μg/L） | recovery (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | slope | intercept | $R^2$ | | | |
| 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) | 3658-77-3 | $SPE_{SIM}$ | IS1 | 128 | 115.570 | –0.508 | 0.9999 | 21.9–1404.0 | 15.8 | 80.5 |
| 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol) | 27538-09-6 | $SPE_{SIM}$ | IS1 | 142 | 2.933 | –0.054 | 0.9938 | 12.6–401.9 | 9.7 | 78.4 |
| *γ*-decalactone | 706-14-9 | SPE | IS1 | 85 | 0.296 | 0.003 | 0.9980 | 0.3–275.0 | 0.2 | 90.1 |
| *γ*-undecalactone | 104-67-6 | SPE | IS1 | 85 | 0.257 | 0.017 | 0.9982 | 0.3–275.0 | 0.5 | 89.5 |
| vinyl guaiacol | 7786-61-0 | SPME | IS1 | 150 | 0.073 | –0.059 | 0.9980 | 3.5–3600.6 | 3.8 | 94.0 |

[a]The quantitative methods used, ‘SPE’ stand for solid-phase extraction-gas chromatography-mass spectrometry, ‘SPME’ stand for headspace solid-phase microextraction-gas chromatography-mass spectrometry, ‘SPMED’ stand for headspace solid-phase microextraction-gas chromatography-mass spectrometry after derivatization. [b]The internal standard used to quantitate the compounds, they were *L*-menthol (IS1), octyl propionate (IS2) and *p*-fluorobenzaldehyde (IS3).

### 3.2.7 Aroma Recombination of Icewine by Descriptive Analysis

Aroma compounds were recombined in odorless icewine and compared with the corresponding real wine. Odorless icewine was prepared as follow. The icewine was extracted by the SPE method until the remaining liquid was odorless, and was freeze-dried to obtain the lyophilisate matrix. Before the recombination, the lyophilisate matrix was dissolved by aqueous solutions containing 10% of alcohol, and was adjust to the icewine concentration level which included 12.2 g/L total acid, 159.0 g/L residual sugar, and 11.0% ethanol by volume, with a pH of 3.4. The aroma compounds with FD≥ 9 (Table 6) of icewine were added into the odorless icewine according to their occurring concentrations (Table 7).

Twelve assessors (seven females and five males, 24 years old on average) were involved in descriptive analysis. They were recruited from Jiangnan University and trained according to the standard (ISO-8586, 2012). '*Le nez du vin*' (Jean Lenoir, Provence, France) was used as aroma standard to help assessors to describe the odor qualities of 54 odorants. After one year trainings and tests, they showed good performance in flavor memory and discrimination, and also showed good ability in consistency, stability and repeatability for giving scores.

After assessors meeting to discuss the lexicon terms and reach consensus, the final lexicon was generated. The six major descriptions were honey, caramel, apricot, rose, tropical fruit and raisin. Then, assessors were given the icewine reconstitution samples and real icewine samples one by one in a randomly order with three-digit-coded. Assessors needed to score the intensity of each attribute on a seven-point scale from 1 (extremely weak) to 7 (extremely strong). During the session, the assessors evaluated these samples with a 5-min break after each sample.

### 3.2.8 Aroma Omission Test by Discrimination Analysis

Seven aroma omission models were prepared based on their aroma descriptions (Table 8). Three glasses of sample (15 mL), including one omission model and two complete models were randomly coded with three digits. The order of the omission model was presented balanced to those 12 assessors. Assessors were asked to follow the order to sniff the samples, and then selected the odd one. The significance of difference between omission model and complete recombination was determined according to the method described (Jellinek, 1985).

## 3.3 Results and Discussions

### 3.3.1 Odor-active Compounds Identification of Icewine

The aromatic extract sample was sniffed by the assessors. It showed that the sample extracted by SPE represented exactly the typical icewine aroma profile. Thus, it was reliable to be used to analyze the icewine key aroma compounds.

A total of 59 aroma compounds were recorded with GC-O analysis on DB-FFAP and HP-5MS columns in three ranks (Table 6). Yellow label icewine hold the highest total FD values among the three ranks. Aroma compounds existing in three ranks were almost the same, except for 2 esters, 3 terpenes and 4 alcohols, which were not detected in Yellow label icewine. The aroma compounds with higher FD values (FD≥ 9) were likely to contribute a lot to

overall icewine type aroma, giving icewine honey, fruity, and caramel flavors. Among these important aroma compounds with the FD≥ 9, *β*-damascenone (FD≥ 2187) was determined as the most important aroma compounds in all ranks, which was also previously reported as an important odorant in Canada Vidal icewine (Bowen & Reynolds, 2012), suggested that it could be the key role in Vidal icewine aroma profile regardless of its origin. *β*-damascenone was frequently descript as a cooked apple flavor, but in this study, it showed a honey-like flavor. This disagreement might result from the stronger aroma intensity of *β*-damascenone in icewine, because aroma characteristic could be different if its intensity greatly changed. In the secondary position was 3-methyl-1-butanol (FD≥ 729 in Yellow label, FD≥243 in the others), followed by furaneol. Some compounds such as ethyl 2-methylbutanoate, ethyl isobutanoate, methional, *cis*-rose oxide, etc. were determined as important odorants by the assessors, with some differences of intensity in three rank samples.

A careful literature inspection showed that 3-(methylthio)-1-propanal (methional) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) were first detected and rated as important aroma compounds in Vidal icewine. Methional was detected as a cooked-potato flavor, with the FD value of 81 in all the samples. It could source from *L*-methionine, and the concentration of *L*-methionine was about 4.5 mg/L in three ranks of year 2010 Chinese icewine (Schrader, 2007; Tang, Li, Wang, Ma, & Xu, 2013). Methional could be formed by Strecker degradation or by sulfur metabolism in *Saccharomyces cerevisiae* yeast (Schrader, 2007; Ugliano & Henschke, 2009). This compound has been detected in red wine, botrytized wine, aged wine and some oxidized wines (Escudero et al., 2000; Falcao, de Revel, Rosier, & Bordignon-Luiz, 2008). This compound plays an ambiguous role. In young white wines it causes unpleasant odors, but in complex wines, such as some Chardonnays or some great red wines, is a net contributor to some appreciated aroma (V. Ferreira, 2010). However, its contribution to icewine aroma profile was not clear, thus, recombination and omission tests should be conducted on it. Furaneol was detected as a caramel flavor, with the FD value of 243, 81, and 243 in the three ranks respectively. It exhibits a pineapple, strawberry-like odor in dilute solutions and a caramel like one in concentrates (Schrader, 2007). It is a very important *O*-heterocyclic compound, commonly come from as a carbohydrate transformation product (Dunkel et al., 2014; Schrader, 2007), as a product of Maillard reaction. Maillard reaction results from a complex series of chemical reactions between an amino acid and a reducing sugar (Schwab, 2013). This chemical reaction might happen in icewine, because it has lots of fructose, glucose, and also rich amino acid (Tang et al., 2013). Besides its chemical formation, furaneol could be biosynthesized by plants and microorganism, and the concentration of it could also be different from grape variety, oak wood addition as well as the usage of pectolytic enzymes with *β*-glucosidic secondary activities (de Pinho & Bertrand, 1995; Genovese, Piombino, Lisanti, & Moio, 2005). In white wines made with hybrid grapes still used in Europe, furaneol was detected in almost all cases (de Pinho & Bertrand, 1995). It is also showed that furaneol is positively linked to the noble-rotten grapes and has been detected as a key-aroma compounds of botrytized wines (Sarrazin, Dubourdieu, & Darriet, 2007; Tosi et al., 2012). Furaneol biosynthesis has been studied in strawberry and key enzymes have been identified (Schiefner, Sinz, Neumaier, Schwab, & Skerra, 2013); However,

formation mechanisms in grapes and wine are still unknown (Robinson et al., 2014a).

**Table 6** Important Aroma Compounds in Vidal Icewine Detected by AEDA and GC-MS

| No. | compounds [a] | odor description | FD | | | DB-FFAP | | HP-5 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Black | Blue | Yellow | LRI[b] | RI | LRI[c] | RI |
| 1 | ethyl acetate | fruity | 27 | 27 | 27 | 839 | 875 | 628 | 669 |
| 2 | ethyl propionate | fruity | | 1 | | 944 | 949 | 717 | 693 |
| 3 | ethyl isobutanoate | pineapple | 243 | 9 | 27 | 973 | 978 | 751 | 734 |
| 4 | 2,3-butanedione | cream | 9 | 243 | 81 | 970 | 984 | 593 | 600 |
| 5 | isobutyl acetate | fruity | 9 | 1 | 1 | 1018 | 1002 | 776 | 744 |
| 6 | ethyl butanoate | melon | 27 | 9 | 27 | 1040 | 1029 | 800 | 769 |
| 7 | ethyl 2-methylbutanoate | apple | 243 | 81 | 9 | 1060 | 1041 | 801 | 826 |
| 8 | ethyl isovalerate | pineapple | 3 | 27 | 9 | 1068 | 1048 | 853 | 839 |
| 9 | isobutanol | pine | 1 | 1 | 3 | 1097 | 1095 | 647 | 686 |
| 10 | isoamyl acetate | banana | 3 | 9 | 9 | 1125 | 1126 | 872 | 849 |
| 11 | ethyl valerate | grape | 9 | 9 | 9 | 1138 | 1127 | 900 | 877 |
| 12 | 1,4-cineole * | pine | 9 | 9 | 3 | 1169 | 1130 | 1018 | 1010 |
| 13 | terpinolene | pine | 3 | 3 | 3 | 1175 | 1130 | 1014 | 1010 |
| 14 | 3-methyl-1-butanol | organic reagent | 243 | 243 | 729 | 1215 | 1192 | 737 | 722 |
| 15 | ethyl hexanoate | apple peel | 9 | 9 | 27 | 1238 | 1232 | 998 | 971 |
| 16 | *γ*-terpinene | pine | 1 | 1 | 1 | 1258 | 1252 | 1062 | 1060 |
| 17 | 1-octen-3-one | mushroom | 27 | 81 | 9 | 1333 | 1320 | 894 | 900 |
| 18 | ethyl heptanoate | prune | 27 | 81 | 3 | 1331 | 1330 | 1095 | 1052 |
| 19 | 2,3-dimethylpyrazine | nutty | 3 | 9 | 3 | 1355 | 1331 | 889 | 853 |
| 20 | *(E)*-3-hexen-1-ol | pine | 9 | 3 | 3 | 1346 | 1340 | 855 | 826 |
| 21 | 1-hexanol | nutty | 9 | 9 | 9 | 1358 | 1349 | 866 | 834 |
| 22 | *cis* -rose oxide | lychee, rose | 27 | 27 | 81 | 1356 | 1378 | 1111 | 1094 |
| 23 | *(Z)*-3-hexen-1-ol | pine | 27 | 9 | 3 | 1361 | 1393 | 847 | 796 |
| 24 | 3-octanol | pine nuts | 1 | | | 1395 | 1413 | 996 | 1041 |
| 25 | ethyl octanoate | fruity | 9 | 27 | 81 | 1419 | 1414 | 1197 | 1146 |
| 26 | 2-octanol | mushroom | | 3 | | 1430 | 1418 | 994 | 974 |
| 27 | 4-mercapto-4-methylpentan-2-one * | blackcurrant bacillus | 1 | 9 | 3 | 1377 | 1426 | 937 | 930 |
| 28 | acetic acid | vinegar | 1 | 3 | 1 | 1435 | 1450 | 600 | 626 |
| 29 | 1-octen-3-ol | mushroom | 9 | 3 | 27 | 1456 | 1450 | 976 | 966 |
| 30 | 1-heptanol | fatty | 27 | 9 | 9 | 1460 | 1464 | 959 | 891 |
| 31 | 3-(methylthio)-1-propanal (methional) | cooked potato | 81 | 81 | 81 | 1458 | 1490 | 909 | 885 |
| 32 | *β*-linalool | lavender | 3 | 9 | 9 | 1457 | 1535 | 1087 | 1070 |
| 33 | 2-ethylhexanol | floral | | 1 | | 1484 | 1542 | 1028 | 1031 |
| 34 | 3-heptyl alcohol * | pine | | 1 | | 1487 | 1543 | 941 | 1022 |
| 35 | terpinen-4-ol | spicy | | 9 | 1 | 1584 | 1615 | 1187 | 1164 |
| 36 | hotrienol * | flowery | 27 | 81 | 81 | 1623 | 1687 | 1103 | 1077 |
| 37 | terpineol | spicy | | 1 | | 1677 | 1728 | 1189 | 1204 |
| 38 | carvenone* | toasty | | 1 | | 1700 | 1743 | 1255 | - |

| No. | compounds [a] | odor description | FD | | | DB-FFAP | | HP-5 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Black | Blue | Yellow | LRI[b] | RI | LRI[c] | RI |
| 39 | epoxylinalool * | floral | 3 | | | 1772 | 1766 | 1163 | 1141 |
| 40 | ethyl phenylacetate | apple | 3 | 3 | 1 | 1785 | 1808 | 1228 | - |
| 41 | phenethyl acetate | flowery | 9 | 9 | 9 | 1831 | 1856 | 1240 | 1183 |
| 42 | *β*-damascenone | honey | 2187 | 2187 | 2187 | 1802 | 1859 | 1381 | 1365 |
| 43 | guaiacol | smoky | 9 | 27 | 9 | 1875 | 1875 | 1087 | 1119 |
| 44 | geraniol | rose | 27 | 27 | 81 | 1847 | 1893 | 1276 | 1330 |
| 45 | phenylethyl alcohol | flowery | 9 | 9 | 27 | 1903 | 1913 | 1107 | 1099 |
| 46 | 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) | caramel | 243 | 81 | 243 | 2043 | 2009 | 1098 | 1115 |
| 47 | octanoic acid | sweat | 1 | 3 | 3 | 2060 | 2075 | 1170 | 1158 |
| 48 | 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol) | caramel | 27 | 9 | 27 | 2112 | 2091 | 1175 | 1173 |
| 49 | *γ*-decalactone | apricot | 9 | 9 | 9 | 2103 | 2116 | 1467 | 1470 |
| 50 | *δ*-decalactone | apricot | 1 | 3 | 3 | 2194 | 2176 | 1493 | 1490 |
| 51 | *γ*-undecalactone | apricot | 9 | 27 | 9 | 2270 | 2196 | 1922 | 1998 |
| 52 | vinyl guaiacol | smoky | 9 | 3 | 9 | 2187 | 2213 | 1312 | 1298 |
| 53 | decanoic acid | acid | | 1 | 1 | 2270 | 2231 | 1386 | - |
| 54 | ethyl hexadecanoate | potpourri | 3 | | | 2252 | 2251 | 1995 | - |
| | extra compound group | | | | | | | | |
| 55 | unknown 1 | nutty | 1 | 3 | 9 | - | 1215 | - | 1045 |
| 56 | unknown 2 | blackcurrant bacillus | 1 | 9 | 1 | - | 1248 | - | 930 |
| 57 | unknown 3 | nutty | | 1 | 1 | - | 1438 | - | 1212 |
| 58 | unknown 4 | caramel | 3 | | 3 | - | 2036 | - | 1549 |
| 59 | unknown 5 | caramel | 9 | 3 | 3 | - | 2147 | - | 1628 |

[a] The odorants were identified by comparing their RI, mass spectra, and aroma attributes with those of pure standards (except for compounds marked with '*'). [b,c] The RI in literature.

Although the use of AEDA approach can highlight main odorants presenting in aroma organic extract, the approach do not provide a representative sampling of those compounds transferred to the vapor phase at very different proportions, depending on their specific volatilities and their interactions with the product matrix (De-La-Fuente-Blanco & Ferreira, 2020). Therefore, it cannot be ruled out that some of the compounds (e.g. polar) considered in the extract might have been overestimated and further studies involving odor threshold and recombination test should be further conducted.

### 3.3.2 Quantitative Analysis in Yellow Label Icewine

Yellow label icewine, which had the highest FD value among three ranks, was selected to be recombined. Thirty aroma compounds with FD≥ 9 were identified as potential important aroma compounds in Chinese Vidal icewine. Twenty-eight of these (except for 1 unknown compound and unavailable hotrienol) were further quantitated (Table 5, Table 7). Among these 28 aroma odorants, 3-methyl-1-butanol, phenylethyl alcohol, 2,3-butanedione, and 1-hexanol had the highest concentration, all of them were above 1 mg/L. Ethyl isobutanoate, ethyl octanoate, ethyl hexanoate, ethyl acetate, 1-octen-3-ol, *β*-damascenone, ethyl butanoate, furaneol, *γ*-decalactone were with the concentration range from 100 *μ*g/L to 1000 *μ*g/L.

There were no reference gave the thresholds of these aroma compounds in icewine matrix, the thresholds given in Table 7 were obtained either from the system of table wine or ethanol solution. Thus, the odor activity values (OAVs) calculated in this study was just an auxiliary approach to understand the contributions of different compounds. Twenty-two of these odorants were further verified as important odorants in Vidal icewine with the OAVs above 1.0. *β*-Damascenone, 1-octen-3-one, 2,3-butanedione, ethyl hexanoate, *cis*-rose oxide, ethyl isobutanoate, ethyl 2-methylbutanoate, 1-octen-3-ol, methional, furaneol, 1-heptanol, *γ*-undecalactone, ethyl isovalerate and ethyl butanoate had higher OAVs (> 10.0). The compound with the highest OAVs of 5580 was *β*-damascenone, its OAVs was much higher than other compounds. *β*-Damascenone also had the highest FD value in GC-O analysis. Thus, it should be the most important key aroma compound. 1-octen-3-one, 2,3-butanedione, ethyl hexanoate, *cis*-rose oxide, ethyl isobutanoate, ethyl 2-methylbutanoate and 1-octen-3-ol were followed with OAVs above 40.0, which might gave the icewine a possible characteristics like fruit and flora. Methional and furaneol also had great influence on icewine aroma profile based on their OAVs of 37.4 and 35.0 respectively. In these results, matrix effects and interactions with aroma compounds were ignored. Icewine has much more sugars than normal wine, and its special wine matrix could affect the aroma perception and threshold, so OAVs< 1.0 does not mean that the aroma compound could not be perceived in the wine. Although it could have some bias to take OAVs into account when evaluate aroma compounds contribution in icewine, but it could be useful for giving some preliminary information.

**Table 7** Concentrations and Odor Activity Values (OAVs) of the Odor-active compounds in Chinese Vidal Icewine

| No. | compounds | avg. ($\mu$g/L) [a] | RSD% [b] | thresholds ($\mu$g/L) | OAVs |
|---|---|---|---|---|---|
| 1 | ethyl acetate | 354.8 | 3.7 | 7500.0 (Guth, 1997) | 0.0 |
| 2 | ethyl isobutanoate | 883.0 | 4.0 | 15.0 (Guth, 1997) | 58.9 |
| 3 | 2,3-butanedione | 9576.0 | 7.3 | 100.0 (Guth, 1997) | 95.8 |
| 4 | ethyl butanoate | 213.4 | 2.2 | 20.0 (Guth, 1997) | 10.7 |
| 5 | ethyl 2-methylbutanoate | 57.8 | 4.9 | 1.0 (Guth, 1997) | 57.8 |
| 6 | ethyl isovalerate | 49.4 | 3.9 | 3.0 (Guth, 1997) | 16.5 |
| 7 | isoamyl acetate | 92.3 | 3.5 | 30.0 (Guth, 1997) | 3.1 |
| 8 | ethyl valerate | 13.5 | 1.3 | 1.5 (Takeoka, Buttery, Turnbaugh, & Benson, 1995) | 9.0 |
| 9 | 3-methyl-1-butanol | 99515.9 | 0.8 | 30000.0 (Guth, 1997) | 3.3 |
| 10 | ethyl hexanoate | 474.7 | 4.9 | 5.0 (Guth, 1997) | 94.9 |
| 11 | 1-octen-3-one | 6.9 | 0.8 | 0.04 (Greger & Schieberle, 2007) | 171.8 |
| 12 | 1-hexanol | 1034.8 | 5.0 | 8000.0 (Guth, 1997) | 0.1 |
| 13 | *cis*-rose oxide | 17.1 | 1.1 | 0.2 (Guth, 1997) | 85.5 |
| 14 | ethyl octanoate | 553.1 | 1.6 | 580.0 (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004) | 1.0 |
| 15 | 1-octen-3-ol | 322.4 | 2.8 | 7.0 (Darriet et al., 2002) | 46.1 |
| 16 | 1-heptanol | 87.0 | 3.5 | 3.0 (Fazzalari, 1978) | 29.0 |
| 17 | 3-(methylthio)-1-propanal (methional) | 18.7 | 1.0 | 0.5 (Escudero et al., 2000) | 37.4 |
| 18 | $\beta$-linalool | 50.5 | 2.8 | 15.0 (Guth, 1997) | 3.4 |
| 19 | phenethyl acetate | 46.0 | 4.2 | 250.0 (Guth, 1997) | 0.2 |
| 20 | $\beta$-damascenone | 279.0 | 1.2 | 0.05 (Guth, 1997) | 5580.0 |
| 21 | guaiacol | 73.7 | 7.8 | 9.5 (V Ferreira, Lopez, & Cacho, 2000) | 7.8 |
| 22 | geraniol | 17.1 | 1.8 | 30.0 (Guth, 1997) | 0.6 |
| 23 | phenylethyl alcohol | 84829.6 | 4.9 | 10000.0 (Guth, 1997) | 8.5 |
| 24 | 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) | 175.2 | 10.4 | 5.0 (V. Ferreira et al., 2002) | 35.0 |
| 25 | 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol) | 71.8 | 0.4 | 125.0 (V. Ferreira et al., 2002) | 0.6 |
| 26 | $\gamma$-decalactone | 155.8 | 9.5 | 88.0 (Etievant, 1991) | 1.8 |
| 27 | $\gamma$-undecalactone | 80.3 | 5.5 | 4.2 (Greger & Schieberle, 2007) | 19.1 |
| 28 | vinyl guaiacol | 71.0 | 3.9 | 1100.0 (V Ferreira et al., 2000) | 0.1 |

[a]avg., average concentration of triplicates. [b]RSD, relative standard deviation of the average concentration.

### 3.3.3 Aroma Recombination and Omission Experiments

To confirm 28 of those compounds (with FD values of≥ 9) contribution in Chinese Vidal icewine, they were recombined in odorless icewine and compared with the corresponding real wine. As shown in Figure 8, the aroma profile of the icewine reconstitution was similar to the original icewine, but still had some intensity difference with the original icewine on apricot, honey and tropical fruit flavor. The values of them were 5.7, 4.6 and 3.3 in icewine respectively, and 4.9, 4.1, 2.7 in icewine reconstitution respectively.

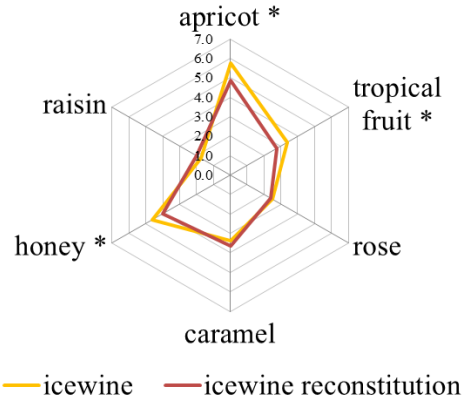


**Figure 8** Aroma profile analysis of Chinese Vidal icewine and the complete reconstitution icewine model. The difference between icewine reconstitution and icewine was analyzed using a paired t-test (*t.test* function). The significance of the difference for each aroma attribute is marked in the figure (*: $p$ <0.05).

To achieve investigation of these compounds contributions, 7 aroma omission models were prepared based on their aroma descriptions (Table 8). Each of the omission models was presented in comparison with the complete model, using a triangle test. The result showed esters were one of the key groups for icewine. Missing these esters, all of assessors could detect the difference, indicating their highly significant influence ($p$≤ 0.001). Icewine recombination without $\beta$-damascenone also could be simple pointed out by all of assessors. Group $M_2$, mainly constituted of terpenes, aromatics, was also significantly ($p$≤ 0.01) infected the models perception. As well as the group $M_5$ (methional only) and group $M_7$ were determined with significant difference ($p$≤ 0.01). $M_3$ and $M_6$ also had little effects ($p$≤ 0.05) on icewine profile; these groups were lactones, ketones, alcohols and phenols. Omission tests indicated that $\beta$-damascenone, methional, furaneol, and some of esters, aromatics and terpenes given in Table 8 were important and contributed a lot to icewine aroma profile.

**Table 8** Omission Tests from the Icewine Complete Models

| No. | flavor | omitted compounds | correct number in all | significance |
|---|---|---|---|---|
| $M_1$ | fruity | ethyl octanoate<br>ethyl hexanoate<br>ethyl isovalerate<br>ethyl butanoate<br>ethyl isobutanoate<br>ethyl 2-methylbutanoate<br>isoamyl acetate<br>ethyl valerate<br>ethyl acetate | 12/12 | *** |
| $M_2$ | floral | *cis*-rose oxide<br>geraniol<br>phenylethyl alcohol<br>*β*-linalool<br>phenethyl acetate | 9/12 | ** |
| $M_3$ | caramel | 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol)<br>4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) | 8/12 | * |
| $M_4$ | honey | *β*-damascenone | 12/12 | *** |
| $M_5$ | cooked potato | 3-(methylthio)-1-propanal (methional) | 9/12 | ** |
| $M_6$ | apricot | *γ*-decalactone<br>*γ*-undecalactone | 8/12 | * |
| $M_7$ | others | guaiacol<br>vinyl guaiacol<br>3-methyl-1-butanol<br>1-heptanol<br>2,3-butanedione<br>1-hexanol<br>1-octen-3-ol<br>1-octen-3-one | 10/12 | ** |

‘*’, ‘**’, and ‘***’ indicated significance at $p < 0.05$, 0.01, and 0.001, respectively

To further explore the aroma contribution of certain compounds to the specific aroma characteristic, descriptive analysis was used. *β*-Damascenone, furaneol and methional omission models were prepared by omitting single of them from the complete reconstitution. *β*-Damascenone was chosen because of its highest FD value, super high OAVs and its importance proved by omission tests. Besides, observation found in red wine showed that although *β*-damascenone has a very low detection threshold and a highest FD in AEDA, it possibly acted as an enhancer of fruity aromas rather than directly affect red wine aroma (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2007). Furaneol and methional were selected

because they were first observed as important odorants in icewine. As shown in Figure 9, compared with the complete icewine reconstitution, without *β*-damascenone was evaluated with a very highly significant difference on apricot, tropical fruit and honey, resulting to lose every icewine typical flavor. Furaneol and methional also showed significant effects on icewine character, especially on apricot, caramel and topical fruit characteristics. It was implied that these two compounds were important to Chinese Vidal icewine.

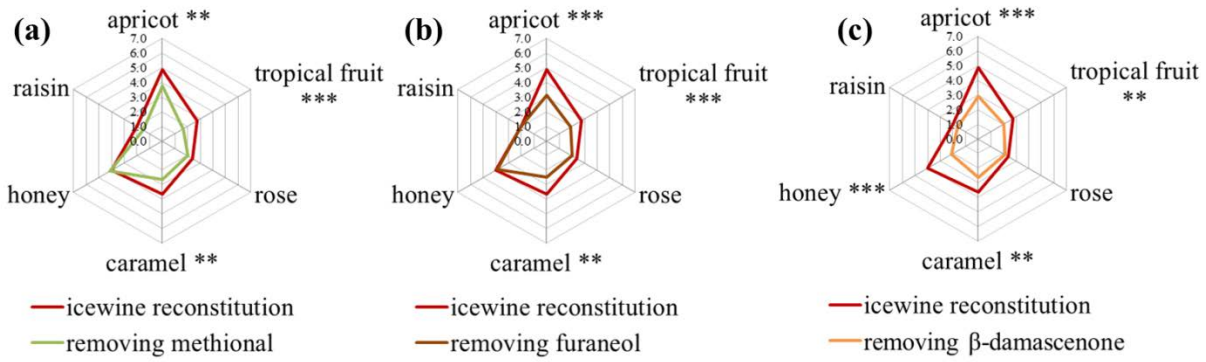


**Figure 9** Aroma profile analysis of the complete reconstitution icewine model and omission models. The difference between icewine reconstitution and each of the omission samples was analyzed using a paired t-test (*t.test* function). The significance of the difference for each aroma attribute is marked in the figure (*: $p$ <0.05, **: $p$ <0.01, ***: $p$ <0.001). **(a)** Aroma profile analysis of the complete reconstitution icewine model and the model removing methional. **(b)** Aroma profile analysis of the complete reconstitution icewine model and the model removing furanel. **(c)** Aroma profile analysis of the complete reconstitution icewine model and the model removing *β*-damascenone.

## 3.4 Conclusions

This study gave a preliminary understanding of odor-active compounds in Chinese Vidal icewine. These odor-active compounds were screened from a volatile extract by GC-O analysis on both polar and nonpolar columns and further identified by GC-MS. The sensory impact of odor-active compounds was ranked by FD value which was obtained from AEDA. In three ranks of Vidal icewines, 59 odor-active aroma compounds were identified, and *β*-damascenone (FD≥ 2187) was determined as the most important aroma compounds in all ranks, followed by 3-methyl-1-butanol, furaneol, ethyl 2-methylbutanoate, ethyl isobutanoate, methional, *cis*-rose oxide, etc. Methional and furaneol were first observed as important odorants in Vidal icewine. To verify the contribution of these aroma compounds, 28 odorants (FD≥ 9) were further quantitated for aroma reconstitution and omission tests. Aroma recombination experiments revealed a good similarity containing the 28 important aromas. Omission tests corroborated the significant contribution of *β*-damascenone and the entire group of esters. Besides, furaneol and methional also had significant effects on icewine character, especially on apricot, caramel, and tropical fruit characteristics. However, there were still subtle differences between aroma reconstruction based on identified key odorants and the original wine, which indicates some limitations in this study. The first limitation may come from the qualitative and quantitative analysis in the studies and the second limitation may come from the neglecting of the contribution of some compounds with subthreshold or low FD or OAV that could be highly influential once embedded in the aroma buffer.

# Chapter IV The Contribution of Aroma Compounds in Icewine Considering Odor Mixture-Induced Interactions

**Related to the publication**

Ma, Y., Béno, N., Tang, K, Li, Y. Y., Simon, M., Xu, Y., & Thomas-Danguin, T. Assessing the contribution of odor-active compounds in icewine considering odor mixture-induced interactions through gas chromatography-olfactometry and Olfactoscan. (submitted)

## Abstract

The sensory impact of odor-active compounds on icewine aroma could be influenced by perceptual interactions with other odor-active compounds. The aim of this study was to evaluate the contribution of odor-active compounds found in icewine considering mixture-induced interactions. By comparing the impact of key odorants detected in icewine following a gas chromatography-olfactometry (GC-O) approach with an Olfactoscan-based methodology using a background odor of icewine, 69 odor zones were detected. The candidate odor-active compounds were identified by comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC-TOFMS). The results revealed that icewine background odor could exert masking or synergy on key odorants when they are considered in the complex wine aroma buffer. Several compounds can induce qualitative changes in the overall wine aroma. This study underlined the efficiency of Olfactoscan-like approaches to screen for the real impact of key odorants and to pinpoint specific compounds that could be highly influential once embedded in the aroma buffer.

**Keywords:** key odorants, Olfactometer, background odor, perceptual interactions

## 4.1 Introduction

Wine flavor is built mostly on the basis of interactions between taste and odor processing (Small & Prescott, 2005), with a major contribution of the numerous odor-active compounds found in wine (Polášková et al., 2008). These odor-active compounds can be screened from a huge body of wine volatiles by gas chromatography-olfactometry (GC-O; Dunkel et al., 2014) and further identified using a variety of separation and spectroscopic techniques, such as comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC-TOFMS; Lyu, Ma, Xu, Nie, & Tang, 2019). The sensory impact of odor-active compounds can be ranked by different GC-O procedures, such as Aroma Extract Dilution Analysis (AEDA; Schieberle, 1995) and Detection Frequency analysis (DF; Pollien et al., 1997). Strong correlations were found between AEDA and DF (Pollien et al., 1997), while DF was reported to be more rapid and more repeatable than AEDA (Delahunty, Eyres, & Dufour, 2006). Although these GC-O procedures help reveal the most intense odor-active compounds when isolated, their actual sensory impact could be influenced not only by interactions with nonvolatile compounds of the wine matrix (Sáenz-Navajas et al., 2010) but also perceptual interactions induced by the olfactory processing of the mixture of odor-active compounds (Thomas-Danguin et al., 2014).

Perceptual interactions between odorants have been observed in wines and other alcoholic beverages. Esters have been shown to play a crucial role in berry fruit odor notes (Escudero et al., 2007) but also to mask or enhance fruity and floral notes at various levels in model wine recombination (Lytra et al., 2013) and to induce synergistic effects on the overall aroma perception of Chinese cherry wines (Niu et al., 2019). Synergistic effects induced by aldehydes such as benzaldehyde, furfural, and vanillin were observed in a *Huangjiu* aroma reconstitution (Yu et al., 2020). Individual *γ*-lactones were unlikely to be key aroma compounds, but combinations of some *γ*-lactones might act additively or synergistically to contribute to the ‘apricot’ aroma of white wine (Siebert, Barker, et al., 2018). Ethylphenols had a masking effect on wine fruity notes even at subliminal concentrations (Tempere et al., 2016). Monoterpenes such as linalool were found to influence the fruity aroma of Pinot Gris wine (Tomasino, Song, & Fuentes, 2020). Furthermore, the complex mixture of the most common wine odor-active compounds, such as ethyl esters, fusel alcohols, volatile phenols, have been suggested to be able to exert an aroma-buffering effect that had both the ability to make unnoticeable the omission of one of its components or the addition of many single odorants, particularly those with fruity characteristics (V. Ferreira, 2010).

Because of the critical impact of perceptual interactions on wine aroma perception, the actual contribution of odor-active compounds should be systematically checked by reconstitution, addition or omission procedures (Grosch, 2001). Nevertheless, the compounds tested in these procedures have usually been selected on the basis of GC-O results that tend to highlight only those single compounds at a concentration above the detection threshold, thus preventing the contribution of subthreshold compounds (Atanasova, Thomas-Danguin, Langlois, et al., 2005; D. Ryan et al., 2008) or other mixture-induced perceptual effects. New methods, such as the Olfactoscan (Béno et al., 2015; Burseg & de Jong, 2009; Thomsen et al.,

2017), OASIS (Hattori, Takagaki, & Fujimori, 2003), InnOscent (Villiere, Le Roy, Fillonneau, & Prost, 2018), and Gas Chromatography-Pedestal Olfactometry (GC-PO) (R. C. Williams, Sartre, Parisot, Kurtz, & Acree, 2009), have been developed to overcome this deficiency. The Olfactoscan and GC-PO techniques enable simultaneous stimulation with a constant background odor (the pedestal) and separated odors eluted from the gas chromatograph of a GC-O system. Thus, it is possible to apply these techniques to evaluate the contribution of each candidate key aroma compound within the aroma buffer of wines.

Icewine is an intensely sweet wine made from grapes naturally frozen on the vine at temperatures below or equal to –7°C. The icewine grape undergoes a unique dehydration process and freeze-thaw cycles, and its must for icewine making, which is pressed from frozen grapes, is a concentrated grape juice with more sugars, acids, and other dissolved solids, resulting in slower-than-normal fermentation. These different winemaking procedures lead to a unique aroma characteristic of icewine (Ma, Xu, & Tang, 2021). The typical aroma of icewine has been described as honey, tropical fruit, apricot, caramel, raisin, nutty and floral (Huang et al., 2018; Nurgel et al., 2004), and more than 80 odor-active compounds were detected by GC-O from different grape varieties (Bowen & Reynolds, 2012; Lan et al., 2019; Ma et al., 2017). The contribution of these odorants was evaluated by comparing the dilution factors (FDs) obtained from AEDA and OAV calculated from odor threshold data and quantification, and the contribution of the most impactful compounds were verified by recombination studies in icewine mixtures (Lan et al., 2019; Ma et al., 2017). However, the research haven’t considered the perceptual impact of the wine complex aromatic buffer which could modify the contribution of some compounds especially the compounds with subthreshold or low FD or OAV.

The aim of this study was to establish a method based on the Olfactoscan technique to evaluate the contribution of odor-active compounds in icewine considering complex odorant mixture-induced effects. We especially compared the impact of odorants detected in icewine using the classical GC-O approach with those identified following Olfactoscan analysis using the icewine odor as the background odor. The results should help reconsider the key status of several odor-active compounds and reveal new compounds, initially considered minor, on the global odor profile of icewine.

## 4.2 Materials and Methods

### 4.2.1 Samples

Commercial icewine was purchased from ChangYu Winery (Yantai, Shandong Province, China). This icewine was made from Vidal grapes harvested in 2019 from the Huanren region (Liaoning Province, China), and its quality meets the standards of the Vintners Quality Alliance system. This icewine was chosen because the Huanren region dominates the major production of icewine in China, and it was selected by wine experts to ensure the presence of typical wine styles in this region. All samples were stored horizontally at 11 °C in the dark before use.

### 4.2.2 Chemicals

Absolute ethanol (≥99.8%, GC grade), dichloromethane (≥99.8%, GC grade), and methanol (≥99.9%, GC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Analytical-grade anhydrous sodium sulfate (for draining trace water after solid-phase extraction) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Some analytical standards, with at least 95% purity, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used for odor identification (see Table 10).

### 4.2.3 Aroma Extraction Methods

Solid-phase extraction (SPE) was used to extract volatile compounds following a procedure modified from the one we conducted previously (Ma et al., 2017). Briefly, the extraction tube (LiChrolut ® EN, Merck; 500 mg of phase) was first rinsed with 10 mL of dichloromethane, then 10 mL of methanol and 10 mL of a water-ethanol mixture (11%, ethanol by volume). Then, 100 mL of sample was filtered through the tube at a flow rate of 1 mL/min. Then, the column was rinsed with 20 mL of ultrapure water to remove sugars, pigment, or other low-molecular-weight polar compounds, and then the column was dried under vacuum before eluting the sorbent. To obtain the icewine aroma extract, 10 mL of dichloromethane was used to elute organic compounds from the extraction tube, and anhydrous sodium sulfate was added to the eluate to remove trace water. Finally, a nitrogen stream was used to concentrate the eluate to a final volume of 0.25 mL for GC-O or Olfactoscan analysis.

### 4.2.4 Gas Chromatography−Olfactometry (GC-O) and Olfactoscan Analysis Conditions

GC-O and Olfactoscan analyses were conducted on an HP 6890A GC (Agilent Technologies, Santa Clara, CA, USA) coupled to a flame ionization detector (FID) and an olfactory detection port (ODP). Both analyses used a dynamic dilution olfactometer (OM4/b, Burghart, Wedel, Germany), in which the outlet was connected to the ODP of the GC by a homemade T-piece to provide a stable airflow (Béno et al., 2015; Barba, Beno, Guichard, & Thomas-Danguin, 2018; Burseg & de Jong, 2009).

For each GC-O and Olfactoscan analysis (Figure 10), 1 µL of icewine aroma extract was injected into the split/splitless inlet of the GC (splitless mode). The GC system was equipped with a 30 m×0.25 mm i.d. fused silica capillary column coated with a 0.5-µm layer of polyethylene glycol (DB-Wax, Agilent Technologies); helium was used as a carrier gas at a constant flow rate of 2 mL/min. The column effluent split to the FID and ODP was 1:1. The injector and transfer line temperatures were set at 250°C. The olfactory port was heated at 240°C to prevent the condensation of high boiling point compounds. The oven temperature was held at 50°C for 2 min, increased to 240°C at 6°C/min, and then held at 240°C for 10 min. Following the GC-O configuration, the olfactometer delivered to the ODP moist air at a constant flow of 155 mL/min and stabilized temperature of 20°C, while in the Olfactoscan configuration, the olfactometer delivered a stable icewine odor background (155 mL/min, 20°C). The background odor or moist air would combine with the separated volatile compounds of the icewine extract eluted from the gas chromatograph at the ODP. To generate

the icewine background odor (Olfactoscan configuration) or moist air (GC-O configuration), one of the olfactometer chambers was filled with icewine or the same level of ultrapure water. The icewine or ultrapure water in the chamber was kept at 20°C. The icewine in the olfactometer chamber was continuously renewed with a peristaltic pump (Gilson, Middleton, USA) at 1 mL/min to keep the icewine background odor intensity and quality stable. Nitrogen went through the chamber at a constant flow (155 mL/min) to generate the wine odor or moist air headspace. The flow at the sniffing port was checked before each sniffing session. The quantitative and qualitative chemical stability of the background odor was checked before the beginning of the experiment, with two replications. The quantitative stability was evaluated by monitoring the total volatile content of the wine background odor using a photoionization detector ppbRAE 3000 (RAE, Lyon, France). The results showed that the total volatile content decreased by less than 5% during a 90-min period of monitoring, while the GC run lasted less than 45 min. The qualitative stability was evaluated by comparing the chromatograms of two odor samples, which were collected from the outlet of GC-O at the beginning and at the end of the GC run. The results showed that there was no significant change in the volatile compound profile (chromatogram) between the two sampling times.

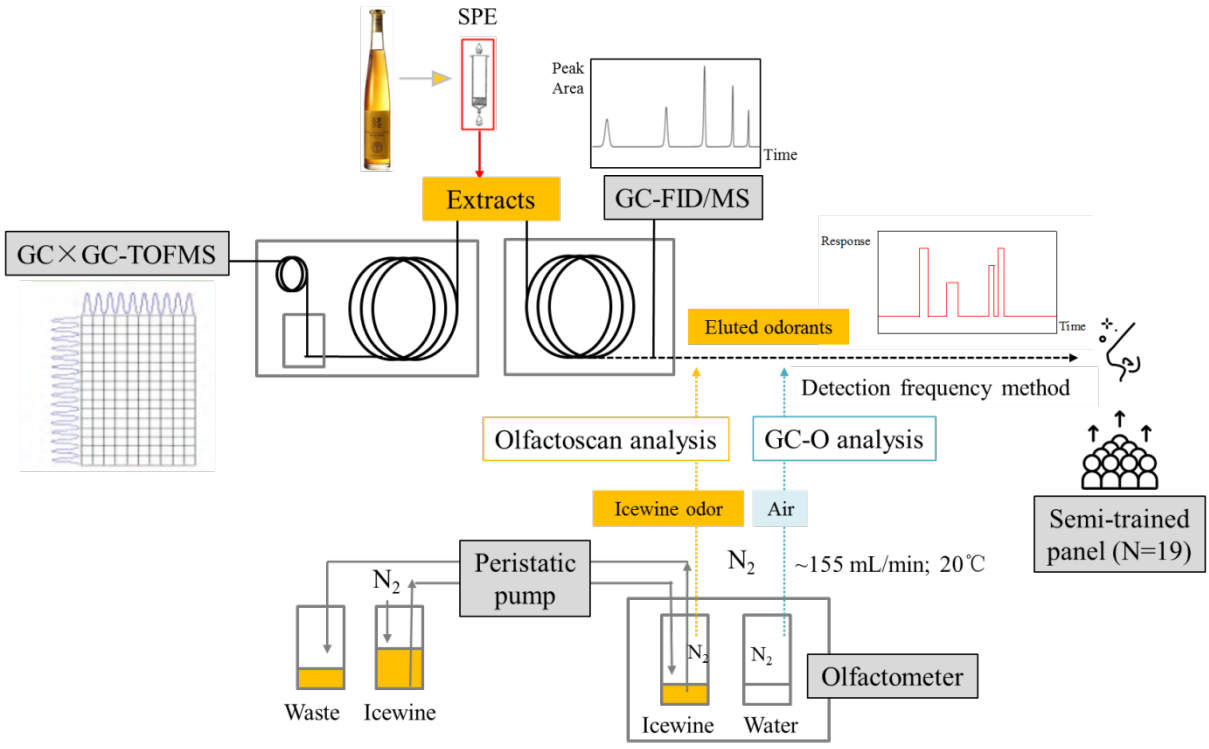


**Figure 10** Schematic representation of the GC-O and Olfactoscan analysis for icewine

### 4.2.5 Subjects

Nineteen healthy subjects (24 to 65 years old) were recruited from the INRAE center and participated in the GC-O/Olfactoscan analyses. These subjects first went through two screening tests to evaluate: (i) their performance in detecting and identifying different odor qualities using the European Test of Olfactory Capabilities (ETOC, Thomas-Danguin et al., 2003), and (ii) their ability to maintain selective attention with time using the Bourdon Test (Boomsma & Bosch, 1978). Before the actual acquisition sessions, they were also asked to

perform one sniffing training session to become familiarized with the GC-O procedure and devices. In this familiarization session, 1 μL of a solution of eight odorants diluted in dichloromethane (Supplementary Table 2) was injected into the GC inlet. Participants were requested not to smoke or eat for 1 h before the session and received one gift for each session.

### 4.2.6 Gas Chromatography-Olfactometry and Olfactoscan Analysis

We conducted three sessions in the formal test. In the first session, we applied traditional gas chromatography-Olfactometry (GCO) analysis to evaluate the contribution of each candidate key aroma compound. In the other two sessions, we applied the Olfactoscan technique to evaluate the contribution of each candidate key aroma compound within the aroma buffer of icewine at high (OLFH, icewine odor at 155 mL/min) and low concentrations (OLFL, icewine odor at 78 mL/min combined with $N_2$ at 77 mL/min). These two levels were determined based on odor intensity as evaluated by 3 experienced internal subjects from the laboratory staff, who tested these levels to ensure that they corresponded to distinct low-to-moderate, and moderate-to-high, but still comfortable, odor intensities. The Detection Frequency (DF) method was selected as the GC-O and Olfactoscan measurement procedure. This method involves untrained assessors and has been shown to be more rapid and more repeatable than AEDA or OSME (Delahunty et al., 2006). Furthermore, this method has been shown to be well correlated with the olfactory intensity generated by given concentrations of the compound (Pollien et al., 1997). During each sniffing, subjects were asked to detect the presence of an odor by pushing a button rapidly as soon as they perceived it and to try to give a descriptor that was as accurate as possible of the perceived odor. The responses were recorded by a Gerstel Olfactory Detection Port Recorder system (Gerstel GmbH & Co., Mülheim, Germany), and audio tracks were recorded via a microphone simultaneously with the response recordings. The duration of each sniffing was 35 min, starting after solvent elution. By comparing the results obtained through GC-O analysis and Olfactoscan, the contribution of each compound to the global odor profile of icewine can be evaluated considering odor mixture-induced effects in icewine.

### 4.2.7 Data Process for Detection Frequency (DF) Method

The data obtained in GC-O and Olfactoscan were processed using the DF method (Pollien et al., 1997) to perform an overall grouping of all the responses given by all the subjects into Odor Zones (OZs) on the basis of their retention time closeness. Because of the background odor in Olfactoscan, it was more difficult to determine OZs; thus, a semiautomatic method was established to define and standardize the OZs between GC-O and Olfactoscan. The GC-O result of the odor cocktail solution (Supplementary Table 2) in the training session and the GC-O result of the icewine extract were used to optimize different parameters of the semiautomatic method to obtain the OZs as precisely as possible. In this semiautomatic method, retention time was first transferred to Kovats retention indices (RIs) by means of *n*-alkane injections ($C_8$–$C_{32}$), and then the detection frequency was calculated from the number of odor events that occurred in a range of 5 RI values. This integration process was applied because of the variability of subject response times. Then, the detection frequency as a function of the RI was analyzed by *R* software (version 4.0.1) using the *findPeak* function of

the *quantmod* package (J. A. Ryan et al., 2020) to determine detection frequency peaks. In this procedure, a noise level of 3 for frequency was chosen as a threshold to consider a significant peak corresponding to an OZ. The obtained OZs were further manually checked in the raw data to evaluate whether any important OZs were missing or duplicated considering the odor descriptors given by the subjects. Finally, OZs from GC-O and Olfactoscan analysis were defined, and each OZ was characterized by: 1) its nasal impact frequency (NIF, %), corresponding to the number or proportion of detection responses that each OZ contained; 2) its odor descriptors given by subjects; and 3) the first, last and average retention indices of the response given by the subjects.

### 4.2.8 Identification of the Impact Compounds

The compounds responsible for OZs were identified by: 1) GC-MS (Ma et al., 2017) and comparing the RI and odor descriptor of a candidate compound with the RI and odor descriptor of its pure standards under the same GC conditions than GC-O; 2) comparing the odor descriptor of a candidate compound with its odor descriptor reported in the database; 3) comparing the experimental RI of a candidate compound with its RI reported in the National Institute of Standards and Technology (NIST) mass spectral library and 4) comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry (GC × GC-TOFMS) analysis.

GC × GC-TOFMS analysis was performed on a LECO Pegasus 4D® GC × GC-TOFMS instrument (LECO Corporation, St. Joseph, MI, USA), basically consisting of an Agilent GC model 7890B, LECO dual nozzle thermal modulator system, and secondary column thermostat connected to a time-of-flight mass spectrometer. A polar column DB-FFAP (60 m × 0.25 mm × 0.25 μm, Agilent Technologies, Palo Alto, CA, USA) was used as the first-dimension ($1^{st}$ D) column, and a medium polarity column Rxi-17Sil MS (1.5 m × 0.25 mm × 0.25 μm, Restek, Bellefonte, PA, USA) was used as the second-dimension ($2^{nd}$ D) column. After optimizing several GC × GC parameters by raising the rate of column temperature and modulation period, the following GC × GC conditions were used. Split injection (1.0 μL) was applied, and the split ratio was set as 5:1. The initial temperature of the primary oven was held at 40°C for 1 min, programmed at 10°C/min to 85°C for 1 min and then raised at 4°C/min to 135°C for 1 min, then at 3°C/min to 210°C for 1 min, and finally programmed at 8°C/min to 240°C for 15 min. The secondary oven temperature was 5°C higher than the primary oven during the chromatographic run. The modulator temperature was offset +15°C from the primary oven, and the modulation time was set at 3 s (0.5 s hot, 1.0 s cold pulses). Helium (99.999%) was used as the carrier gas at a constant flow of 1.0 mL/min. The temperatures of the GC injector and the transfer line were set to 240 °C. The ion source was programmed at 230°C and EI voltage at 70 eV. An electron multiplier at 1400 V, a mass range of 30−400 m/z and an acquisition frequency of 100 spectra/s were programmed. LECO ChromaTOF® Workstation (version 4.44) was used for acquisition control and data processing. Automated peak detection and spectral deconvolution were employed. The baseline signal was drawn just above the noise and the segmented signal-to-noise (S/N) for peak picking was set at 200:1 for a minimum of 2 apexing masses. Within individual chromatograms, subpeaks

in the 2nd dimension were required to meet a S/N≥6 and a minimum spectral similarity match of 650 (65%) to be combined. The reference peak was determined by the unique mass ion and the overall purity and shape of the peak. All chromatograms were compared spectrally with the reference peak chromatogram from the NIST Mass Spectral Library and Wiley Registry™ of Mass Spectral Data Library. The mass spectra of a reference peak with similarity scores greater than 700 were selected as candidate peaks, and its name was assigned to the automated peak detection result. Kovats retention indices (RIs) of peaks were calculated by injection of a reference solution of *n*-alkanes under the same GC × GC conditions ($C_8$−$C_{29}$). The RI of each peak was compared with its RI reported in the NIST library, and peaks with RI differences exceeding 20 units were excluded from the peak identification.

#### 4.2.9 Data Analysis

Statistical analyses were performed with *R* software (version 4.0.1). Principal component analysis (PCA) was carried out on the nasal impact frequency (NIF, %) of every odor descriptor for the high impact odor peaks over the icewine background odor level by using the *prcomp* function of the *tempR* package (Castura, 2016). The PCAs were used to provide a global representation of the trajectory of impact odor peaks in relation to the evolution of odor descriptors based on the first and second principal components. The categorized odor descriptors trajectories in each impact odor peak are illustrated by connecting three different icewine background odor levels. The icewine background odor levels were none for GC-O analysis, low level for Olfactoscan analysis (OLFL) and high level for Olfactoscan analysis (OLFH).

## 4.3 Results and Discussions

### 4.3.1 Odor Zone Defined in GC-O and Olfactoscan Analysis by the Detection Frequency (DF) Method

A total of 2430 odor events were recorded from 19 subjects during all the analysis methods. These events were distributed as follows: GC-O analysis (GCO, 820), Olfactoscan analysis at a low background odor level (OLFL, 870) and Olfactoscan analysis at a high background odor level (OLFH, 740). The raw detection frequency data are reported in Figure 11a for each analysis method.

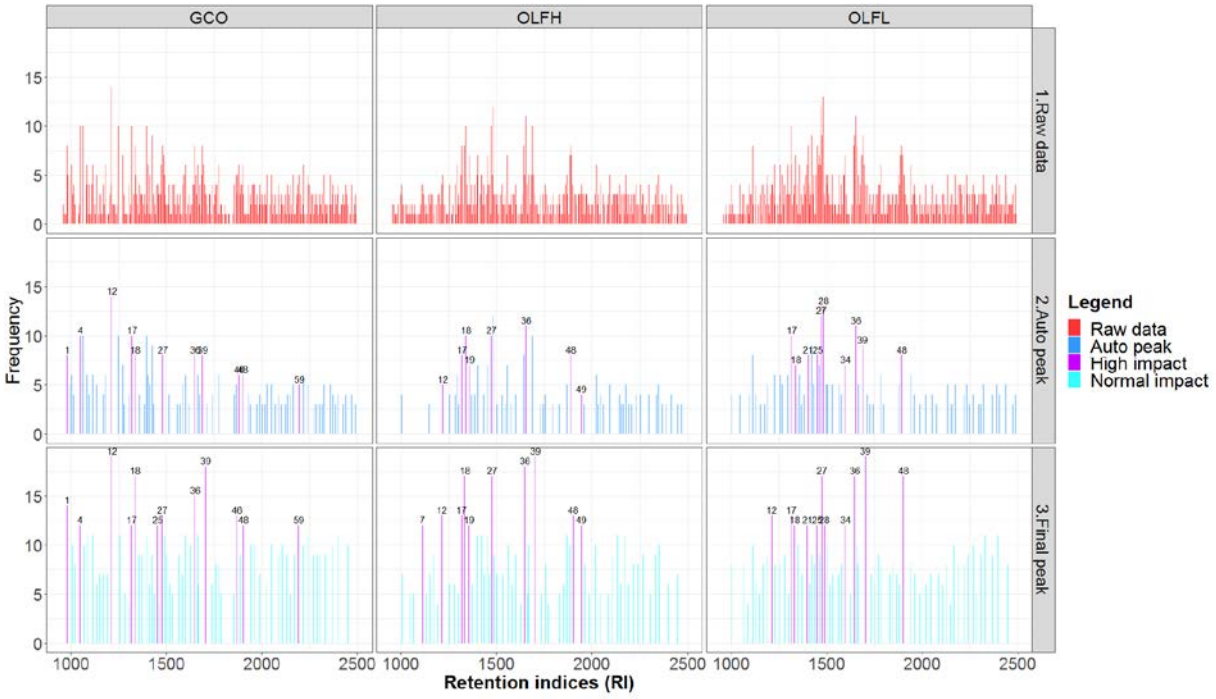


**Figure 11** Results of detection frequency data processing for data obtained in GC-O and Olfactoscan analysis. Graphs were arranged according to analysis methods (column) and data processing methods (row). For each column of graphs: GCO refers to GC-O analysis; OLFH refers to Olfactoscan analysis within the aroma buffer of icewine at a high concentration; OLFL refers to Olfactoscan analysis within the aroma buffer of icewine at a low concentration. The top graphs **(a)** illustrate the detection frequency raw data for each analysis method; the middle graphs **(b)** illustrate the frequency of the highest peaks for odor zones (OZs) based on average RIs, which were defined in a semiautomatic method for each analysis method; and the bottom graphs **(c)** illustrate the final OZs based on average RIs after manual checking. Only OZs with frequency≥ 4 were considered in the final OZ data, and the OZs with frequency≥ 12 were marked as high impact (in purple color); otherwise, they were marked as normal impact (in light blue).

A first observation is that the number of odor events in the OLFH method is lower than in other methods, suggesting a mixture-induced masking effect of the icewine background odor on the detection of odorants. A semiautomatic method was applied to define the odor zones (OZs) in each analysis condition. First, an automatic peak detection function led to the identification of 75 OZs in GCO, 65 OZs in OLFL and 56 OZs in OLFH. The frequency of the highest peaks for these OZs is illustrated in Figure 11b based on the average RI. The OZs identified following automatic detection were then manually checked to ensure that no important OZs were missing or that duplicated OZs were mistakenly considered. This manual check was conducted for two main reasons. First, there can be coelution of odorants in a narrow RI range so that two different odor events generated by the same subject can be grouped into a single OZ. In that case, the OZs were separated or pooled on the basis of the events RI and odor descriptors. For example, the OZ with an RI range from 1470 to 1500 was manually separated into two OZs (1470–1485 and 1485–1500). Second, there can be an intense odor that might be lasting for a long time so that more than one odor event would be generated by the same subject. Thus, the OZs that had close RIs (± 10) and were described with the same odor descriptor were combined into a single OZ. For example, the two OZs (1345–1355 and 1355–1365) were combined into one OZ (1345–1365). The RI range (± 10)

was selected on the basis of the GC-O analysis of the odor cocktail solution performed in the training session (Supplementary Table 2), which showed that for an intense odor, the RI range can be from 15 to 30. A threshold frequency above or equal to 4, corresponding to a proportion of 20%, was used to remove noise from the results. In previous reports (Barba et al., 2018; Machiels, Istasse, & van Ruth, 2004), various threshold values from 12.5% to 40% were selected as the noise level. In the absence of any clear recommendations and based on the GC-O result of the odor cocktail solution performed in the training session, a threshold of 4 was chosen to avoid excluding too many OZs. After manual checking, a total of 69 OZs were considered from all the analysis methods and distributed as follows: GCO (66), OLFL (65), and OLFH (60). The final OZ data are represented in Figure 11c based on average RIs and reported in Table 9.

**Table 9** Odor Zones (OZs) of Vidal Icewine Defined in GC-O and Olfactoscan Analysis by Detection Frequency (DF) Method

| OZ | Retention indices (RI) | | | NIF(%)[a], n=19 | | | Odor descriptor [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| number | Average | Start | End | GCO | OLFH | OLFL | GCO | OLFH | OLFL |
| 1 | 980 | 970 | 990 | 73.7 | 15.8 | 15.8 | strawberry, strawberry, fruity, potato | honey | change |
| 2 | 1008 | 1000 | 1015 | 52.6 | 36.8 | 42.1 | plastic, solvent | increase, rubber | new odor, nut, plastic |
| 3 | 1020 | 1015 | 1025 | 42.1 | 10.5 | 15.8 | flowery, pineapple | change | cassis |
| 4 | 1048 | 1040 | 1055 | 63.2 | 26.3 | 15.8 | fruity, plastic, solvent | change | fruity |
| 5 | 1068 | 1060 | 1075 | 52.6 | 26.3 | 42.1 | strawberry | fruity increase | prune, strawberry |
| 6 | 1088 | 1080 | 1095 | 57.9 | 15.8 | 21.1 | cabbage, caramel, fruity, orange, solvent | change | change |
| 7 | 1115 | 1105 | 1125 | 57.9 | 63.2 | 52.6 | plastic, nut | alcohol, apple, fruity, plastic | nut, plastic |
| 8 | 1135 | 1130 | 1140 | 31.6 | 26.3 | 31.6 | banana, cabbage | metallic | change |
| 9 | 1153 | 1145 | 1160 | 36.8 | 36.8 | 26.3 | flowery, fruity | flowery, fruity increase | change |
| 10 | 1170 | 1160 | 1180 | 36.8 | 47.4 | 42.1 | banana, caramel, chocolate, fruity, strawberry | flowery, fruity increase, red wine, sour | change |
| 11 | 1193 | 1185 | 1200 | 36.8 | 21.1 | 47.4 | baked, baked vanilla | change | caramel increase, sweet |
| 12 | 1215 | 1200 | 1230 | 100.0 | 68.4 | 68.4 | cheese, flowery, caramel, chocolate, sour | ethanol, fruity increase, increase, strawberry jam | flowery, increase |
| 13 | 1235 | 1230 | 1240 | 15.8 | 15.8 | 42.1 | fruity | change | change |
| 14 | 1255 | 1245 | 1265 | 57.9 | 31.6 | 42.1 | fruity, strawberry | fruity increase | flowery increase |
| 15 | 1283 | 1275 | 1290 | 26.3 | 31.6 | 47.4 | caramel | alcohol | flowery increase |
| 16 | 1305 | 1300 | 1310 | 21.1 | 26.3 | 26.3 | apple peel, fruity | flowery | nut |
| 17 | 1318 | 1310 | 1325 | 63.2 | 68.4 | 68.4 | mushroom | mushroom, potato | mushroom, fruity |
| 18 | 1335 | 1325 | 1345 | 89.5 | 89.5 | 63.2 | baked cocoa, bread, fruity, nut, roasted nut, sour | curry, fruity, meaty soup, nut roasted | mushroom, new odor, nut, plastic |
| 19 | 1355 | 1345 | 1365 | 47.4 | 63.2 | 52.6 | baked | pine | change |
| 20 | 1373 | 1365 | 1380 | 47.4 | 31.6 | 36.8 | flowery, menthol | cheese, tablet | fruity, rose |

| OZ | Retention indices (RI) | | | NIF(%)[a], n=19 | | | Odor descriptor [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| number | Average | Start | End | GCO | OLFH | OLFL | GCO | OLFH | OLFL |
| 21 | 1400 | 1390 | 1410 | 57.9 | 57.9 | 63.2 | cake, grass, herb | alcohol, passion fruit, plastic, strawberry | grass |
| 22 | 1415 | 1410 | 1420 | 31.6 | 10.5 | 31.6 | fruity | change | rose |
| 23 | 1425 | 1420 | 1430 | 47.4 | 57.9 | 47.4 | mushroom | flowery, fruity, mushroom, plastic | cabbage, nut |
| 24 | 1438 | 1430 | 1445 | 26.3 | 36.8 | 42.1 | solvent | fruity change, increase | change |
| 25 | 1453 | 1445 | 1460 | 63.2 | 57.9 | 63.2 | baked, coffee, coffee | flowery, nut increase, roasted hazelnuts, toast | fruity, nut |
| 26 | 1465 | 1460 | 1470 | 21.1 | 36.8 | 47.4 | unknown | malty, plastic, roasted | fruity, new odor, nut |
| 27 | 1478 | 1470 | 1485 | 68.4 | 89.5 | 89.5 | potato, cooked potato, soy sauce | potato, cooked potato | animal food, cooked potato, potato |
| 28 | 1490 | 1485 | 1495 | 57.9 | 47.4 | 63.2 | animal, curry, sweet | pine | fruity, mint candy, plastic, potato |
| 29 | 1503 | 1495 | 1510 | 47.4 | 36.8 | 52.6 | plastic, coffee, fruity, pine | grapefruit, plastic | acid, another plastic, potato |
| 30 | 1518 | 1510 | 1525 | 31.6 | 15.8 | 31.6 | unknown | sweet | fruity, soy sauce |
| 31 | 1533 | 1525 | 1540 | 26.3 | 36.8 | 36.8 | unknown | animal, soy sauce | bad soy sauce |
| 32 | 1565 | 1555 | 1575 | 47.4 | 52.6 | 36.8 | flowery, fruity with something | fruity, soy sauce increase | hay, increase |
| 33 | 1583 | 1575 | 1590 | 36.8 | 31.6 | 42.1 | caramel, fruity, vanilla | fruity change | change |
| 34 | 1600 | 1590 | 1610 | 57.9 | 47.4 | 63.2 | flowery, animal, mint candy | nut change, peach, red wine | mint candy |
| 35 | 1628 | 1615 | 1640 | 52.6 | 21.1 | 26.3 | bread, cereal, sugar, sweet | change | change |
| 36 | 1650 | 1640 | 1659 | 78.9 | 94.7 | 89.5 | cheese, hay, solvent | cheese, hay | bad odor, flowery, hay |
| 37 | 1665 | 1660 | 1670 | 57.9 | 26.3 | 52.6 | almond, baked cocoa, caramel | animal, smoky | cheese, new odor |
| 38 | 1668 | 1670 | 1685 | 31.6 | 52.6 | 42.1 | herb, solvent acid | flowery, fruity | rose |
| 39 | 1705 | 1685 | 1720 | 94.7 | 100.0 | 100.0 | cheese, acid, bad odor | cheese, sweaty, unpleasant | cheese, new odor, strong sweaty |
| 40 | 1738 | 1725 | 1750 | 31.6 | 26.3 | 36.8 | nut | change | nut |

| OZ | Retention indices (RI) | | | NIF(%)[a], n=19 | | | Odor descriptor [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| number | Average | Start | End | GCO | OLFH | OLFL | GCO | OLFH | OLFL |
| 41 | 1758 | 1750 | 1765 | 42.1 | 42.1 | 31.6 | cereal, cheese, nut | honey increase | caramel increase |
| 42 | 1773 | 1765 | 1780 | 42.1 | 21.1 | 47.4 | alcohol, red fruit, sweet | change | mint, new odor |
| 43 | 1785 | 1780 | 1790 | 26.3 | 15.8 | 42.1 | unknown | change | honey caramel |
| 44 | 1833 | 1825 | 1840 | 10.5 | 26.3 | 36.8 | roasted | increase | caramel change |
| 45 | 1853 | 1845 | 1860 | 26.3 | 31.6 | 42.1 | unknown | citrus, metallic | honey increase, vegetable |
| 46 | 1870 | 1860 | 1880 | 68.4 | 57.9 | 36.8 | fruity, fruity jam, honey | animal | mint cold, new odor, sweet |
| 47 | 1885 | 1880 | 1890 | 47.4 | 52.6 | 31.6 | baked, fruity alcohol, fruity, honey | sweet | mint cold increase |
| 48 | 1903 | 1890 | 1915 | 63.2 | 68.4 | 89.5 | flowery, smoky, solvent, wine, wood | flowery, sweet | honey, plastic, smoky, sweet |
| 49 | 1945 | 1935 | 1955 | 52.6 | 63.2 | 42.1 | fruity, plastic, rose, sweet, wine | flowery, sweet | sweet |
| 50 | 1963 | 1955 | 1970 | 52.6 | 42.1 | 36.8 | alcohol, rose | sweet, wine | prune |
| 51 | 1990 | 1980 | 2000 | 36.8 | 26.3 | 26.3 | honey, fruity alcohol | increase | change |
| 52 | 2023 | 2010 | 2030 | 31.6 | 52.6 | 31.6 | honey | apple, grapefruit, honey, increase, sweet increase | fruity |
| 53 | 2050 | 2040 | 2060 | 52.6 | 26.3 | 36.8 | alcohol, fruity alcohol, fruity, honey with something | apple, apricot | smoky |
| 54 | 2088 | 2080 | 2095 | 47.4 | 26.3 | 36.8 | alcohol, fruity alcohol, red fruit, vegetable | apricot | honey increase |
| 55 | 2108 | 2095 | 2120 | 52.6 | 47.4 | 31.6 | apricot, bread | apricot, fruity, red | change |
| 56 | 2133 | 2120 | 2145 | 47.4 | 57.9 | 42.1 | fruity, vegetable | apricot, mushroom, rotten, sugar | flowery, sweet increase |
| 57 | 2153 | 2145 | 2160 | 26.3 | 31.6 | 21.1 | caramel milk tea, jam | candy, pineapple | sweet increase |
| 58 | 2170 | 2160 | 2185 | 47.4 | 57.9 | 52.6 | baked, bread, caramel milk tea, honey | jam | apricot, fruity, sweet |

| OZ | Retention indices (RI) | | | NIF(%)[a], n=19 | | | Odor descriptor [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| number | Average | Start | End | GCO | OLFH | OLFL | GCO | OLFH | OLFL |
| 59 | 2193 | 2185 | 2205 | 63.2 | 31.6 | 42.1 | candy, caramel milk tea, caramel, cereal, jam | caramel, fruity candy, peach, sugar | apricot |
| 60 | 2220 | 2210 | 2230 | 52.6 | 42.1 | 42.1 | caramel, caramel baked, peach candy, sugar | increase, peach increase, strawberry | soy sauce, sweet |
| 61 | 2243 | 2230 | 2255 | 57.9 | 42.1 | 47.4 | baked caramel, cake, caramel | red fruit, strawberry | fruity candy |
| 62 | 2270 | 2260 | 2280 | 47.4 | 47.4 | 52.6 | cake | increase, peach increase, smoky, strawberry | caramel increase, fruity |
| 63 | 2288 | 2280 | 2295 | 47.4 | 15.8 | 26.3 | baked caramel, bread | peach | flowery, red fruit |
| 64 | 2308 | 2295 | 2320 | 47.4 | 26.3 | 47.4 | caramel, fruity | increase | caramel, fruity |
| 65 | 2333 | 2320 | 2345 | 47.4 | 47.4 | 57.9 | caramel, fruity, vegetable, fruity baked | increase | mango, new odor |
| 66 | 2353 | 2345 | 2360 | 10.5 | 52.6 | 10.5 | fruity | increase, peach, strawberry | change |
| 67 | 2370 | 2360 | 2380 | 52.6 | 15.8 | 47.4 | alcohol, baked sauce, fruity | fruity | new odor, papaya, smoky |
| 68 | 2400 | 2390 | 2410 | 57.9 | 31.6 | 57.9 | caramel, caramel baked, sugar wine | increase | fruity candy increase |
| 69 | 2450 | 2440 | 2460 | 52.6 | 36.8 | 42.1 | baked, spicy, sugar wine | increase, nut | prune |

Compounds listed in the table were ranked by their appearances from 1 to 69, and each OZ was featured by 1) the first, the last and the average RI of the response given by all subjects; 2) [a] Nasal impact frequency (NIF%), corresponding to the proportion of detection response that each OZ contained; 3) [b] Odor descriptors given by subjects, the odor descriptor was ordered by frequency from high to low. The descriptor 'change' was used by the panelists when they qualified an OZ related to a modification (i.e. a 'change') of the background odor, but they did not provide additional descriptors to qualify the 'change'.

### 4.3.2 Peak Identification and Odor-active Compound Contribution in GC-O and Olfactoscan Analysis

To identify the odor-active compounds responsible for each OZ obtained in GC-O and Olfactoscan analysis, GC-MS and GC × GC-TOFMS analyses were conducted. The identification of several compounds was further checked through injection in GC-MS of pure standards under the same GC conditions as in GC-O (marked by 'S' in Table 10). Finally, 57 OZs were associated with 63 compounds identified by GC-MS and GC × GC-TOFMS analysis; there was coelution for 4 OZs. These results were confirmed by injection of pure standards under the same GC conditions (Ma et al., 2017). The OZs that failed to be related to the compounds identified by GC × GC-TOFMS analysis were defined by at least two of the following methods: 1) comparing the RI and odor descriptor of a candidate compound with the RI and odor descriptor of its pure standards under the same GC condition; 2) comparing the odor descriptor of a candidate compound with its odor descriptor reported in The Good Scents Company database; and 3) comparing the experimental RI of a candidate compound with the RI reported in the NIST Mass Spectral Library. The OZ identification results are given in Table 10. Due to different GC conditions in the GC-MS and GC × GC-TOFMS analyses, the RI of several compounds calculated from the detection response obtained in the GC-MS analysis was different from the RI calculated from the GC × GC-TOFMS analysis. To highlight these compounds with different RI but double-checked with the injection of standard compounds, we tagged them with a '*' in Table 10.

**Table 10** Identification of Odor Zones (OZs) in Vidal Icewine by GC-O, GC-MS and GC × GC-TOFMS

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds[d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 1 | 980 | 961 | 955 | ethyl isobutanoate | sweet, ethereal, fruity, alcoholic, fusel, rummy | MS;RI;O;S | 97-62-1 | 71 |
| 2 | 1008 | 977 | 970 | 2,3-butanedione* | butter, sweet, creamy, pungent, caramel | MS;RI;O;S | 431-03-8 | 86 |
| 3 | 1020 | 1025 | 1015 | isobutyl acetate | sweet, fruity, ethereal, banana, tropical | MS;RI;O;S | 110-19-0 | 43 |
| 4 | 1048 | 1044 | 1028 | ethyl butanoate | fruity, juicy, pineapple, cognac | MS;RI;O;S | 105-54-4 | 71 |
| 5 | 1068 | 1062 | 1050 | ethyl 2-methylbutanoate | sharp, sweet, green, apple, fruity | MS;RI;O;S | 7452-79-1 | 102 |
| 6 | 1088 | 1067 | 1060 | ethyl isovalerate | fruity, sweet, apple, pineapple | MS;RI;O;S | 108-64-5 | 88 |
| 7 | 1115 | 1088 | 1099 | 2-methyl-1-propanol | ethereal, winey, cortex | MS;TOFMS;RI;O;S | 78-83-1 | 42 |
| 8 | 1135 | 1127 | 1117 | isoamyl acetate | sweet, fruity, banana, solvent | MS;TOFMS;RI;O;S | 123-92-2 | 70 |
| 9 | 1153 | 1140 | 1133 | ethyl valerate | sweet, fruity, apple, pineapple, green, tropical | MS;TOFMS;RI;O;S | 539-82-2 | 88 |
| 10 | 1170 | 1181 | 1176 | pentyl acetate | ethereal, fruity, banana, pear, banana, apple | TOFMS;RI;O | 628-63-7 | 61 |
| 11 | 1193 | 1196 | 1183 | 2-heptanone | fruity, spicy, sweet, herbal, coconut, woody | TOFMS;RI;O | 110-43-0 | 58 |
| 12 | 1215 | 1209 | 1205 | 3-methyl-1-butanol | fuel oil, alcoholic, whiskey, fruity, banana | MS;TOFMS;RI;O;S | 123-51-3 | 39 |
| 13 | 1235 | 1220 | 1216 | 2-hexanol | chemical, winey, fruity, fatty, terpene, cauliflower | TOFMS;RI | 626-93-7 | 45 |
| 13 | 1235 | 1237 | 1235 | 2-pentylfuran | fruity, green, earthy, beany, vegetable, metallic | TOFMS;RI | 3777-69-3 | 81 |
| 14 | 1255 | 1239 | 1220 | ethyl hexanoate | sweet, fruity, pineapple, waxy, green, banana | MS;TOFMS;RI;O;S | 123-66-0 | 88 |

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds [d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 15 | 1283 | 1280 | 1267 | ethyl pyruvate | ether, fruity, sweet, sharp, rum, vegetable, caramel | TOFMS;RI;O | 617-35-6 | 43 |
| 16 | 1305 | 1297 | 1285 | 2-octanone | earthy, weedy, natural, woody, herbal | TOFMS;RI | 111-13-7 | 58 |
| 17 | 1318 | 1314 | 1313 | 1-octen-3-one | herbal, mushroom, earthy, musty, dirty | TOFMS;RI;O;S | 4312-99-6 | 70 |
| 18 | 1335 | | 1331 | 2-acetyl-1-pyrroline | popcorn, toasted, grain, malty | RI;O | 85213-22-5 | |
| 19 | 1355 | 1351 | 1360 | 1-hexanol | ethereal, fuel oil, fruity, alcoholic, sweet, green | MS;TOFMS;RI;O;S | 111-27-3 | 43 |
| 20 | 1373 | 1363 | 1358 | *cis*-rose oxide | green, red rose, spicy, fresh, geranium | MS;TOFMS;RI;O;S | 16409-43-1 | 139 |
| 21 | 1400 | 1384 | 1386 | (*Z*)-3-hexen-1-ol | fresh, green, cut grass, foliage, vegetable, herbal, oily | MS;TOFMS;RI;O;S | 928-96-1 | 67 |
| 22 | 1415 | 1439 | 1436 | ethyl octanoate | fruity, wine, waxy, sweet, apricot, banana, brandy, pear | MS;TOFMS;RI;O;S | 106-32-1 | 88 |
| 23 | 1425 | 1447 | 1447 | 1-octen-3-ol* | mushroom, earthy, green, oily, fungal, raw chicken | MS;TOFMS;RI;O;S | 3391-86-4 | 57 |
| 24 | 1438 | 1448 | 1451 | linalyl oxide | earthy, floral, sweet, woody | MS;TOFMS;RI;O | 5989-33-3 | 59 |
| 25 | 1453 | | 1449 | 2-ethyl-3,5-dimethylpyrazine | burnt almonds, roasted nuts, coffee | RI;O | 13925-07-0 | |
| 26 | 1465 | 1453 | 1460 | 1-heptanol | musty, leafy, violet, herbal, green, sweet, woody, peony | MS;TOFMS;RI;S | 111-70-6 | 70 |
| 27 | 1478 | 1476 | 1458 | methional | musty, potato, tomato, earthy, vegetable, creamy | TOFMS;RI;O;S | 3268-49-3 | 47 |
| 28 | 1490 | 1480 | 1479 | nerol oxide | green, weedy, cortex, herbal, diphenyl, oxide, narcissus, celery | MS;TOFMS;RI;O | 1786-08-9 | 68 |
| 29 | 1503 | 1489 | 1484 | 2-ethylhexanol | citrus, fresh, floral, oily, sweet | MS;TOFMS;RI;O | 104-76-7 | 57 |

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds [d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 30 | 1518 | 1523 | 1524 | ethyl 3-hydroxybutanoate | fruity, green, grape, tropical, apple skin | MS;TOFMS;RI | 5405-41-4 | 88 |
| 31 | 1533 | 1544 | 1545 | ethyl 2-hydroxy-4-methylvalerate | fresh blackberry | TOFMS;RI | 10348-47-7 | 87 |
| 32 | 1565 | 1553 | 1537 | *β*-linalool | citrus, floral, sweet, bois de rose, woody, green, blueberry | MS;TOFMS;RI;O;S | 78-70-6 | 71 |
| 33 | 1583 | | 1561 | ethyl 3-methylthiopropionate | sulfur, metallic, pineapple, fruity, ripe pulpy tomato | RI;O | 13327-56-5 | |
| 34 | 1600 | 1613 | 1620 | hotrienol | sweet, tropical, fennel, ginger | MS;TOFMS;RI;O | 29957-43-5 | 71 |
| 35 | 1628 | 1662 | 1648 | benzeneacetaldehyde | green, sweet, floral, hyacinth, clover, honey, cocoa | TOFMS;RI;O | 122-78-1 | 91 |
| 36 | 1650 | 1670 | 1662 | 2-methylbutanoic acid | pungent, acid, cheese | MS;TOFMS;RI;O;S | 116-53-0 | 74 |
| 36 | 1650 | 1673 | 1680 | acetophenone | sweet, pungent, hawthorn, mimosa, almond, acacia, chemical | TOFMS;RI;O | 98-86-2 | 105 |
| 37 | 1665 | | 1660 | 2-acetyl thiazole | earthy | RI;O | 932-16-1 | |
| 38 | 1668 | 1679 | 1687 | diethyl succinate | mild, fruity, cooked apple | TOFMS;RI;O;S | 123-25-1 | 101 |
| 39 | 1705 | | 1665 | 3-methylbutanoic acid* | sour, stinky, feet, sweaty, cheese, tropical | MS;RI;O;S | 503-74-2 | |
| 40 | 1738 | 1724 | 1738 | 3-(methylthio)-1-propanol | sulfurous, onion, sweet, soup, vegetable | TOFMS;RI;O;S | 505-10-2 | 106 |
| 41 | 1758 | 1741 | 1732 | linalool oxide (trans-pyranoid) | woody | MS;TOFMS;RI;O | 39028-58-5 | 68 |
| 42 | 1773 | 1797 | 1779 | ethyl phenylacetate* | sweet, floral, honey, rose, balsam, cocoa | MS;TOFMS;RI;O;S | 101-97-3 | 91 |
| 43 | 1785 | 1798 | 1794 | 1-(4-methylphenyl)ethanone | hawthorn, sweet, mimosa, cherry | TOFMS;RI | 122-00-9 | 119 |

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds[d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 44 | 1833 | 1823 | 1817 | *γ*-heptalactone | sweet, coconut, nutty, caramel, hay | MS;TOFMS;RI;O | 105-21-5 | 85 |
| 45 | 1853 | 1827 | 1829 | phenethyl acetate* | floral, rose, sweet, honey, fruity, tropical | MS;TOFMS;RI;S | 103-45-7 | 104 |
| 46 | 1870 | 1832 | 1840 | *β*-damascenone* | natural sweet, fruity, rose, plum, grape, raspberry, sugar | MS;TOFMS;RI;O;S | 23696-85-7 | 69 |
| 46 | 1870 | 1845 | 1853 | 3-mercaptohexanol | sulfurous, fruity, tropical | TOFMS;RI;O;S | 51755-83-0 | 100 |
| 46 | 1870 | 1848 | 1840 | geraniol* | sweet, floral, fruity, rose, waxy, citrus | MS;TOFMS;RI;O;S | 106-24-1 | 69 |
| 47 | 1885 | 1854 | 1857 | *p*-cymen-8-ol | sweet, fruity, cherry, floral, camphor | MS;TOFMS;RI;O | 1197-01-9 | 135 |
| 48 | 1903 | 1871 | 1859 | guaiacol* | phenolic, smoke, spice, vanilla, woody | MS;TOFMS;RI;O;S | 90-05-1 | 109 |
| 49 | 1945 | 1918 | 1925 | phenylethyl alcohol* | floral, rose, dried rose, flower, rose water | MS;TOFMS;RI;O;S | 60-12-8 | 92 |
| 50 | 1963 | 1936 | 1923 | *γ*-octalactone | sweet, coconut, waxy, creamy, dairy, fatty | MS;TOFMS;RI;O | 104-50-7 | 85 |
| 51 | 1990 | 1988 | 1988 | *δ*-octalactone | sweet, fatty, coconut, tropical, dairy | MS;TOFMS;RI;O | 698-76-0 | 99 |
| 52 | 2023 | 2013 | 2008 | phenol | phenolic, plastic, rubber | MS;TOFMS;RI;O | 108-95-2 | 94 |
| 53 | 2050 | 2047 | 2042 | *γ*-nonalactone | coconut, creamy, waxy, sweet, buttery, oily | MS;TOFMS;RI;O | 104-61-0 | 85 |
| 54 | 2088 | 2066 | 2056 | 4-hydroxy-2,5-dimethyl -3(2H)-furanone (furaneol)* | sweet, cotton candy, caramel, strawberry, sugar | TOFMS;RI;O;S | 3658-77-3 | 85 |
| 55 | 2108 | | 2031 | 4-ethylguaiacol | spicy, smoky, bacon, phenolic, clove | MS;RI;S | 2785-89-9 | |

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds [d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 56 | 2133 | 2146 | 2127 | ethyl cinnamate | sweet, balsam, fruity, spicy, powdery, berry, plum | MS;TOFMS;RI;O;S | 103-36-6 | 131 |
| 57 | 2153 | 2091 | 2088 | 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (homofuraneol) | sweet, caramel, bready, maple, brown sugar, burnt | MS;TOFMS;RI;O;S | 27538-09-6 | 43 |
| 58 | 2170 | 2161 | 2144 | $\gamma$-decalactone | fresh, oily, waxy, peach, coconut, buttery, sweet | MS;TOFMS;RI;O;S | 706-14-9 | 85 |
| 59 | 2193 | 2178 | 2167 | eugenol | sweet, spicy, clove, woody | MS;TOFMS;RI;O;S | 97-53-0 | 164 |
| 60 | 2220 | 2208 | 2203 | 4-vinylguaiacol | dry, woody, fresh, amber, cedar, roasted, peanut | MS;TOFMS;RI;O;S | 7786-61-0 | 135 |
| 61 | 2243 | 2213 | 2208 | $\delta$-decalactone | fresh, sweet, oily, coconut, fruity, peach, creamy, dairy | MS;TOFMS;RI;O | 705-86-2 | 99 |
| 62 | 2270 | 2216 | | thymol | herbal, thyme, phenolic, medicinal, camphor | TOFMS;RI;O | 89-83-8 | 115 |
| 63 | 2288 | 2276 | 2270 | $\gamma$-undecalactone | fruity, peach, creamy, fatty, apricot, coconut | TOFMS;RI;O | 104-67-6 | 85 |
| 64 | 2308 | 2281 | 2296 | syringol | smoky, phenolic, balsamic, bacon, powdery, woody | TOFMS;RI;O | 91-10-1 | 154 |
| 65 | 2333 | 2319 | 2311 | 4-methyl-5-thiazoleethanol | fatty, cooked beef juice | TOFMS;RI | 137-00-8 | 112 |
| 66 | 2353 | 2333 | 2336 | 9-decenoic acid | waxy, green, fruity, fatty, soapy | TOFMS;RI | 14436-32-9 | 69 |
| 66 | 2353 | 2340 | 2347 | geranic acid | dry, weedy, acidic, green, moldy, feet, woody | TOFMS;RI | 459-80-3 | 100 |
| 66 | 2353 | 2359 | 2327 | isophytol | mild, floral, herbal, green | TOFMS;RI | 505-32-8 | 71 |
| 67 | 2370 | 2363 | 2350 | isoeugenol | sweet, spicy, clove, woody, carnation, floral | MS;TOFMS;RI;O | 97-54-1 | 164 |
| 68 | 2400 | 2415 | 2415 | $\gamma$-dodecalactone | fatty, peach, sweet, metallic, fruity | MS;TOFMS;RI;O | 2305-05-7 | 85 |

| OZ number | Retention indices (RI) GCO [a] | TofMS [b] | NIST [c] | Compounds [d] | Odor descriptors [e] | Identification [f] | CAS. | Quantitative mass |
|---|---|---|---|---|---|---|---|---|
| 69 | 2450 | 2447 | 2445 | *δ*-dodecalactone | fresh sweet metallic peach oily coconut buttery | TOFMS;RI | 713-95-1 | 99 |

[a] RI calculated from GC-O and Olfactoscan analysis; [b] RI calculated from GC × GC-TOFMS analysis; [c] RI reported in NIST library on similar column; [d] Comounds tagged with an '*' were found to have different RI calculated from the GCO analysis and from the GC × GC-TOFMS analysis; the identification of these odor zones have been verified by injecting pure standards; [e] descriptors obtained from the database of The Good Scents Company (http://www.thegoodscentscompany.com/); [f] Peak identified by: 1) GC × GC-TOFMS analysis (TOFMS), the first six odorants were not identified by TOFMS due to the setting of solvent delay; 2) GC-MS (MS) and comparing the RI and odor descriptor of a candidate compound with the RI and odor descriptor of its pure standards under the same GC conditions as in GC-O (S); 3) comparing the odor descriptor of a candidate compound with its odor descriptor reported in the database (O); and 4) comparing the experimental RI of a candidate compound with the RI reported in the NIST Mass Spectral Library (RI).

Detection frequency (DF) or nasal impact frequency (NIF, %) was used to evaluate the contribution of OZs identified in icewine by GC-O analysis without background odor (GCO) or Olfactoscan analysis with background odor (OLFH, OLFL). Although the NIF value is not a direct measurement of the perceived odor intensities, it increases with intensity and concentration (Pollien et al., 1997). Therefore, the NIF can be used to compare peak intensities between different compounds. Based on GC-O results of the odor cocktail solution performed in the training session, the compounds with DF≥ 12 or NIF> 60% were considered as high impact compounds; they were marked in purple in Figure 11b and 11c.

There were 12 OZs, 10 OZs, and 11 OZs considered to have a high impact in the GCO, OLFH and OLFL analyses, respectively. Among these OZs, 7 OZs were in common in the three analyses. The compounds associated with these peaks were 3-methyl-1-butanol (peak 12), 3-methylbutanoic acid (peak 39), 2-acetyl-1-pyrroline (peak 18), 2-methylbutanoic acid (peak 36), acetophenone (peak 36), methional (peak 27), 1-octen-3-one (peak 17) and guaiacol (peak 48). For peak 36, there might be two compounds for the OZ since they were eluted at very close RI based on the GC × GC-TOFMS result. Among other high odor impact compounds, 2-ethyl-3,5-dimethylpyrazine (peak 25) was identified in GCO and OLFL analyses with the same NIF (63.2%), but it was detected in OLFH analysis with a lower value (NIF=57.9%). Ethyl isobutanoate (peak 1, NIF=73.7%), geraniol (peak 46, NIF=68.4%), *β*-damascenone (peak 46, NIF=68.4%), 3-mercaptohexanol (peak 46, NIF=68.4%), eugenol (peak 59, NIF=63.2%) and ethyl butanoate (peak 4, NIF=63.2%) were only identified as high impact compounds in GCO. Interestingly, most of these compounds had fruity or sweet-like odors that would likely be masked by the wine background odor in OLF analyses. For peak 46, there might be three compounds for the OZ since they were eluted at very close RI based on the GC × GC-TOFMS result (see Table 10). The high-impact odorants found only in OLFH (phenylethyl alcohol, 2-methyl-1-propanol, 1-hexanol) and OLFL (hotrienol, nerol oxide, (Z)-3-hexen-1-ol) were also detected in GCO analysis, but at lower NIF values (from 47.4% to 57.9%).

Focusing on the GCO method, we compared the odor-active compounds identified by the DF method with the results previously obtained by the AEDA method (Ma et al., 2017). We found that 76% of odor-active compounds with a flavor dilution factor above or equal to 9 in AEDA were well detected by DF analysis with NIF values above or equal to 47.4%, and 21% of odor-active compounds with NIFs from 21.1% to 36.8%. Only one compound, ethyl acetate, was not detected by DF analysis, which can be explained by the fact that this compound was eluted before the solvent and thus, not delivered at the olfactory port in the present study. Compared to AEDA, the DF method detected more OZs, and some of these OZs showed a high contribution, such as peak 1 (ethyl isobutanoate, NIF=73.7%), peak 18 (2-acetyl-1-pyrroline, NIF=89.5%), peak 25 (2-ethyl-3,5-dimethylpyrazine, NIF=63.2%), peak 36 (2-methylbutanoic acid/acetophenone, NIF=78.9%), peak 39 (3-methylbutanoic acid, NIF=94.7%) and peak 59 (eugenol, NIF=63.2%). The identification of these compounds might be due to the difference in the experimental samples between the two studies (same icewine but different vintages) or to the limited number of subjects involved in the AEDA method, namely, from 2 to 4 (Ma et al., 2017). Therefore, the sensitivity, discrimination ability,

risk of inattention and specific anosmia of the sniffers could result in missed peaks (Pollien et al., 1997). Another difference between AEDA and DF concerned peak 46. In AEDA, the flavor dilution factor of this peak was the highest, as large as 2187; however, in the DF method, its NIF was not the highest, only 68.4%. This difference might be explained by subjects' sensitivity, since the AEDA method is based on detection thresholds, and/or by suprathreshold sensitivity as reflected by Steven's power function slope, which can be low, meaning that the increase of odor intensity as a function of concentration is small. Notably, *β*-damascenone was identified as a putative odor-active compound responsible for peak 46. This compound has both a very low detection threshold (0.002 μg/l in water; (Buttery, Teranishi, Flath, & Ling, 1989) and a low Steven's power function slope (V. Ferreira, 2010). Nevertheless, GC × GC-TOFMS analysis indicated that geraniol and 3-mercaptohexanol were also candidate odorants for peak 46 since they were eluted at very close RI.

### 4.3.3 Mixture-induced Effect of Icewine Background Odor on the Detection and Identification of Odor-active Compounds

The mixture-induced effect of icewine background odor on the detection of odor-active compounds was evaluated by comparing the NIF value between GCO analysis (without icewine background odor) and Olfactoscan analysis (with icewine background odor at high, OLFH, and low levels, OLFL). Since a threshold value (20%) was applied to consider significant NIF in the identification of OZs, the same threshold (DF=4) was applied to consider a significant NIF difference between GC-O and Olfactoscan. If an OZ's NIF in Olfactoscan was significantly lower than the NIF in GCO, the icewine background odor induced a masking effect for this OZ. Conversely, if an OZ's NIF in Olfactoscan was significantly higher than the NIF in GCO, the icewine background odor induced a synergy effect for this OZ.

The results showed that with a high level of icewine background odor (OLFH), the NIF value of 18 OZs decreased significantly (from -21.1% to -57.9%), which indicated that these OZ were masked by the icewine odor. The NIF of 4 OZs increased significantly (+21.1% to +42.1%), which indicated a synergy effect of the icewine odor on these OZs. The contrast between OLFH and GCO data is illustrated in Figure 12a, thus highlighting the influence of the icewine odor on each OZ. The compounds associated with the most importantly masked OZs were ethyl isobutanoate (peak 1, NIF decrease in OLFH -57.9%), ethyl isovalerate (peak 6, -42.1%), ethyl butanoate (peak 4, -36.8%), isoeugenol (peak 67, -36.8%), 3-methyl-1-butanol (peak 12, -31.6%), eugenol (peak 59, -31.6%), 2-acetyl thiazole (peak 37, -31.6%), benzeneacetaldehyde (peak 35, -31.6%), *γ*-undecalactone (peak 63, -31.6%) and isobutyl acetate (peak 3, -31.6%). The compounds associated with OZs that benefited from synergy with the icewine odor were methional (peak 27, +21.1%), diethyl succinate (peak 38, +21.1%) and phenol (peak 52, +21.1%). Moreover, peak 66 was considered nonsignificant in GCO since its NIF value was 10.5%, but in OLFH, its NIF was 52.6%. Three compounds, namely, 9-decenoic acid, geranic acid, and isophytol, might be related to this peak based on GC × GC−TOFMS analysis, RIs and odor descriptors.

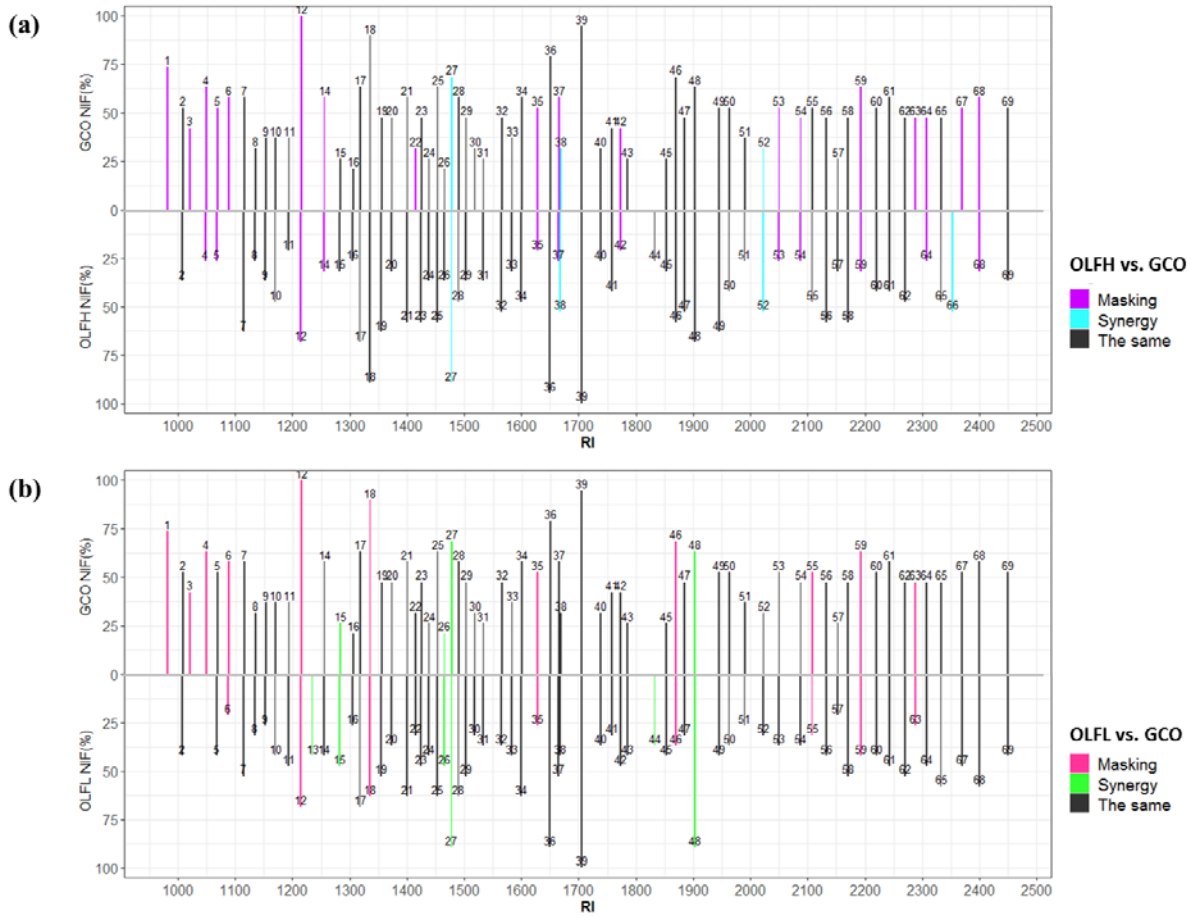


**Figure 12** Nasal impact frequency (NIF, %) comparisons between GC-O and Olfactoscan analysis. An NIF difference above 20% (4/19) was considered a threshold for a significant mixture-induced effect for a peak. **(a)** The NIF difference between GC-O analysis and Olfactoscan analysis within the aroma buffer of icewine at a high concentration. If the NIF for OLFH was significantly lower than the NIF for GCO, a masking effect (in purple color) occurred; if the NIF for OLFH was significantly higher than the NIF for GCO, synergy effect (in light blue color) occurred. **(b)** The NIF difference between GC-O analysis and Olfactoscan analysis within the aroma buffer of icewine at a low concentration.

At a low level of icewine background odor (OLFL), a masking effect occurred for 11 OZs, with a decrease in NIF values compared to GCO in the range of 21.1% to 57.9%. Synergy occurred for 6 OZs with an increase in NIF of 21.1% to 26.3% (Figure 12b). The compounds associated with the OZs that were masked in OLFL were ethyl isobutanoate (peak 1, -57.9%), ethyl butanoate (peak 4, -47.4%), ethyl isovalerate (peak 6, -36.8%), 3-methyl-1-butanol (peak 12, -31.6%), geraniol (peak 46, -31.6%), *β*-damascenone (peak 46, -31.6%) and 3-mercaptohexanol (peak 46, -31.6%). The compounds associated with OZs in synergy with the icewine odor were guaiacol (peak 48, +26.3%), 1-heptanol (peak 26, +26.3%), *γ*-heptalactone (peak 44, +26.3%), ethyl pyruvate (peak 15, +21.1%) and methional (peak 27, +21.1%). Peak 13 was also found to benefit from synergy (+26.3%), and 2 odorants (2-pentylfuran and 2-hexanol) might contribute on the basis of GC × GC−TOFMS analysis, RIs and odor descriptors.

We observed that 8 OZs were masked at both icewine background odor levels (peaks 1, 3, 4, 6, 12, 35, 59, 63; red color Figure 13a) and that 1 OZ was synergized at both levels (methional, peak 27); 39 OZs were not influenced by the background odor regardless of the level (black color Figure 13a). Nevertheless, the results also showed that the mixture-induced

effects caused by the icewine background odor were level-dependent (Figure 13b). Indeed, between OLFH and OLFL, as the concentration of icewine background odor mixture decreased, the DF of 10 OZs increased, while the DF of 6 OZs decreased. In OLFH, 10 OZs were masked only at high concentration (peaks 5, 14, 22, 37, 42, 53, 54, 64, 67, 68, purple color Figure 13a); 2 OZs were synergized only at high concentration (peaks 38, 52; light blue color Figure 13a); 3 OZs were masked at low concentration (peaks 18, 46, 55; rose color Figure 13a); and 5 OZs were synergized at low concentration (peaks 13, 15, 26, 44, 48; light green color Figure 13a). We did not observe any OZ that was masked at one concentration but synergized at the other concentration. This comparison between GCO and Olfactoscan is visualized in Figure 13.

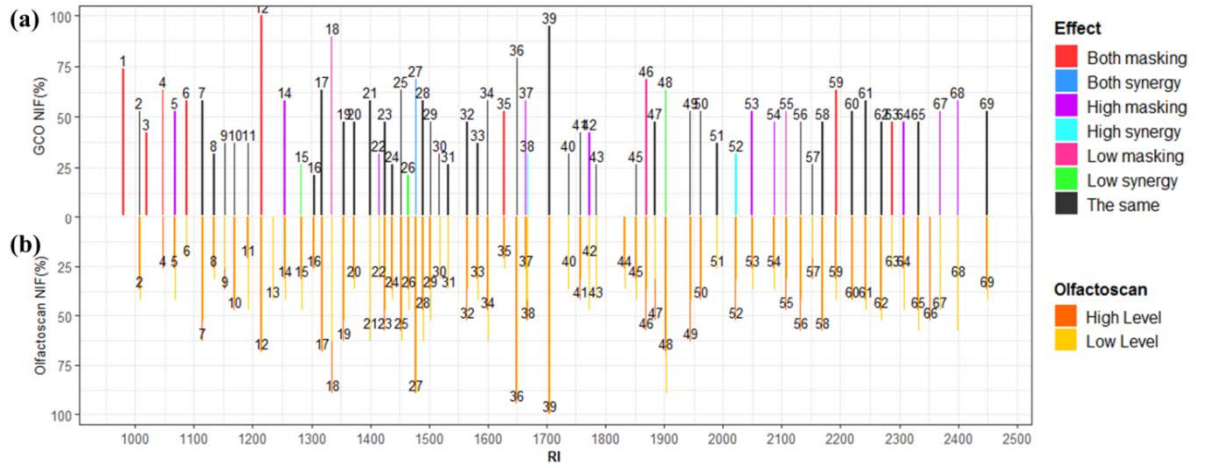


**Figure 13** The NIF difference between GC-O analysis and Olfactoscan analysis at both high and low concentrations. **(a)** The peak of GC-O analysis and the effect occurring within the aroma buffer of icewine for each peak are marked. The effects including masking occurring at both concentrations (in red); synergy occurring at both concentrations (in dark blue); masking occurring at high concentration (in purple); synergy occurring at high concentration (in light blue); masking occurring at low concentration (in rose); synergy occurring at low concentration (in light green); and no significant effect occurring at either concentration (in black). **(b)** Peak of Olfactoscan analysis within the aroma buffer of icewine at high (deep orange) and low (light orange) concentrations. E.g.: For the NIF of peak 11, the aroma buffer of icewine at high level was marked in deep orange and the aroma buffer of icewine at low level was marked in light orange, the NIF at low level (47.4%) is higher than the NIF at high level (21.1%).

In addition to mixture-induced intensity effects such as masking and synergy, the Olfactoscan approach provides cues about the modification of odor quality of odor-active compounds once embedded in the icewine odor. To investigate these odor quality modifications, the descriptors provided by the subjects during GCO and Olfactoscan runs were categorized into 10 categories based on an adapted version of the wine aroma wheel (Supplementary Figure 1) proposed by Noble (Noble et al., 1987). The categories are as follows: caramelized, chemical, earthy, floral, fruity, microbiological, nutty, spicy, vegetative and woody. Two categories of the original wine aroma wheel (pungent and oxidized) were not considered relevant for icewine. When no descriptor was provided by subjects for an odor event, a category ‘not identified’ was used, and when the OZ was not detected, it was categorized as ‘not detected’. Individual responses within GCO, OLFH and OLFL analyses were dispatched in the 10 categories and expressed as percentages. Principal Component

Analysis (PCA) was conducted to follow odor quality modification induced by the background odor for the high impact OZ (NIF> 60%). The first 2 dimensions of PCA accounted for 34% of the total variance, which increased to 55.7% when the first 4 dimensions were considered. The PCA maps are presented in Figure 14 as trajectories of odor quality evolution as a function of odor background level. The starting point was the GCO analysis, i.e., with no background odor of icewine, then was the low level of icewine odor (OLFL) and finally was the high level of background odor (OLFH).

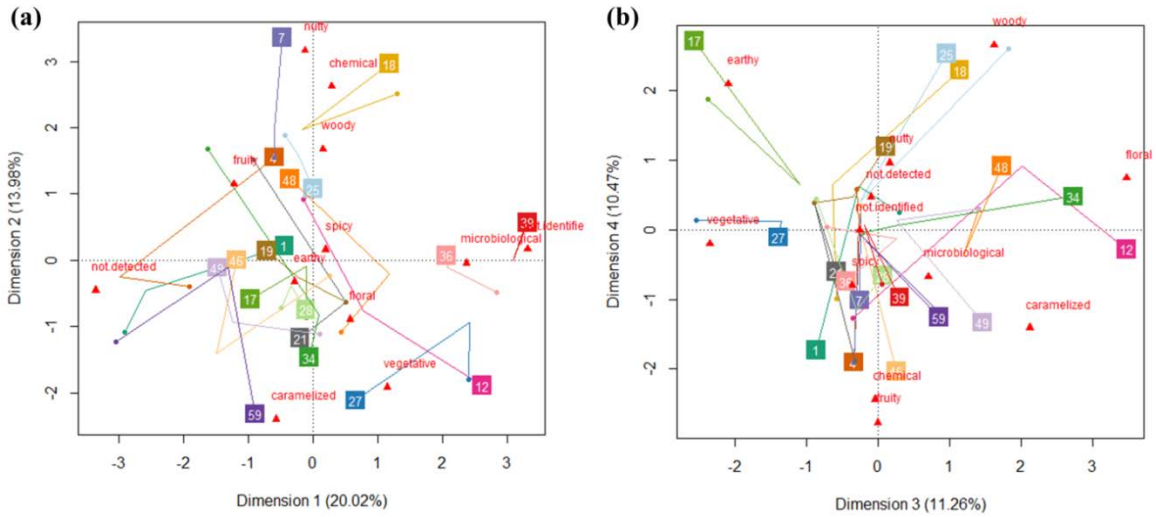


**Figure 14** Principal component analysis (PCA) biplot showing the descriptor trajectories of highly impacted odor peaks (NIF> 60%) over icewine background odor levels: zero level (GCO), low level (OLFL) and high level (OLFH). The beginning of the trajectory was GCO data (position at the peak number), the end of the trajectory was OLFH data (position at the solid dots), and the turning point was OLFL data. **(a)** The first 2 dimensions of the PCA map of odor descriptors. **(b)** The 3$^{rd}$ and 4$^{th}$ dimensions of the PCA map of odor descriptors.

As a first observation, peak 7 (2-methyl-1-propanol), peak 17 (1-octen-3-one), peak 19 (1-hexanol), peak 28 (nerol oxide), peak 36 (2-methylbutanoic acid, acetophenone), and peak 39 (3-methylbutanoic acid) did not move widely on the first 2 planes of PCA, meaning that the odor of these compounds was not very affected by the icewine background odor and that their characteristic odor was still highly recognizable even with a high level background icewine odor. The same conclusion can be suggested for peak 18 (2-acetyl-1-pyrroline), peak 25 (2-ethyl-3,5-dimethylpyrazine) and peak 49 (phenylethyl alcohol) since their trajectories are at least in the first PCA plan. The trajectories for peak 1 (ethyl isobutanoate), peak 4 (ethyl butanoate), peak 12 (3-methyl-1-butanol) and peak 59 (eugenol) obviously changed from right to left in Figure 14a, which confirmed the masking of the odor of these compounds by increasing levels of the background odor as previously observed. Therefore, it is likely that the odor of these compounds blended with the aromatic buffer of the icewine odor that contained relatively high concentrations of ethanol, ethyl esters, fusel alcohols, and volatile phenols (Escudero et al., 2004). Interestingly, for 3-methyl-1-butanol (peak 12), not only did the increasing levels of icewine odor mask the perception of its characteristic odor, but it seems that its odor quality also changed from floral-sweet to fruity. Conversely, the trajectories of peak 48 (guaiacol) and peak 27 (methional) changed from left to right in Figure

14a, in line with the previously observed synergy effect for these peaks. For peak 27, the vegetative odor of methional seemed to be maintained in the icewine aroma buffer, while the woody odor of guaiacol likely changed for a more floral or caramelized odor. For peak 21 ((*Z*)-3-hexen-1-ol) and peak 34 (hotrienol), as the background icewine odor level increased, their descriptors changed to a fruity aspect.

### 4.3.4 General Discussion

As a major result, our study showed that although odor-active compounds can be considered to have a significant aroma contribution when they are separated, their perception can be influenced by a mixture-induced effect (V. Ferreira, 2012a; Ma, Tang, Xu, & Thomas-Danguin, 2021; Thomas-Danguin et al., 2014) , so that their odor contribution might be very different when they are embedded in the complex aroma of icewine. Roughly, we observed that 57% of the odor-active compounds were not highly affected by mixture effects, while 30% were masked and 13% benefited from synergy. Previous research based on binary mixture models showed that synergy, or hyper-addition, is rare but may occur mostly at low intensity levels (V. Ferreira, 2012a). In our study, we observed only a few cases of increase in the NIF for a compound when it is added to the complex odor mixture formed by the icewine aroma delivered in the OLF conditions; we considered that such an NIF increase would be indicative of a synergy effect. However, based on our experimental protocol, we cannot affirm that a hyper-addition occurred since partial addition can also explain our observations. Indeed, partial addition could have been induced by the amount of the target compound actually present in the icewine background odor. Nevertheless, our results indicated that synergy appeared mostly in OZ, which had a relatively low NIF ($\leq$ 31.6%), and in the OLFL condition (67% of cases), in which a low level of icewine background odor was delivered. Among the compounds for which synergy was observed, the Olfactoscan analysis highlighted several odorants that were not considered in the GCO analysis because their contribution was below the noise threshold. 2-Pentylfuran and/or 2-hexanol, $\gamma$-heptalactone and 9-decenoic acid and/or geranic acid and/or isophytol benefited from synergy and were thus only considered impact odorants under the mixture conditions. Interestingly, these compounds were not considered icewine key odorants before because they had not been detected in AEDA (Ma et al., 2017). Strikingly, only one compound (methional, peak 27) benefited from synergy with the icewine background odor at both low and high levels. This compound was already considered a high-impact odorant in GCO, but its impact likely increased when embedded in icewine aroma buffer. Moreover, the vegetative usually cooked potato-like odor of methional seemed to be maintained in the icewine aroma buffer. This odorant, which is related to oxidation or aging in fermented beverages (Escudero et al., 2000), was found to be involved in perceptual interactions in binary mixtures (Burseg & de Jong, 2009). However, its detection probability in such simple mixtures was already proven to be strongly dependent on the compound with which it was mixed, suggesting highly intricate interactions in the case of complex mixtures. Guaiacol is another odorant that benefited from synergy with the icewine odor, but in contrast with methional odor, we observed a shift in odor quality in the OLFL condition, suggesting that this compound interacted with the icewine odor at low intensity to

contribute to a floral or caramelized character. This compound associated with the woody character of wine was found to develop perceptual interactions with the fruity component of wine (Atanasova, Thomas-Danguin, Langlois, Nicklaus, & Etievant, 2004). In particular, at low background concentration level, guaiacol could boost fruity character, while at higher concentration level, the woody odor could be perceived at the expense of fruity odor (Atanasova, Thomas-Danguin, Langlois, et al., 2005).

Wine aromatic buffer has previously been reported to be able to suppress the effect of many odorants added to it, particularly those with fruity characteristics (Escudero et al., 2004; V. Ferreira, 2010). Our results confirmed that several odorants carrying a fruity or floral-like odor were masked once in the wine background odor. Several of these compounds had a relatively high NIF in GCO (3-methyl-1-butanol, ethyl isobutanoate, ethyl butanoate and eugenol), meaning that they can be identified as high impact odorants. However, once in the complex wine mixture, their impact would be much lowered, or they may have a similar odor quality contribution to the overall fruity/floral icewine odor. Such a general contribution has been proposed following the concept of aroma vectors (V. Ferreira et al., 2016), supported, for instance, by the idea that the contribution of several ethyl esters can be mimicked by only one of them (De-La-Fuente-Blanco, Sáenz-Navajas, Valentin, & Ferreira, 2020). Synergy has also been reported to be able to occur between these ethyl esters (Lytra et al., 2013; Niu, Liu, & Xiao, 2020), which reinforces the idea that they contribute to a general fruity character. In the case of 3-methyl-1-butanol, we found that it remained a high impact odorant even in the icewine odor but that in the complex mixture, the odor quality associated with its OZ changed to a more fruity-sweet character. This result is fully in line with previous reports demonstrating, in model solutions, that higher alcohols contribute to the fruity aroma expression of esters from red wine (Cameleyre et al., 2015).

## 4.4 Conclusions

This study is among the very first attempts to evaluate the contribution of odor-active compounds considering the mixture-induced effect on a complex aroma (here icewine). This study relies on the Olfactoscan setup, which allowed us to consider the impact of a single odorant on the global aroma online during GCO analysis. To analyze the data, a semiautomatic method was used to allow the identification of odor zones in a similar way both in GCO and Olfactoscan approaches based on the detection frequency method. The results showed that considering a key odorant in the background odor of icewine could reveal mixture-induced effects such as masking or synergy resulting in a lower or higher detection probability of the characteristic odor of this compound or in a modification of the overall wine aroma supporting qualitative perceptual interactions. In that sense, the Olfactoscan approach can lead to reconsider the impact of key odorants and reveal specific compounds that could be highly influential once embedded in the aroma buffer. Nevertheless, this study also stressed the high complexity of perceptual odor interactions in real food and beverages, which advocates for the development of systematic research studies to better understand the impact of a compound, or a group of compounds, in complex aroma mixtures.

# Chapter V Factors Influencing the Perception of Odor Mixtures in Icewine

**Related to the publication**

Ma, Y., Guilbert, A., Béno, N., Tang, K, Xu, Y., & Thomas-Danguin, T. The effect of odor elements and perceptual interactions on the perception of icewine odor mixtures. (In progress)

## Abstract

The influence of elements, namely key odorants of icewine, on odor mixtures perception and perceptual interactions was explored in a set of 150 mixtures of odorants, which varied in their number of components, combinations and odor intensities. Stimuli were delivered using a computer-controlled multi-channel dynamic dilution olfactometer to a panel of 36 trained subjects, who performed a RATA evaluation. The results showed that (1) the perception of odorants such as 1-hexanol and ethyl 2-methylbutanoate in the mixture was affected by the intensity of the odorant. This influence affected not only the characteristic odor of the odorant, but also other odor characteristics in the mixture. (2) When one to six odorants were combined with the complex icewine odor, regardless of the combination, there were at most only two odor characteristics of the elements that can be distinguished in the mixture, and the olfactory system was more sensitive to the 'addition' than the 'omission' of odorants, especially when there were less than three odorants manipulated. (3) The addition and omission of combinations containing guaiacol significantly increased or reduced the intensity of its related smoky attribute. In contrast, the addition of 3-methylbutanoic acid significantly increased the perception of its dominant cheese attribute in the mixtures, but its omission did not significantly reduce the cheese attribute intensity. (4) Several specific perceptual interactions between were observed, such as a possible synergistic effect in the combination of 3-methylbutanoic acid and guaiacol, and the combination of ethyl 2-methylbutanoate and furaneol, and a possible masking effect between $\beta$-damascarone, furaneol, ethyl 2-methylbutanoate and 3-methylbutanoic acid. These effects highlighted the complexity of the perception of odor mixtures and their intricate effects on food complex aroma processing.

**Keywords:** wine aroma vector, odor quality, olfactory processing, odor combination

## 5.1 Introduction

Key odorants of wine aroma have been identified in many different wines (Ma, Xu, et al., 2021). However, it is still impossible to predict the final odor perception of wine based on the composition of odorants. The odor perception of wine, as of food in general, is based on the perception of odor mixtures. However, the olfactory processing of the complex chemical signal carried by odor mixtures is still poorly understood. Indeed, highly intricate perceptual interactions have been observed because of the binding and encoding of the chemical signal by the peripheral olfactory system and the synthetic processing (Livermore & Laing, 1998) or elemental processing (Berglund & Engen, 1993) of these signals, which lead the odor of mixtures to be perceived as homogeneous or heterogeneous and different effects to arise, such as synergy, masking and blending (Thomas-Danguin et al., 2014). Thus, the perception of an odor mixture is not a linear combination of the elemental perception of the single odorants (Thomas-Danguin et al., 2017).

The wine odor diversity is mainly due to the changes in the concentration and combination of numerous odor-active compounds (Polášková et al., 2008). However, it has been observed that only a small variation in the concentration of one odorant in a complex mixture can modify the overall odor quality (Le Berre, Beno, et al., 2008), and that specific combinations of a few odorants play an important role in the perceptual outcome of a mixture (Barkat et al., 2012; Lindqvist, Hoglund, & Berglund, 2012). In some cases, a single compound, such as linalool in Moscatel wines, is capable of transmitting its own odor, but in most situations, various odorants influence and interact in the perception of a particular odor attribute (San-Juan et al., 2011; Siebert, Barker, et al., 2018; Tomasino et al., 2020). Since the odor profile of a wine is determined by not only the key odorants but also the perceptual interactions between key odorants or other odorants, exploring the rules and mechanisms of perceptual interaction between odorants is one of the most critical steps to achieve the goal of predicting the final odor perception of wine based on the composition of odorants.

Perceptual interactions between odorants belonging to different categories have been described. The results showed that esters (Lytra et al., 2012; Lytra et al., 2013), norisoprenoids, dimethyl sulfide (Escudero et al., 2007) and some volatile fatty acids (San-Juan et al., 2011) could enhance the perception of fruity odors. Contrariwise, diacetyl, acetoin, acetic acid, $\gamma$-butyrolactone (Lytra et al., 2012), 4-ethylphenol, acetic acid, phenylacetaldehyde, methional (San-Juan et al., 2011), and mixture of higher alcohols were found to have a masking effect on fruity odors (Cameleyre et al., 2015). Ethyl decanoate and methyl salicylate at subthreshold concentrations were also likely to contribute to the overall odor of Chinese cherry wines (Niu et al., 2019) and various concentrations of ethyl isobutanoate and ethyl isovalerate gave additive or synergistic odor effects for its mixtures (Niu, Zhu, et al., 2020). Aldehydes such as benzaldehyde, furfural, and vanillin could induce synergistic effects in the aroma reconstitution of Chinese *Huangjiu* wine (Yu et al., 2020). Acetic and butyric acids at subthreshold concentrations could enhance the rated intensity of moderately intense sensations of coffee aroma compounds (Miyazawa et al., 2008a). Combinations of some $\gamma$-lactones might act additively or synergistically to contribute to the

‘apricot’ odor of white wine (Siebert, Barker, et al., 2018). Binary mixtures of monophenols could be involved in different perpetual interactions ranging from partly additive to strong synergistic for almost all combinations of monophenols (Sterckx et al., 2011). Ethylphenols had a masking effect on wine fruity notes even at subliminal concentrations (Tempere et al., 2016). All these results highlighted the complexity of perceptual interactions between odorants. However, few studies have systematically assessed the contribution of different odorant combinations to the odor profile of real products or complex odor mixtures.

To assess such a contribution, the main challenge is to identify odorants and odorant combinations that would be relevant in the context of a product. Icewine is a good example of an odor mixture. It makes from grapes naturally frozen on the vine at temperatures below or equal to –7°C and undergoes a special dehydration process and freeze-thaw cycles. Since the must for icewine making is pressed from frozen grapes, the grape juice is concentrated, leading to a rich odor after a slower than normal fermentation (Ma, Xu, et al., 2021). The typical odor of icewine has been described as honey, tropical fruit, apricot, caramel, raisin, nutty and floral (Huang et al., 2018; Nurgel et al., 2004), and more than 80 odor-active compounds were detected from different grape varieties (Bowen & Reynolds, 2012; Lan et al., 2019; Ma et al., 2017). Wine has been described as a sensory buffer containing the most common wine odor-active compounds, such as ethyl esters, fusel alcohols, volatile phenols, which are able to counterbalance the addition or omission of several odorants without any significant change in the overall odor (V. Ferreira, 2010). However, certain ‘impact’ odorants or families of odorants sharing chemical and odor properties can break the sensory buffer and form an aroma vector specific to the odor features of a real wine. By constructing a wine model from a common base supplemented by six target odor vectors, it has been shown that sensory interactions between the six common aroma vectors can explain four main red wine aroma nuances (V. Ferreira et al., 2016). Moreover, it is reasonable to assume that aroma vectors can be represented by a single odorant, since the results showed that ethyl 2-methylbutanoate could replace 14 ester compounds (De-La-Fuente-Blanco et al., 2020). Following this line of reasoning, the number of odorants can be reduced while still considering the potential interactions among main aroma nuances. In this study, we developed an original approach and constituted more than 150 odor mixtures to determine which odorants and odorants combinations have an impact on the overall complex mixture odor. The aim was to highlight the rules that drive perceptual interactions and the cause of the different perceived aromatic notes. We especially considered mixture composition factors such as the number of components, the specific associations, and the odor intensity. The expected results should be beneficial to understanding the interactions between different odorants in icewine, promoting quality control in the production of fast-moving consumer goods such as wine, and gaining knowledge of the information processing mechanism of the olfactory system.

## 5.2 Materials and Methods

### 5.2.1 Chemicals and Samples

Eleven odorants were selected for this study (see details in Supplementary Table 3). The odorants and their purity were 3-methyl-1-butanol (CAS. 123-51-3, > 98%), ethyl 2-methyl

butanoate (CAS. 7452-79-1, ≥ 98%), *β*-damascenone (CAS. 23696-85-7, 1.1-1.4 wt. % in 190 proof ethanol), 3-methylbutanoic acid (CAS. 503-74-2, ≥ 98%), guaiacol (CAS. 90-05-1 , ≥ 99%), furaneol (CAS. 3658-77-3, ≥ 98%), 1-hexanol (CAS. 111-27-3, ≥ 98%), phenylethyl alcohol (CAS. 60-12-8, ≥ 99%), *γ*-decalactone (CAS. 706-14-9, ≥ 97%), methional (CAS. 3268-49-3, ≥ 97%), 1-octen-3-one (CAS. 4312-99-6, 50 wt.% in 50 % 1-octen-3-ol). Propylene glycol (≥99.9%, food grade) and food-grade odorants were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Ultrapure water was obtained with a Milli-Q purification system (Millipore, Bedford, MA). Commercial icewine was purchased from ChangYu Winery (China), and its quality meets the standards of the Vintners Quality Alliance system. Icewine bottles were stored horizontally at 11 °C in the dark before use.

### 5.2.2 Stimuli and Delivery Apparatus

The 11 odorants were chosen because they were key aroma compounds in icewine based on a previous study (Ma et al., 2017) and were also found to impact the whole icewine odor in a study based on an Olfactoscan approach (Ma et al., submitted). Besides, six of the 11 compounds were considered as part of the common odor of wine (N, norisoprenoids; A, branched acids; F, enolones; E, branched ethyl esters; L, fusel alcohols, M, wood compounds) (V. Ferreira et al., 2016). The number of odorants used was limited to 11 because of the capacity of the odor delivery system (olfactometer).

For the formal experiment, all the stimuli were delivered by a computer-controlled dynamic-dilution 12-channels olfactometer (OL023; Burghart Instruments, Holm, Germany). The olfactometer allows for the application of rectangular-shaped chemical stimulus embedded into a constant flow of odorless air (Kobal, 1981). The air was humidified, and the temperature stabilized at 25 °C. The total airflow at the outlet of the olfactometer (olfactory port) was kept constant at 3000 mL/min. Stimulus duration was 10 s, the inter-stimulus interval (ISI) was ranged and randomized between 40 and 45 s. A brief illustration of the entire experimental setup was given in Figure 15.

Each odorant solution or icewine was poured into a dedicated chamber of the olfactometer and renewed before each session. Each chamber was connected to a dedicated channel. The left and the right channels were connected to a single outlet at the olfactory port (Figure 15). A channel was dedicated to icewine odor, for which, real icewine was used; the 11 other channels were used for odorants. For 1-hexanol and phenylethyl alcohol, pure compounds were used; for the other nine odorants, they were diluted in propylene glycol (see details in Supplementary Table 3). A total of 159 stimuli were delivered in three formal test sessions, and 52 stimuli were used in the training session. The stimuli were designed for 4 different blocks of the formal experiment by combining one to eleven odorants (Supplementary Table 4). To deliver the different odor stimuli, odorless dry air went through the chamber that contained the liquid odor sample to produce odorized air. Different flows of odorized air coming from the different chambers were combined and diluted with humidified odorless air at the outlet of the olfactometer to produce the final odor stimulus. The odorized air flow/total air flow ratio of each stimulus was given in Supplementary Table 3. The flow ratio for each stimulus was determined in a pretest involving experienced lab members, then if necessary, it

was adjusted after the training session involving the selected external panel to obtain an odor intensity that matched 3 levels (low, medium, high) and that was similar for each odor within each level.

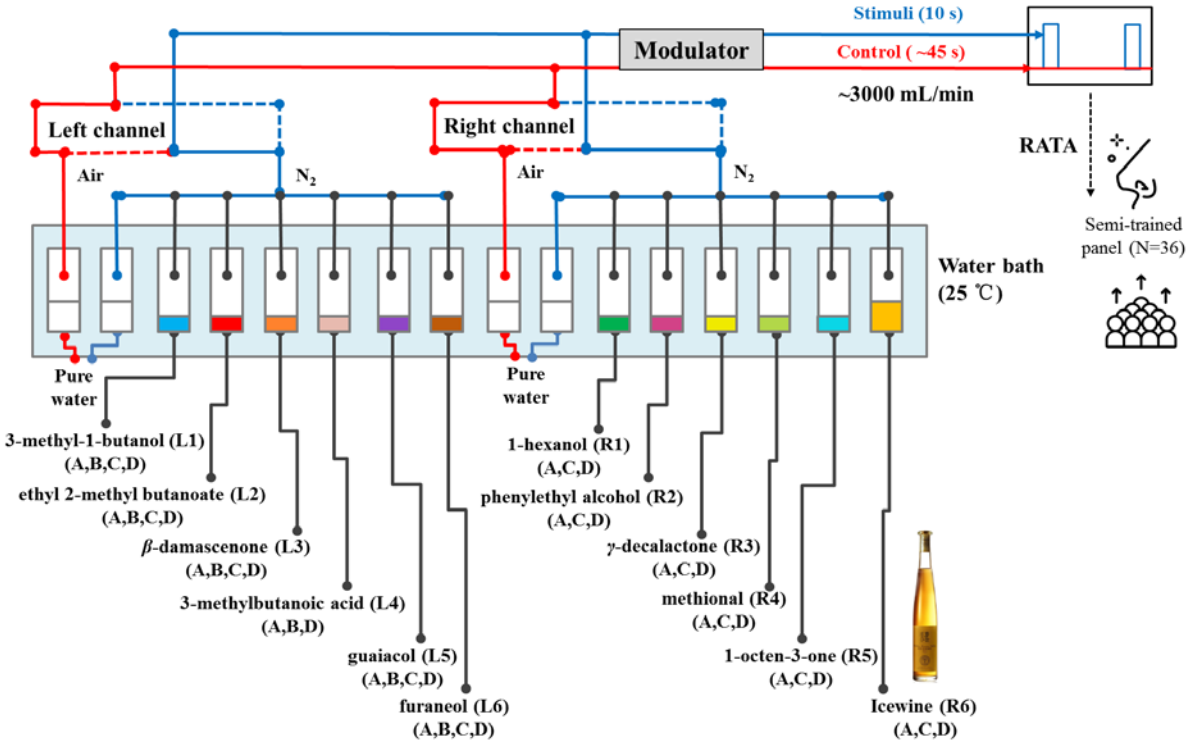


**Figure15** Illustration of computer-controlled olfactometer and the olfactometer experiment

The stability of the device was checked. The airflow at the outlet for each olfactometer chamber at different air flow/total air flow ratio condition was measured before the first experiment and after the last experiment, with two replicates. The result showed no significant deviation of the flow during the whole experiment.

The chemical composition stability of each odor stimulus delivered by the olfactometer was evaluated by monitoring the total volatile content at different air flow/total air flow ratio conditions using a photoionization detector PpbRAE 3000 (RAE, Lyon France) during 1 to 4 run sequences. The results showed that the total volatile content of the 11 odorants and the icewine stimuli was stable within 3 run sequences in two repetitions, which indicated that each odor stimuli could be considered stable during the whole experiment.

### 5.2.3 Subjects

Thirty-six healthy subjects (50% women 50% men; age range 20 to 64 years old) were selected three weeks before the main experiment from eighty healthy subjects. Selection was based on two screening tests to evaluate subjects' (i) ability to maintain selective attention with time using the Bourdon Test (Boomsma & Bosch, 1978) and (ii) performance in detecting and identifying different odors at low to medium intensity. For the screening test (ii), 2 samples of icewine, icewine supplemented with an odorant (2 samples), and the eleven odorants (11 samples), which were involved in the formal experiment, were used. For the 4 icewine samples, 1 mL icewine solution was absorbed by a sniffing strip (7*140 mm, Granger Veyron, France) and the sniffing strip was placed in a 60 mL brown glass bottle. For the 11

odorant samples, pure food grade odorant was first diluted in propylene glycol to obtain a low to medium intensity based on a pretest with internal expert staff (Supplementary Table 5). Then 60 µL of diluted odorant was deposited on a sniffing strip, which was placed in a 60 mL brown glass bottle. To evaluate the samples, the subjects were asked to evaluate the perceived odor characteristics by using the same process of rate-all-that-apply (RATA) analysis (Ares et al., 2014) as the process used in the formal experiment (see below). The 36 subjects who obtained the highest scores on both tests were selected to participate in this study. All of the subjects confirmed that they were in good health and had normal smell during the experiment. The study was conducted with respect to the recommendations of the Declaration of Helsinki and in accordance with the French regulations concerning research involving human volunteers (‘étude hors-RIPH ne visant pas le développement des connaissances biologiques ou médicales’). Subjects provided a written consent after being informed about the aims and potential risks of the study. Subjects were instructed not to wear any perfume and not eat or drink anything for at least 1 hour before the study. Subjects received a 10 € gift card for each hour of participation.

### 5.2.4 Experimental Procedure

The experiment included one training session and three formal sessions. All the stimuli in these sessions were delivered by the olfactometer (Figure 15). All the sessions were conducted in an air-conditioned room for each subject separately. During the experiment, the subjects were seated in a booth, isolated from odor, noise or visual pollution, and in which the olfactometer olfactory port was located. They received a light cue when each stimulus was delivered and they were asked to evaluate perceived odor characteristics following a rate-all-that-apply (RATA) procedure (Ares et al., 2014). Subjects used a computer system running the FIZZ sensory analysis software (version 2.51, Biosystemes, Couternon, France) to record their responses.

Every selected subject participated first in a training session prior to the formal sessions to be acquainted with the olfactometer, the different odor descriptors and the details of the experimental procedure. The training session included three parts. In the first part, the 11 basic (unmixed) odors were delivered at medium odor intensity, and their target descriptors were provided to subjects in a fixed order. The subjects were asked to remember the descriptor dedicated to each odor (Supplementary Table 3). In the second part, the 11 basic odors were delivered again at medium odor intensity but in random order. The subjects were asked to recognize the odor by selecting the expected descriptor from the list provided on the computer screen. In the third part, the procedure of rate-all-that-apply (RATA) was introduced, and they were asked to evaluate 30 odor stimuli (Supplementary Table 4) using the RATA scales (see below) displayed on the computer screen. This last procedure was exactly the one used during formal sessions.

The formal sessions comprised a total of 156 different odor stimuli dispatched in 4 separate blocks (blocks A, B, C, D; Supplementary Table 4); icewine odor stimuli were also delivered in block A and B. The odor stimuli and their corresponding sample codes were provided in Supplementary data/ sheet 1 (The data of block C were not analyzed). The stimuli

were dispatched in the blocks evenly to ensure that each session comprised the same number of samples (52 odor stimuli and one odor of icewine). The order of the blocks (or sessions) was balanced across subjects to minimize the order effect. In each session (the odor stimuli and their corresponding blocks for each session were provided in Supplementary data /sheet 2), the subject began with 2 warm up stimuli and then the 53 stimuli. For the 2 warm up stimuli, the first warm up stimuli was a single odorant and the second was a real icewine. The presentation order of the odor stimuli was random and different for each subject. The duration for each odor stimulus was 10 s, and the interstimulus interval ranged randomly between 40 and 45 s to maintain vigilance. During a session, the subject was given a 2-minute break every 15 stimuli. Each session lasted approximately 1 h.

### 5.2.5 Rate-All-That-Apply (RATA) Procedure

In the RATA procedure, subjects sniffed the odor stimuli in front of the sniff port of the olfactometer when the red light cue is on. Then they were asked to check different terms they considered appropriate for describing the odor stimuli and then to rate the intensity of the selected terms using a 5-point structured scale (‘very low’, ‘low’, ‘medium’, ‘high’, ‘very high’). There were 13 odor descriptors provided (in French): alcoholic (alcool), apricot (abricot), caramel (caramel), cheese (fromage), cooked potato (patatate cuite), green (vert/ herbe coupée), honey (miel), mushroom (champignon), nut (noisette), raisin (raisin sec), rose (rose), smoky (fumé), tropical fruit (fruits tropicaux). These terms were selected based on the descriptor for each odorant obtained in GC-O analysis or previous descriptive analysis using similar icewines (Huang et al., 2018; Ma et al., 2017).

### 5.2.6 Data Processing

All statistical analyses were performed with the *R* software (version 3.5.3, R Foundation for Statistical Computing, Vienna, Austria.).

Subjects’ ratings following the RATA procedure were transformed into numerical values from 1.0 (very low) to 5.0 (very high). The value for the terms that were not selected was assigned at 0.

The quality assessment of the sensory data was achieved by using ANOVA (*panelperf* function from the *SensoMineR* package (Husson & Le, 2008); on two data subsets, in which the dependent variables were the thirteen attributes and the independent variables were the factors associated with the sample effect, the panelist effect, the session effect, and eventually all their first-order interactions. The results of ANOVA were used to test the sensory panel’s ability to differentiate the samples (discrimination) consistently (repeatability) and consensually (agreement). If the sample effect was significant, it meant that the panel had discriminated the samples with respect to the target sensory attribute (discrimination); if the sample × subject effect was significant, it meant that the subjects did not have the same perception of the samples with respect to the target sensory attribute (no agreement); if the sample × session effect was significant, it meant that the panel was not repeatable from one session to the other.

The odor perceptual impact of the stimuli in terms of odor Intensity Modification was assessed through the calculation of an index called *IM*. *IM* values were calculated using

Equation 6, where *F* was the percentage of subjects who selected a given attribute and *A* the average intensity value calculated from the rating of the subjects who selected the attribute. *IM* value was calculated for each odor attribute and each sample. *IM* values were used to estimate odor perceptual impact, instead of intensity mean values, because it can account for both the selection frequency and intensity for a given descriptor. *IM* values were used to draw the odor profile of each sample, and to run further multivariate statistical analyses. The odor profile of each sample was visualized using the *radarchart* function from the *fmsb* package (Nakazawa & Nakazawa, 2017).

$$IM=\sqrt{F \times A} \quad \text{(Equation 6)}$$

For each odor attribute, the difference of between the value of 1) icewine (R6) and icewine + senary combination (sample B6-R6L1L2L3L4L5L6); 2) icewine (R6) and other icewine + odorants (addition condition); 3) icewine + senary combination (sample B6-R6L1L2L3L4L5L6) and other icewine + odorants (omission condition) in test A and B, was analyzed by two side paired Wilcoxon test using the *wilcox.test* function from the *ddply* package (Wickham, 2011).

The relationship and classification between sample attributes and samples was analyzed by means of a cluster analysis and visualized through the *Heatmap* function from the *ComplexHeatmap* package (Gu, Eils, & Schlesner, 2016). The data were the *IM* value of the 13 attributes for each sample. The Euclidean distance was used in the clustering process.

## 5.3 Results and Discussions

### 5.3.1 Sensory Data Quality Assessment

The quality of the sensory data was assessed by using ANOVA to test repeatability on two subsets of data. In the first subset, we evaluated the panel repeatability using the replications of the same icewine sample, carried out in 3 different sessions. In the second subset, we evaluated the discrimination ability of the panel, using the data obtained for 6 different odor mixtures rated in 2 different sessions. The ANOVA results (Supplementary Table 6) showed that the sample effect was statistically significant for none of the attributes, which suggested that the panel was repeatable in its evaluation of the same sample of icewine across sessions. The sample × subject effect was significant for the rose ($p$= 0.006) and honey ($p$= 0.027) descriptors, which suggested that the panel members evaluated all the descriptors, but these two ones, in a similar way. The sample × session effect was significant only for the rose ($p$= 0.026) descriptor, which suggested that the panel was repeatable in its across-session evaluation of all descriptors, except for rose.

*IM* values of each attribute of the icewine sample in each session and the average across sessions were used to draw the icewine odor profile (Supplementary Figure 2). The average profile indicated that icewine was mainly characterized by odors of tropical fruit, alcoholic, honey, and apricot, a result in line with previous data, except for alcoholic, which wasn't selected in the previous study (Ma et al., 2017). A paired two-sided Wilcox test was performed to compare the *IM* value of each attribute by session to the average across session. The results (Supplementary Figure 2) revealed only a few likely inconsistent differences for the descriptors nut, rose and apricot. Overall, this first analysis highlighted a good

repeatability and consistency of the panel.

In the second analysis, we evaluated the discrimination ability of the panel, using a second subset of data obtained for 6 different icewine odor mixtures rated in 2 different sessions. The samples were prepared by separately combining six medium-intensity single odorants (3-methyl-1-butanol, ethyl 2-methyl butanoate, *β*-damascenone, 3-methylbutanoic acid, guaiacol, furaneol) with the icewine odor channel. The ANOVA results (Supplementary Table 7) showed a statistically significant sample effect on 7 descriptors among the 13 (alcoholic, tropical fruit, honey, cheese, smoky, caramel, and apricot). Since the six odorants added to the icewine odor corresponded respectively to these 6 attributes, with the exception of apricot that was not part or the target descriptor, it can be concluded that the sensory panel was able to differentiate the odor mixtures samples but also to correctly identify the odor quality of the odorant added in the icewine odor. The sample × subject effect was significant for 4 descriptors (cheese, smoky, cooked potato and raisin) and the sample × session effect only on the raisin descriptor, which suggested that the panel provided consistent results and that the subjects are in agreement the most for the descriptors. Considering these two analyses related to panel performance, it can be concluded that the panel was able to correctly perform the RATA task and provided repeatable, consistent, and rather consensual data.

### 5.3.2 Influence of the Addition of Single Odorants on the Perception of Icewine Odor

Experiment A (see details in Supplementary Table 4) was designed to test the influence of the odor intensity of single odorants addition on the perception of icewine odor. In this test, the 11 odorants (Supplementary Table 3) were added, one at a time at low, medium, and high intensity, to the icewine odor. The RATA data for the thirteen attributes were compared between the odor mixtures and the basic icewine odor using a paired two-sided Wilcox test. The difference was defined as significant if $p< 0.05$. *IM* values were further used to draw odor profiles for each of the 33 samples (11 odorants × 3 intensity levels, Supplementary Figure 3). Moreover, the odor profiles for each of the 22 single odorants (11 odorants at low and high intensity, obtained during training sessions) were provided in Supplementary Figure 3 to be compared to the 33 mixture samples.

The results showed that the addition of an odorant to the icewine odor stimulus can lead to several outcomes: 1) the intensity of the attribute mostly related to the odorant added increased in the profile; 2) intensity of other attributes, less or not related to the added odorant, increased or decreased; 3) no significant change was observed whatever the attribute.

In the first case, the results showed that when 3-methylbutanoic acid (L4, cheese), guaiacol (L5, smoky), *γ*-decalactone (R3, apricot), methional (R4, cooked potato) and1-octen-3-one (R5, mushroom) were combined with the odor of icewine (R6), the intensity of the target attribute of the added odorant increased significantly regardless of intensity level. For ethyl 2-methyl butanoate (L2, tropical fruit), *β*-damascenone (L3, honey or apricot), 1-hexanol (R1, green) and phenylethyl alcohol (R2, rose), the target attribute's intensity increased significantly only at medium or high intensity of the added odorant. For 3-methyl-1-butanol (L1, alcoholic or cheese) and furaneol (L6, caramel), no significant modification of the target attribute intensity was observed, regardless of the intensity level.

In the second case, we observed that the addition of an odorant in the icewine odor stimulus can lead to the modification of attributes not directly related to the added odorant. For instance, adding furaneol at medium or high intensity enhanced the intensity of the *raisin* descriptor, while adding 1-hexanol at low intensity or ethyl 2-methyl butanoate at medium intensity enhanced *apricot*; the addition of *γ*-decalactone at high intensity enhanced *alcoholic* and methional at medium or high intensity enhanced *cheese*. Some odorants, added at a specific intensity level to the basic icewine odor, were able to decrease the intensity of some attributes: 3-methyl-1-butanol at low or high intensity decreased *rose* and *tropical fruit*; ethyl 2-methyl butanoate at medium or high intensity decreased *green*; ethyl 2-methyl butanoate at high intensity decreased *cheese* and *smoky*; low intensity level of *β*-damascenone decreased *alcoholic*; *β*-damascenone and furaneol decreased *cheese* at all intensity levels; 3-methylbutanoic acid at medium or high intensity decreased *tropical fruit* and *honey*; guaiacol at high intensity and 1-hexanol at low or high intensity decreased *tropical fruit*; phenylethyl alcohol at high intensity decreased *tropical fruit* and *alcoholic*; *γ*-decalactone at medium or high intensity decreased *smoky*, methional at medium or high intensity decreased *rose*, *alcoholic* and *tropical fruit*; 1-octen-3-one at any intensity decreased *alcoholic*. The third case was less often observed since only the addition of 3-methyl-1-butanol at medium intensity and phenylethyl alcohol at low intensity induced no significant change in the attributes' intensity.

To provide an overall picture of the effects induced by the addition of each 11 odorants to the icewine odor, we performed a cluster analysis using the *IM* values of each attribute compared after to before the odorant addition (Figure 16). The results showed that the profile of the icewine odor was modified in a similar way after the addition ethyl 2-methyl butanoate, *β*-damascenone, and *γ*-decalactone, which increased the intensity of the attributes *raisin*, *honey*, *apricot*, *tropical fruit*, and *apricot*, and decreased the intensity of *nut*, *green*, *cooked potato*, *cheese* and *smoky*. It is also clear from this analysis that the addition of 3-methylbutanoic acid, guaiacol, or 1-octen-3-one only increased the perception of their own odor (resp. *cheese*, *smoky*, and *mushroom*), but adding 1-octen-3-one also decreased *alcoholic*, *caramel* and *honey* odor notes. Similarly, adding furaneol, 1-hexanol, phenylethyl alcohol or methional at medium or high intensity led to an increased perception of their own odor (resp. *caramel*, *green*, *rose*, and *cooked potato*), while the addition of 1-hexanol also decreased fruity notes (*apricot*, *tropical fruit* and *raisin*). Moreover, it is worth noting that the addition of methional led to the decrease in the intensity of all the fruity and floral odor notes. The addition of furaneol, 1-hexanol, phenylethyl alcohol and methional at low intensity affected similarly the icewine odor profile with slight modifications of the descriptors *alcoholic*, *nut*, *green*, *rose* and *cooked potato*.

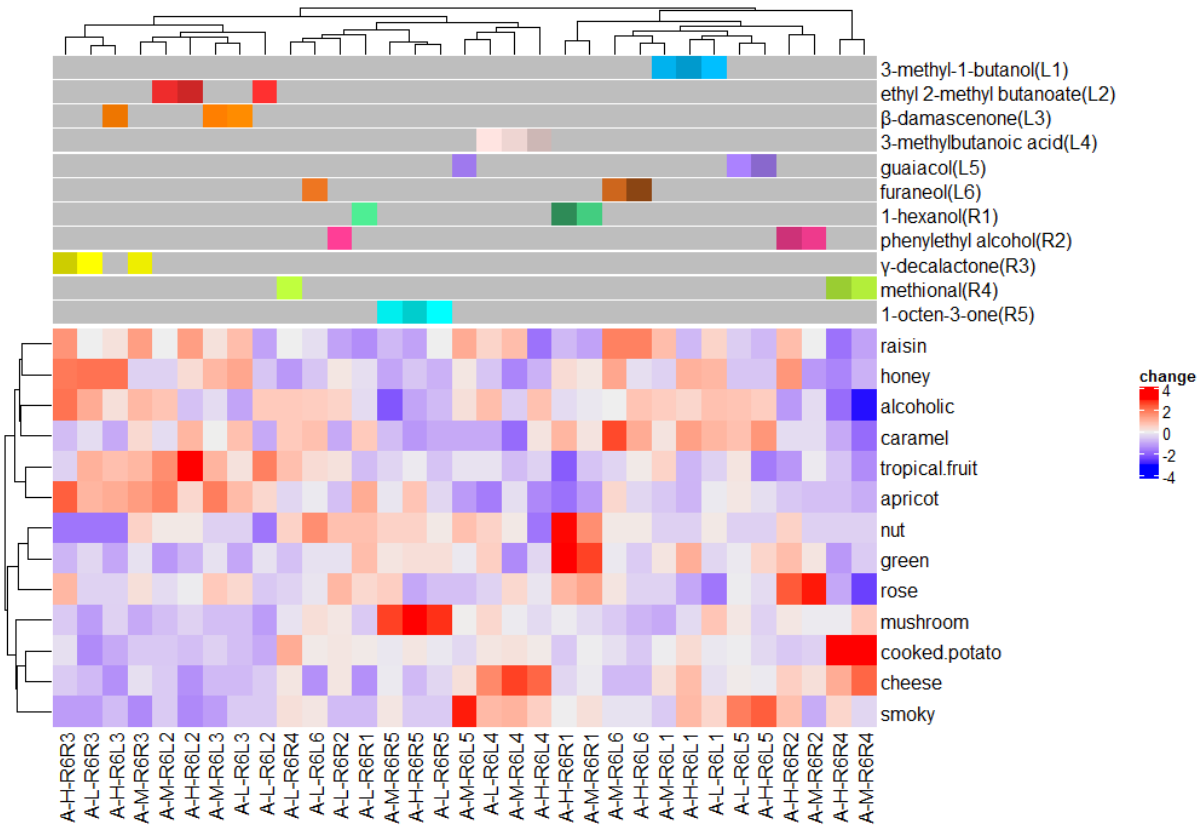


**Figure 16** Cluster analysis and visualization heatmap of the *IM* value of each attribute for samples of icewine odor mixed with a target odorant. The bottom part of the figure represents the cluster analysis results based on the *IM* values after standardization and centralization. Euclidean distance was used in the cluster analysis. Each column represents an odor mixture sample. For example, code A-L-R6R5 represents the sample of a low-intensity odorant R5 mixed with R6 (icewine); code A-M-R6R5 represents the sample of a medium-intensity odorant R5 mixed with R6 (icewine); code A-H-R6R5 represents the sample of a high-intensity odorant R5 mixed with R6 (icewine). The upper part of the figure represents the odorant manipulated and their intensity, the darker the color the stronger the odor intensity.

### 5.3.3 Influence of the Combined Addition of Odorants on the Perception of Icewine Odor

Experiment B (see details in Supplementary Table 4) was set up to determine the perceptual influence of the addition of mixtures of odorants (binary to senary) to the icewine odor stimulus. Six odorants, identified by Ferreira et al. (V. Ferreira et al., 2016) to contribute to wine aroma vectors, were added at medium intensity to the icewine odor stimulus. Considering Experiment B and part of Experiment A (for single odorant addition), a total of 63 combinations of 1 to 6 odorants were tested. For the 13 odor attributes, the difference between the odor mixture in icewine samples and the basic icewine was compared by a paired two-sided Wilcox test. The difference was defined as significant if $p< 0.05$. *IM* values of each attribute for the 63 samples were used to draw odor profiles (Supplementary Figures 4-8). The first result to highlight is related to the combination with the most complex senary mixture of odorants. This sample included the icewine odor in which was added 3-methyl-1-butanol, ethyl 2-methyl butanoate, *β*-damascenone, 3-methylbutanoic acid, guaiacol, and furaneol at a medium intensity (Supplementary Table 4). The odor profile of this sample (Supplementary

Figure 8) showed that except for the attribute *nut* (but with *IM* values almost 0 and thus not further considered), there was no significant difference ($p > 0.05$, Wilcoxon test) compared to the basic icewine sample. Therefore, it is likely that the combination of these 6 odorants would reflect the roughly overall icewine odor so that this mixture did not distort the overall profile when added to the complex icewine odor mixture.

Considering the addition of mixtures including 2 to 5 odorants, the results are similar to the addition of only one odorant in the sense that 3 main outcomes can be observed: 1) increase of the intensity of the odor notes related to the added odorants, but with at most only two attributes significantly modified whatever the added-mixture complexity, 2) modification of other attributes and 3) no major modification of the icewine odor profile. Overall, the different effects induced by odorants addition could be classified in 6 different categories, which occurrence is reported in Table 11. It was difficult to extract general trends but it seemed that the most frequent situation was the significant modification of only one odor descriptor associated to one of the added odorants (One-D, Table 11) and, especially for less complex mixtures (1 to 3 odorants), accompanied by the modification of unrelated odor descriptors (One-D & O). With mixtures including more than 3 odorants, the modification of only two target attributes (Two-D) disappeared to the profit of the modification of two target attributes accompanied by the modification of other unrelated attributes (Two-D & O).

It is possible to consider these data not in terms of addition but of omission of 1 to 6 odorants. In that case, the reference sample for the comparison was not the basic icewine but the mixture including icewine in which all the 6 odorants were added (coded B6-R6L1L2L3L4L5L6); the basic icewine being the mixture in which the 6 compounds were omitted. Considering the 6 categories of perceptual effects mentioned (Table 11), it can be seen that when 1 to 6 odorants were removed from the sample B6-R6L1L2L3L4L5L, the percentages in the category in which only one odorant-related attribute changed (One-D) were lower compared with ‘addition’ situation. However, the categories in which one odorant-related attribute changed simultaneously with changes of other attributes (One-D&O), or in which other attributes not related to the omitted odorants (O) or in which no changed was observed (N), were the most frequently observed. It should be emphasized that we observed only once a modification of 3 odorant-related attributes. This case, not reported in Table 11 was observed for sample L1L2L3L5L6 in which 3-methyl-1-butanol, ethyl 2-methyl butanoate, *β*-damascenone, guaiacol, furaneol were omitted. In that case, the three attributes related to ethyl 2-methyl butanoate (tropical fruit), *β*-damascenone (honey), and furaneol (caramel) had a reduced intensity.

**Table 11** Classification Statistics of the Influence of the Number of Odorant Combinations on the Odor profile

| Test | Number of compound added/omitted | Number of stimuli | The category of attribute changes and the percentage of occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Two-D | Two-D &O | One-D | One-D &O | O | N |
| addition | 1 | 6 | 0.0% | 0.0% | 16.7% | 33.3% | 33.3% | 16.7% |
| | 2 | 15 | 13.3% | 0.0% | 33.3% | 40.0% | 0.0% | 13.3% |
| | 3 | 20 | 5.0% | 15.0% | 15.0% | 35.0% | 0.0% | 30.0% |
| | 4 | 15 | 0.0% | 13.3% | 40.0% | 26.7% | 6.7% | 13.3% |
| | 5 | 6 | 0.0% | 16.7% | 33.3% | 16.7% | 0.0% | 33.3% |
| | 6 | 1 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |
| omission | 1 | 6 | 0.0% | 0.0% | 0.0% | 33.3% | 16.7% | 50.0% |
| | 2 | 15 | 6.7% | 6.7% | 6.7% | 6.7% | 33.3% | 40.0% |
| | 3 | 20 | 0.0% | 15.0% | 5.0% | 15.0% | 35.0% | 30.0% |
| | 4 | 15 | 0.0% | 0.0% | 0.0% | 33.3% | 26.7% | 40.0% |
| | 5 | 6 | 16.7% | 16.7% | 0.0% | 0.0% | 16.7% | 33.4% |
| | 6 | 1 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |

Note: The value in the table is the percentage of different situations occurred in 'addition' type of test and 'omission' type of test. In 'addition' test, the difference was compared between $IM_{sample}$~$IM_{icewine}$; in 'omission' test, the difference was compared between $IM_{sample}$~$IM_{B6\text{-}R6L1L2L3L4L5L6.}$ Significant difference ($p< 0.05$) between odors attributes was counted in situations of 'Two-D', 'Two-D & O', 'One-D', 'One-D&O' and 'O', no significant difference was included in N. Two-D represents two dominant odor attributes related to the odorant were significantly different; Two-D & O represents two dominant odor attributes related to the odorant and other odor attributes were significantly different; One-D represents one dominant odor attribute related to the odorant was significantly different; One-D&O represents one dominant odor attribute related to the odorant and other odor attributes were significantly different; O represents only odor attributes that were not mainly related to the odorant were significantly different. The changes of nut odor were neglected because it was very weak.

A visual summary of the comparison between the 'addition' and 'omission' point of view is presented in Supplementary Figure 9. We observed that the 'omission' of certain odorants or combinations had less effect on the profile of the icewine odor than their 'addition'. The main influence of 'omission' was observed on attributes other than the ones related to the manipulated odorants. In contrast, the 'addition' of certain odorants leaded to the modification of not only the odorant-related attribute, but also on other attributes. This result suggested that the olfactory system was more sensitive to the 'addition' than the 'omission' of odorants, especially when there were less than three odorants manipulated.

In order to explore the relationships between the overall odor profile of the icewine and the overall odor profile of the icewine supplemented with odorants, A cluster analysis was performed on the *IM* values, and the results were shown in Figure 17.The results showed that the samples were roughly divided into three groups according to the evolution of *IM* values (increase or decrease) for the different attributes. The odor profiles of the samples in the group on the left part of Figure 17 were closer to the odor profile of the basic icewine odor, and the number of added odorants in these samples was between 3 and 6. This result suggested that

when more than three odorants were added to the icewine odor, the effect on the overall odor profile was low. The odor profiles of the samples in the group located in the middle of Figure 17 showed relatively high IM values for attributes such as cheese, cooked potato, smoky and green. In this group were gathered the samples that included 3-methylbutanoic acid and/or guaiacol, which led to the enhanced perception of at least one descriptor related to one of these odorants, which suggested that these compounds were able to make their odor quality dominant in the mixture. The odor profiles of the group on the right side of Figure 17 showed relatively high IM values for the attributes tropical fruit, apricot, honey and caramel. In that case, the samples included mostly binary and ternary combinations that especially excluded 3-methylbutanoic acid and/or guaiacol, which suggested that these compounds tended to mask the fruity sweet attributes. Figure 17 also showed that among the samples with the closest odor profile, there were only five pairs of samples which were formed by adding the same number of odorants, and theses pairs were listed in Supplementary Table 8.

Among all the 13 odor attributes, cheese and smoky changed the most frequently (Supplementary Figure 10). In the ‘addition’ situation, when an odorant combination contained 3-methylbutanoic acid (L4) or guaiacol (L5), the other odorants in the odorant combination were normally masked by these odorants, and the odorants combination led to a significant enhancement of cheese or smoke odor in most cases (66%). However, in all the quinary combinations and senary combination containing 3-methylbutanoic acid and *β*-damascenone (L3), the odorants combination didn’t lead to a significant increase of the cheese odor intensity, which implied that the effect of 3-methylbutanoic acid in the combination should be weakened as the number of odorants increased in the mixture and the cheese odor is likely masked by *β*-damascenone (L3) in quinary combinations. Furthermore, some combinations containing ethyl 2-methyl butanoate (L2) and furaneol (L6) were also able to significantly reduce the cheese attribute intensity, as can be observed in the combinations L2L4L5, L1L2L4, L3L4L5, L2L4L5L6, L1L2L4L5, and L1L4L5L6. Combinations containing guaiacol (L5) were able to significantly alter the smoky attribute, especially when 3-methylbutanoic acid was present in the combination. However, there were 11 combinations containing guaiacol that didn’t significantly modulate the smoky attribute, and all of these combinations contained at least ethyl 2-methyl butanoate, or *β*-damascenone, or furaneol. According to these results, it can be concluded that the smoky and cheese attribute are highly related to the presence of 3-methylbutanoic acid and guaiacol, which can promote each other in some specific combination, and that ethyl 2-methyl butanoate, *β*-damascenone or furaneol can modulate theses odor notes in some specific combination.

The attributes apricot, honey, and tropical were also likely modulated by perceptual interactions (Supplementary Figure 10). The ‘addition’ or ‘omission’ of some combinations containing ethyl 2-methyl butanoate or *β*-damascenone or both significantly enhanced or reduced the apricot intensity. Some combinations containing guaiacol and 3-methylbutanoic also modulated the apricot attribute. Unlike apricot, tropical fruit and honey attribute intensity was hardly significantly enhanced by odorants, but these two attribute intensity was significantly reduced by a combinations containing 3-methylbutanoic acid.

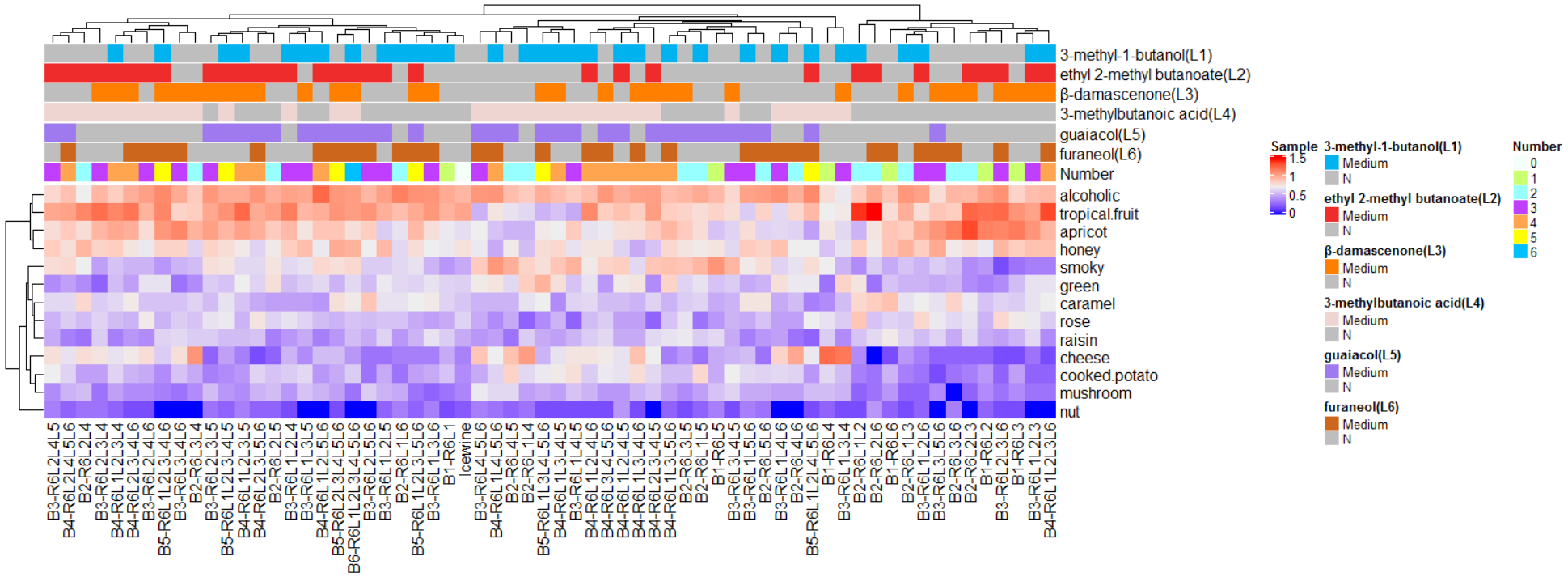


**Figure 17** Cluster analysis and visualization heatmap of the *IM* value of each attribute for samples of icewine odor mixed with target odorants or combinations of 1 to 6 odorants. The bottom part of the figure represents the cluster analysis results based on the *IM* value. Euclidean distance was used in the cluster analysis. Each column represents an odor mixture sample. For example, code B2-R6L5L6 represents the sample of a medium-intensity odorant L5, L6 mixed with R6 (icewine). The upper part of the figure represents the odorants manipulated, different colors represent different odorants, and gray represents no addition. The line indicated as 'Number' represents the number of odorants mixed with the icewine odor.

### 5.3.4 Odor Perception of Binary Odor Mixtures Composed of the 11 Key Odorants

Experiment D (Supplementary Table 4) was designed to explore the odor perception of binary odor mixtures, which were composed from the 11 key odorants. In order to have an overview of the most general trends regarding perceptual interactions in binary mixtures, a cluster analysis was performed on the *IM* values for each attribute. The results are summarized in Figure 18. A first result is that most of the 11 single odorants had a similar odor profile at low and high intensity, except for 3-methyl-1-butanol (L1), phenylethyl alcohol (R2) and ethyl 2-methyl butanoate (L2). Secondly, we observed that the odor profile of binary mixtures were usually not similar to the odor profile of their components, except for all the binary mixtures contained 1-octen-3-one (R5) and most binary mixtures contained 3-methylbutanoic acid (L4) or phenylethyl alcohol (R2). The binary mixture containing 1-octen-3-one (R5) had an odor profile dominated by its mushroom odor as previously observed. Mixtures including 3-methylbutanoic acid (L4), had an odor profile dominated by its cheese odor, except when mixed with 1-octen-3-one (R5), thus confirming that the mushroom odor masked the cheese odor. Nevertheless, when 3-methylbutanoic acid (L4) was mixed with ethyl 2-methyl butanoate (L2), or $\beta$-damascenone (L3), or $\gamma$-decalactone (R3), or phenylethyl alcohol (R2) the odor profile of the mixtures was rather different than the odor profile of 3-methylbutanoic acid (L4) alone.

To further extend the analysis, the mixture effect on an attribute (A) of a component (XO1/XO2) perceived in a binary mixture (X) in test D was determined by comparing the *IM* value of the component's maximum and minimum *IM* out of mixture under Low ($IM_{XOLA}$) and High ($IM_{XOHA}$) level intensity and that within mixture ($IM_{XA}$). $IM_{XA}$ represents the *IM* value of a binary mixture sample for an attribute; $IM_{XO1LA}$ or $IM_{XO2LA}$ represents the *IM* value of one of the binary mixture's component obtained at low concentration level for an attribute; $IM_{XO1HA}$ or $IM_{XO2HA}$ represents the *IM* value of one of the binary mixture's component obtained at high level concentration for an attribute. If $IM_{XA} >$ max ($IM_{XO1LA}$, $IM_{XO1HA}$), the attribute' intensity of the target component was increased, that is *synergy* (+) occurred; if $IM_{XA} <$ min ($IM_{XO1LA}$, $IM_{XO1HA}$), the attribute' intensity of the target component was reduced, that is *masking* (-) occurred; and if min ($IM_{XO1LA}$, $IM_{XO1HA}$) < $IM_{XA}$ < max ($IM_{XO1LA}$, $IM_{XO1HA}$), it was classified into a group named not sure (n), where *independence* might have occurred. A visual result of this approach was shown in Figure 19. The results showed that most frequently, the *IM* value of a binary mixture for a given fall in between the *IM* values of the components (marked as '+-' in Figure 19). This was especially the case for mixtures including 3-methyl-1-butanol (L1), $\beta$-damascenone (L3), guaiacol (L5), furaneol (L6), 1-hexanol (R1), $\gamma$-decalactone (R3) and 1-octen-3-one (R5). However, for mixtures including methional (R4), phenylethyl alcohol (R2), 3-methylbutanoic acid (L4) and ethyl 2-methyl butanoate (L2), this rule did not fully apply. When one of these odorants was mixed with another odorants, the *IM* value of the binary mixture for a given attribute might be similar to the *IM* values of the components (marked as '+' in Figure 19). Moreover, in some cases the perceptual effect might be even more pronounced. For example, in the binary mixture of 1-octen-3-one and guaiacol, the *IM* value of the mushroom attribute was higher than the *IM* values of 1-octen-3-one alone

(marked as '++' in Figure 19). Conversely, in the binary mixture of *β*-damascenone and *γ*-decalactone, the *IM* value for the apricot, honey and tropical fruit attributes were lower (marked as '--' in Figure 19) than the *IM* values of the unmixed *β*-damascenone and *γ*-decalactone.

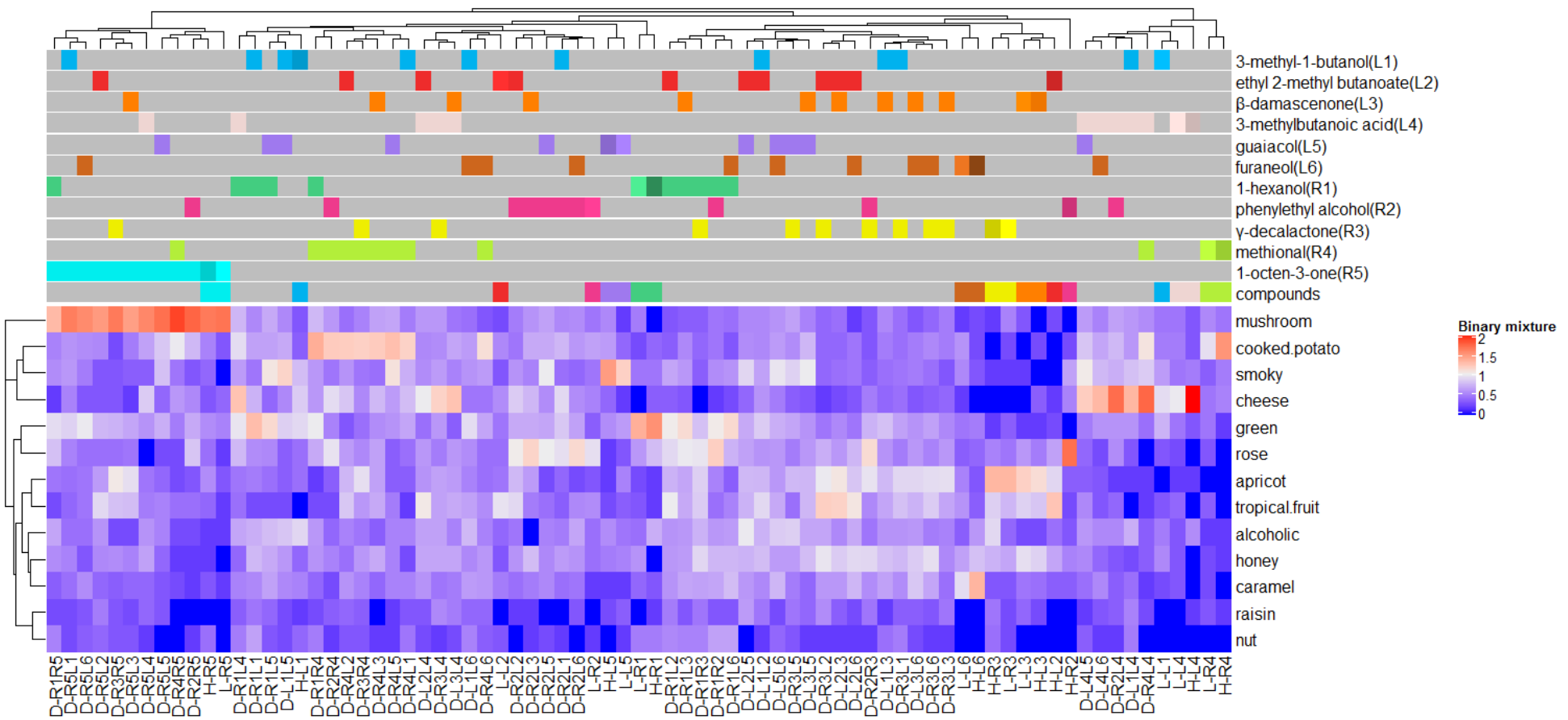


**Figure 18** Cluster analysis and visualization heatmap of the *IM* value of each attribute for samples of the 11 different single odorants and different binary mixtures. The bottom part of the figure represents the cluster analysis results based on the *IM* value. Euclidean distance was used in the cluster analysis. Each column represents an odor mixture sample. Each column represents a sample. For example, code D-R4R5 represents the sample of a medium-intensity odorant R4 (methional) mixed with R5 (1-octen-3-one); code L-R5 represents the sample of a low-intensity odorant R5; code H-R5 represents the sample of a high-intensity odorant R5. The upper part of the figure represents the odorants manipulated, different colors represent the mixed odorant and their intensity, the darker the color is, and the stronger the odor is. The colored samples in the 'compound' row represent the samples of single odorant.

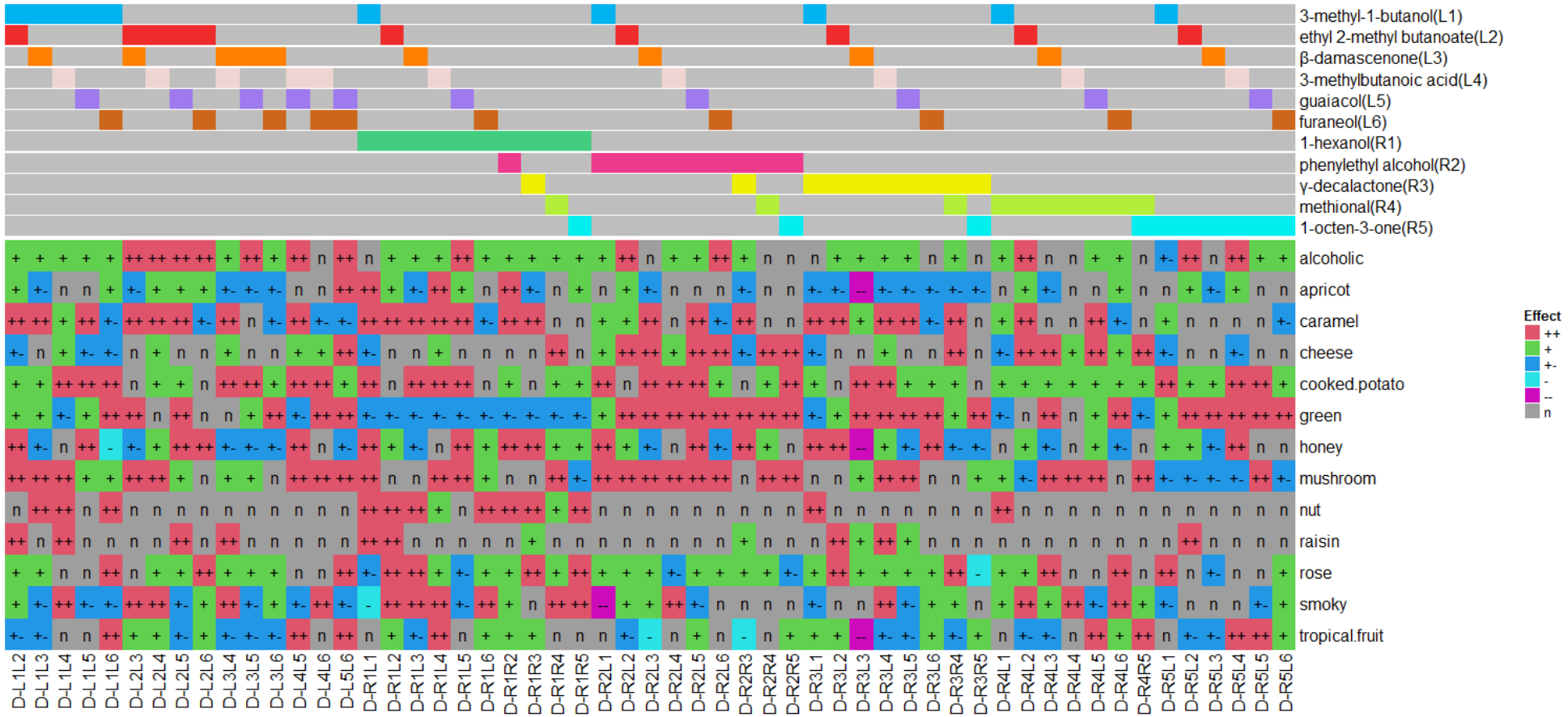


**Figure 19** The effect of perceptual interaction between different odorant combinations on odor attributes in binary mixtures. The mixture effect on an attribute (A) of a component (XO1/XO2) perceived in a binary mixture (X) in test D was determined by comparing the *IM* value of the component's maximum and minimum *IM* out of mixture under Low ($IM_{XO1LA}$) and High ($IM_{XO1HA}$) level intensity and that within mixture ($IM_{XA}$). If $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$), the attribute' intensity of the target component is increased, that is synergy (+) occurs; if $IM_{XA}$ < min ($IM_{XO1LA}$, $IM_{XO1HA}$), the attribute' intensity of the target component is reduced, that is masking (-) occurs; and if min ($IM_{XO1LA}$, $IM_{XO1HA}$)< $IM_{XA}$ < max ($IM_{XO1LA}$, $IM_{XO1HA}$), it was classified into a group namely not sure (n), where *independence* might occur. Attribute changes with *IM* values below 0.4 are also included in (n). In the figure:'++' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA}$ > max ($IM_{XO2LA}$, $IM_{XO2HA}$); '+' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)< $IM_{XA}$ < max ($IM_{XO2LA}$, $IM_{XO2HA}$); '+-' represents $IM_{XA}$ > max ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA}$ < min ($IM_{XO2LA}$, $IM_{XO2HA}$); '-'

represents $IM_{XA} <$ min ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)$< IM_{XA} <$ max ($IM_{XO2LA}$, $IM_{XO2HA}$); '--' represents $IM_{XA} <$ min ($IM_{XO1LA}$, $IM_{XO1HA}$) and $IM_{XA} <$ min ($IM_{XO2LA}$, $IM_{XO2HA}$); 'n' represents min ($IM_{XO1LA}$, $IM_{XO1HA}$)$< IM_{XA} <$ max ($IM_{XO1LA}$, $IM_{XO1HA}$) and min ($IM_{XO2LA}$, $IM_{XO2HA}$)$< IM_{XA} <$ max ($IM_{XO2LA}$, $IM_{XO2HA}$).

### 5.3.5 General Discussion

The ability of a given compound to impact the odor of wine has been studied before. It has been observed that components such as fusel alcohols, acids, esters, $\beta$-damascenone, and some volatile phenols didn't affect individually the odor of wine even if they were present at concentrations well above their odor thresholds due to the sensory buffer effect (Escudero et al., 2004). Nevertheless, a single odorant or a combination of odorants 1) at a concentration large enough, 2) with close similarity in chemical and aromatic properties, 3) with some similarity in a generic (non-specific) odor descriptor, 4) as an odor enhancer, would be able to break the buffer (V. Ferreira, 2010). Our results were consistent with some of these statements, since adding most of the studied impact odorants into icewine odor can affect the overall odor of wine. However, it should be noted that different odorants induced different effects on the odor of icewine. Some odorants such as 3-methyl-1-butanol (alcoholic) and furaneol (caramel) were unable to transmit their specific odor to the icewine odor even if they were added at high intensity. Our study showed that the addition of odorants at low-intensity usually affected the odorant's specific attribute more than other attributes, but the addition of odorants at high-intensity was able to significantly change the overall odor profile.

Several studies have shown the influence of the mixture complexity in terms of number of odorants in a mixture on the perception or discrimination of the mixture. The results showed that humans were not able to identify more than three or four odorants in a mixture that included up to eight odorants, and the identification of odorants was significantly more accurate for the 'poor' blenders than for the 'good' ones if the stimulus contains one to three odorants (Laing & Francis, 1989; Livermore & Laing, 1998). This limitation is likely the result of physiological or processing constraints within the olfactory processing pathway and leads to the loss of most odorant's major characteristic once in a complex mixture (Laing, 1991; Thomas-Danguin et al., 2017). Moreover, it was suggested that the olfactory processing pathway followed other rules in processing olfactory signals which balanced the two main perceptual necessities, which are stability (for recognition despite missing elements) and discrimination (for relevant choice) (Barnes et al., 2008). It showed that some individual piriform cortex neurons easily 'refilled' one missing element in an odor mixture, especially a familiar odor mixture, by processing some 'pattern completion', and clearly detected the novel element by processing some 'pattern separation', which suggested that one compound replacement in an odor mixture should be more easily detected than one compound removed (Barnes et al., 2008; Wilson & Sullivan, 2011). Our study did not directly evaluated the effects of addition or omission of odorants, but the results showed that the olfactory discrimination ability in human was more accurate for added elements, especially for mixture with less than three elements, which is in line with the hypothesis of perceptual stability under certain restrictions. Our results also showed that the main influence of 'omission' was observed on attributes not directly related to the manipulated odorants while the 'addition' of certain odorants leaded to a change in not only the odorant-related attribute of the added odorants but also other attributes. This finding is different from the conclusion of previous studies (V. Ferreira, 2010), which may be explained by the difference in concentration and

intensity of the added odorants. Besides, our results showed that regardless of the number of odorants in a mixture, at most only two attributes were found to be modulated. This finding should reflect the limitation of humans to process information about odors perceived simultaneously, which may have a major relevance with configural and elemental perception (Romagny et al., 2018).

In the experiment exploring the influence of odorants combination on odor profiles, we observed surprising phenomena, especially for the attributes cheese, smoky, and caramel. Indeed, the addition and omission of combinations containing guaiacol or 3-methylbutanoic acid was, as expected, able to significantly modulated the odorant-related smoky or cheese attribute. However, considering the omission of such combination did not affected these attributes, thus revealing a marked asymmetry in the mixture effects. This finding might be something challenging for neurophysiologists and be very meaningful for the way we should understand the hierarchical relationships between odorants. For the attribute caramel, its perception was rarely enhanced by adding ethyl 2-methyl butanoate or furaneol separately, but it was for the combination of these two compounds. This finding suggested that the two odorants can promote each other, or might even induce a synergy effect through blending, since the odor of strawberry and caramel, which match with the odor of ethyl 2-methyl butanoate and furaneol, have been showed to produce a pineapple-like odor (Barkat et al., 2012; Le Berre et al., 2010; Le Berre, Thomas-Danguin, et al., 2008). This study also addressed other perceptual interactions between different odorants or between the 6 common wine aroma vectors (N, norisoprenoids; A, branched acids; F, enolones; E, branched ethyl esters; L, fusel alcohols, M, wood compounds) at medium intensity. Nevertheless, our results cannot be easily compared with previous data (V. Ferreira et al., 2016), due to the difference in mixture samples and concentrations of odorants.

## 5.4 Conclusions

In this study, we investigated the perception of more than 150 odor mixtures to determine which odorants and odorant combinations showed perceptual interactions, and to identify the underpinning rules. The result showed that 1) the addition of impact odorants into icewine odor can affect several odor notes of the overall wine odor, and the impact became more significant as the intensity of the target odorants increased; 2) the olfactory discrimination ability was more sensitive for the addition of elements, especially in mixtures of less than three elements, compared to omission, which proved further evidence of perceptual stability under certain restrictions; Besides, some possible perceptual interaction between different odorants or between the 6 common wine aroma vectors were proposed, which could be verified or assessed in the future through psychological or neurobiological techniques. The result should be beneficial to understanding the interaction pattern between different odorants in icewine or other foods, to promoting quality control in the production of fast-moving consumer goods such as wine, and to gaining knowledge of the information processing mechanisms within the olfactory system.

# Chapter VI General Law of Olfactory Interaction in Binary Odor Mixtures

**Related to the publication**

Ma, Y., Tang, K., Xu, Y., & Thomas-Danguin, T. (2021). A dataset on odor intensity and odor pleasantness of 222 binary mixtures of 72 key food odorants rated by a sensory panel of 30 trained assessors. *Data in Brief*, 36, 107143.

Ma, Y., Tang, K., Xu, Y., & Thomas-Danguin, T. (2021). Perceptual interactions among food odors: Major influences on odor intensity evidenced with a set of 222 binary mixtures of key odorants. *Food Chem.*, 353, 129483. (refer to dataset I)

Ma, Y., Tang, K., Thomas-Danguin, T., & Xu, Y. (2020). Pleasantness of binary odor mixtures: rules and prediction. *Chem. Senses*, 45(4), 303-311. (refer to dataset II)


## Abstract

This study explored the general laws of olfactory interactions and their influence on intensity and pleasantness. The study selected 72 representative odorants in food and beverage systems based on the differences in their structural parameters, and 222 sets of binary odor mixtures were designed among the 2556 possible combinations. By measuring the intensity and pleasantness of the binary odor mixtures and of their two unmixed components, general laws of perceptual interactions, as well as hypotheses on their putative influencing factors in a simple system, were established. For odor intensity, the results showed that in most cases, the components' odor was perceived within the mixture and their intensity remained the same as in the unmixed situation in 54.3% of cases. Masking was the second major effect (44.8%) and occurred more frequently when components' pleasantness was significantly different. Synergy occurred in a small number of cases (0.9%) and only for four compounds in the tested set. The overall odor intensity of the mixture was determined to be equal to the strongest component in most cases (73.9%), while partial addition was observed as the second most frequent effect (21.7%), especially when the components had equal intensity. For odor pleasantness, in most cases, the pleasantness of the binary mixtures was driven by the pleasantness and intensity of its components. Nevertheless, a significant pleasantness partial addition was observed in 6 binary mixtures consisting of 2 components with similar pleasantness ratings. A mathematical model involving the pleasantness of the components as well as τ-values reflecting components' odor intensity proportions, was applied to predict mixture pleasantness. Using this model, the pleasantness of mixtures including two components with contrasting intensity and pleasantness could be efficiently predicted at the panel level ($R^2$ > 0.80, Root Mean Squared Error< 0.67). Overall, the results of this study should contribute to a better prediction of the outcome to expect when mixing key components of food odors.


**Keywords:** wine aroma vector, odor quality, olfactory processing, odor combination

## 6.1 Introduction

There is considerable overlap between odorants (i.e. odor-active compounds) found in different food products because they are produced from common precursors (e.g. amino acids, fatty acids, phenylpropanoids). Nevertheless, the overall odor perceived from these food products, namely from the corresponding mixtures of overlapping odorants, can be very different. This suggests that the variety of odor among the diversity of food products might be caused by subtle variations in the chemical composition of the volatile fraction of food. Indeed, it has been shown that a very small variation in the concentration of one odorant in a complex mixture can modify its odor quality (Le Berre, Beno, et al., 2008). Previous reports also pointed to the importance of specific combinations of a few odorants in the perceptual outcome of a mixture (Barkat et al., 2012; Lindqvist et al., 2012), which led to the definition of key associations in addition to key compounds of food odors (Romagny et al., 2018).

It is now widely accepted that the influence of odorants' concentration and odor quality on the perceptual features of an odor mixture is the result of various qualitative and quantitative perceptual interactions that could occur at different stages of the olfactory system (V. Ferreira, 2012b; Thomas-Danguin et al., 2014). Perceptual interactions between odorants have been observed in foods and beverages (Lytra, Tempere, Marchand, de Revel, & Barbe, 2016; San-Juan et al., 2011). These perceptual interactions are still a major lock in food flavor understanding and analysis since there are millions of possibilities for combining different food odorants at various concentration levels. Factors including the relative intensities of odorants, mixture complexity, component salience, trigeminal interactions (Walliczek-Dworschak et al., 2018), chemical structure, and possible peripheral interactions can influence odor mixture perception (Kay, Lowry, & Jacobs, 2003), and revealing the influencing factors and regular patterns of these interactions is crucial to understanding odor mixture perception, which has major relevance for food flavor perception and its underlying chemistry. In this way, an analysis of binary mixture perception is the first step in understanding the perception of more complex odors.

Early studies of the qualitative and quantitative odor properties of binary mixtures that were mixed at various intensity levels showed that hyper-addition is rare but may occur when both components are weak (V. Ferreira, 2012a). Moreover, these studies showed that the overall intensity of a mixture is mostly less than the sum of the intensities of the components (hypo-addition) but never less than the intensity of the weakest component (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012a; Laing et al., 1984; Laing & Willcox, 1983). These studies also suggested that the intensity ratio of the mixture constituents is the main driver of the odor mixture intensity and quality (Laing et al., 1984; Laing & Willcox, 1983; Olsson, 1994). When two compounds with dissimilar intensities are mixed, the overall odor intensity of the mixture will be well approximated by the intensity of the strongest component, and the quality of the most intense odorant tends to dominate the overall odor of the mixture (Berglund & Olsson, 1993b; Laing & Willcox, 1983). Considering the mixtures of two compounds with similar odor intensities, previous reports suggested that most binary mixtures follow a relatively similar pattern that is characterized by symmetry,

meaning that both odorants suppress each other equally (Laing et al., 1984). However, some odor pairs were observed to behave differently, and showed a marked asymmetry (Atanasova, Thomas-Danguin, Chabanet, et al., 2005). In these mixtures, one of the components induces a perceptual dominance over the other component (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012a, 2012b). The dominance phenomenon indicated that the intensity ratio is the primary factor but not the single factor that determines odor mixture interaction in binary mixtures, especially in isointense mixtures. Other perceptual features, such as odor pleasantness or odor familiarity could intervene (Livermore & Laing, 1996; Ma, Tang, Thomas-Danguin, & Xu, 2020; Sinding et al., 2015). It is also likely those psychophysical parameters, such as odor thresholds or Stevens' law exponent, could have a role but experimental demonstrations or replications are still lacking (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b; Laffort et al., 1989).

Different from odor quality and intensity, at present, only a few studies have attempted to investigate the pleasantness of binary mixtures. In the odor mixture literature, it is widely accepted that the pleasantness of a binary mixture tends to be an intermediate value between the pleasantness values of its components (Moskowitz & Barbe, 1977; Spence & Guilford, 1933) and that the perceived pleasantness is highly dependent on intensity (Laing, Eddy, & Best, 1994; Lawless, 1977). H. Lawless studied two binary mixtures, each composed of a pleasant and an unpleasant odorant at various concentration levels. He proposed a prediction model for the pleasantness of binary mixtures from the pleasantness of their constituents, weighted by their intensity (Lawless, 1977). However, until now, only a limited number of binary odor mixtures have been evaluated with regard to their pleasantness, calling into question the applicability of these rules in a wider range of odorants. Indeed, in natural products, a large range of odorants that span stimulus space have been identified. For instance, a total of 226 key food odorants were identified in 227 food samples (Dunkel et al., 2014). The latest available research on the pleasantness of binary mixtures was conducted by Lapid et al. (Lapid, Harel, & Sobel, 2008). By ranking the pleasantness and intensity of 5 distinct binary mixtures constructed with different ratios of the separate constituent odors, a prediction model with good performance was established (Lapid et al., 2008). This model proposed the possible prediction of the pleasantness of binary mixtures from the pleasantness of their separate constituents weighted by their respective perceived intensities. Interestingly, in this study, the authors observed a partial addition effect (V. Ferreira, 2012a; Thomas-Danguin et al., 2014) for the mixture of *L*-carvone and linalool at the 50–50% concentration ratio, meaning that the pleasantness of the mixture was higher than the pleasantness of the individual constituents (Lapid et al., 2008). These results underlined the key role of odor intensity in pleasantness and suggested that mixtures made of two components with similar intensities might show interesting pleasantness effects.

This study aimed to explore the impact of perceptual interactions on the odor intensity and odor pleasantness of binary mixtures while considering different odor properties of the components. This study was initiated because much of the recent psychophysical work on odor mixtures, even binary mixtures, has been obtained with a limited number of mixtures. In most instances, the odorants used in mixtures are not key food odorants, so the roles of odor

properties in food aroma-related perceptual interactions are not widely tested. Thus, a large set of 222 binary mixtures based on 72 odorants that span the stimulus space and occur in food products was selected to test the generalizability of previous findings and to identify the factors that are critical in odor mixture perceptual interactions. Besides, a mathematical model was adapted and then was applied to our dataset to predict the pleasantness of the 222 binary mixtures.

## 6.2 Materials and Methods

### 6.2.1 Subjects

One hundred twenty-five healthy subjects between the ages of 18 and 25 were recruited from Jiangnan University. Sixty-six of these subjects went through screening tests that evaluated their performance in discriminating between different odors qualities and different odor intensity levels, as well as their performance in logic scaling. To test their ability to evaluate odor quality and intensity, six samples comprising three different odorants at two concentration levels were provided. The subjects needed to sort these six samples into three groups based on their odor quality similarity and then rank the odor intensity of the samples within the same group. Only the subjects who answered both parts correctly, i.e., gathered the samples with the same odor quality into a group and then correctly ranked the odor intensity within the groups, were selected for the experiment. To further test subjects' scaling abilities, we provided six pictures proposed by Meilgaard et al. (Meilgaard, Carr, & Civille, 2006). These pictures had different shadowed areas, and subjects had to evaluate the approximate area using a linear scale. The values given by subjects were compared to the correct values, and only subjects who gave substantially incorrect ratings were not selected.

All subjects provided informed consent in line with the Helsinki Declaration, and six subjects quit after the training session, leaving 60 subjects (41 female) to participate in the experiment. Before the main experiment, subjects participated in 2 training sessions that aimed to provide standards for intensity scale use (see below). During the main experiment, not all the subjects evaluated all the samples (hereafter called trials because each trial included 3 odorized vials); for a given sample, 30 subjects performed the evaluation. Trials were randomly assigned to the subjects, who participated in a minimum of 3 and a maximum of 15 sensory sessions, with a maximum of 3 sessions occurring per week. During a session, participants evaluated 8 to 10 trials. Subjects were paid for their participation.

### 6.2.2 Stimuli

Odor-active compounds occurring in natural products were the focus. To select these odor-active compounds, we included the 226 key food odorants (KFOs) identified in Dunkel et al. (Dunkel et al., 2014) and added 548 different odor-active compounds collected in the Flavornet database (http://www.flavornet.org/). Among the total of 774 compounds, we finally selected 72 (Table 12) that covered the odorant physicochemical space (Weiss et al., 2012) and were easily available from providers. The idea of physicochemical space has been explained by (Snitz et al., 2013). and used by the same group in (Weiss et al., 2012). The so-called 'physicochemical odor space' is based on the fact that odorants can be described by

a large set of structural and physicochemical descriptors. It is thus possible to map large set of odorants using these descriptors that measure various properties and to obtain a 'space' of odorants based on their physicochemical properties. Applied to our study, we considered 226 key food odorants (KFOs) identified in (Dunkel et al., 2014) and 548 different odorants collected in the Flavornet database (http://www.flavornet.org/). For this set of 774 odorants we obtained circa 4000 physicochemical descriptors using the Dragon® software (Talete, Milan, Italy). We mapped the set of odorants using the physicochemical descriptors and we selected 72 odorants that cover the whole map. Doing this, we aimed to cover a large range of structural and physicochemical properties, but using a limited set of odorants.

Most odorants were purchased from Sigma-Aldrich China Co. (Shanghai, China) in the highest available purity, except for *p*-anisaldehyde (obtained from Fluka) and 3-mercaptohexanol (obtained from ACROS Organics). Ultimately, 198 different binary odor mixtures (Supplementary Table 9), plus 24 duplicated binary odor mixtures, made from the 72 odorants were designed for the experiments based on their odor characteristics.

All odorants were diluted with odorless solvents which were 1,2-propanediol, or mineral oil or deionized distilled water depending on odorant solubility. To avoid large differences in intensity and to keep it in a narrow range for all samples, odorants were first diluted to a point approximately equal to the odor intensity of ethyl 2-methylbutanoate at a concentration of 3.9 g/L, as estimated by experienced lab members. Then, we prepared a set of solutions of odorants varying around the obtained concentration. These solutions were presented to 6 subjects who did not participate in the main experiment and who were instructed to provide a number between 0 and 7 reflecting the solution odor intensity. For each odorant, the final concentration (Table 12) was set after the rough iso-intensity and was defined following the procedure described in Weiss et al. (Weiss et al., 2012).

**Table 12** Information and Final Concentration of Odorants Used in Each Trial

| CAS. | Odorant | Odor | Cons. (mg/mL) | Solvent | Purity | Trial number* |
|---|---|---|---|---|---|---|
| 4180-23-8 | 1-methoxy-4-[(*E*)-prop-1-en-1-yl ]benzene(trans-anethol) | anise | 4.40 | mineral oil | ≥99% | 35, 84, 90 |
| 100-52-7 | benzaldehyde | almond | 3.82 | 1,2-propanediol | 99% | 33, 135 |
| 7452-79-1 | ethyl 2-methylbutanoate | apple | 3.99 | 1,2-propanediol | 99% | ***5***, 16, ***22***, ***32***, ***36***, 58, ***59***, ***60***, 61, ***62***, ***63***, ***64***, 67, ***72***, 87, 92, ***94***, 95, 100, 104, 105, ***119***, ***134***, ***145***, 151, ***202***, ***215***, 219, 222 |
| 6378-65-0 | hexyl hexanoate | apple peel | 7.41 | 1,2-propanediol | >98% | 18, 133, 171, 181 |
| 104-67-6 | *γ*-undecalactone | apricot | 33.5 | 1,2-propanediol | 99% | 49, 118, 125, 162, 174, 189 |
| 123-35-3 | myrcene | balsamic | 1.62 | mineral oil | ≥97% | 38, 44, 45, ***60***, 82, ***94***, 103, 106, 107, ***116***, 136, 137, 205, ***210***, 218 |
| 431-03-8 | diacetyl | butter | 9.89 | 1,2-propanediol | 99% | ***5***, ***32***, 169, 194 |
| 513-86-0 | acetoin | butter | 25.2 | water | ≥97% | 68, 146 |
| 3658-80-8 | dimethyl trisulfide | cabbage | 0.000805 | 1,2-propanediol | ≥97% | 65 |
| 96-48-0 | *γ*-butyrolactone | caramel | 143 | 1,2-propanediol | ≥97% | 79 |
| 103-36-6 | ethyl cinnamate | cinnamon | 8.25 | 1,2-propanediol | 98% | 30, 82, 88, 177, 178, 187, 193 |
| 4630-07-3 | valencene | citrus | 4.91 | mineral oil | ≥65% | ***36***, 37, ***71***, 77, ***124***, 126, ***130***, 195, ***201***, ***202***, 203, 209, 216 |
| 99-87-6 | *p*-cymene | citrus | 3.27 | mineral oil | ≥97% | 2, 7, 11, ***13***, ***14***, ***15***, 16, 17, 18, ***25***, ***28***, 43, 46, ***116***, 200, 208, 209, ***210***, ***211***, 214 |
| 97-53-0 | eugenol | clove | 0.467 | 1,2-propanediol | 99% | 21, 56, 80, ***86***, ***175***, 176, 185, 191, 217 |
| 105-21-5 | *γ*-heptalactone | coconut | 4.30 | 1,2-propanediol | ≥98% | 4, 8, ***14***, 20, ***26***, ***28***, ***29***, 30, 51, 52, 53, ***54***, ***62***, 85, 98, 108, 109, 110, ***114***, 118, ***119***, 120, 144, 149, 150 |
| 3268-49-3 | methional | cooked potato | 0.0680 | 1,2-propanediol | ≥97% | 4, 156, 157, ***163***, 167, ***182***, 197 |
| 695-06-7 | 4-hexanolide | coumarin | 3.43 | 1,2-propanediol | ≥97% | 58 |

| CAS. | Odorant | Odor | Cons. (mg/mL) | Solvent | Purity | Trial number* |
|---|---|---|---|---|---|---|
| 13623-11-5 | trimethylthiazole | earth | 1.01 | 1,2-propanediol | ≥98% | 100, 128 |
| 106-33-2 | ethyl laurate | fat | 97.9 | 1,2-propanediol | 99% | 19 |
| 111-13-7 | 2-octanone | fat | 1.11 | 1,2-propanediol | ≥97% | 40, 81, 121, 197, 203, 206, 217 |
| 111-70-6 | 1-heptanol | fat | 11.8 | 1,2-propanediol | ≥99% | 12, 41, 56, 144 |
| 124-13-0 | octanal | fat | 2.44 | 1,2-propanediol | 99% | 47, 57 |
| 106-25-2 | nerol oxide | flower | 2.66 | mineral oil | ≥97% | 53, 133, 170, 180 |
| 140-11-4 | benzyl acetate | flower | 8.08 | 1,2-propanediol | 99% | 39, 83, 89 |
| 78-70-6 | linalool | flower | 6.12 | 1,2-propanediol | ≥80% | 21, 123, ***163***, 172, ***182***, 206, 214, 220 |
| 551-93-9 | *o*-aminoacetophenone | foxy | 49.8 | 1,2-propanediol | 98% | 97, 136 |
| 101-97-3 | ethyl phenylacetate | fruit | 1.18 | 1,2-propanediol | 99% | 27, 55, 57, 81, 87, 176, 177, 186, 192, 218 |
| 105-37-3 | ethyl propionate | fruit | 1.30 | 1,2-propanediol | 99% | 51 |
| 105-54-4 | ethyl butanoate | fruit | 8.14 | 1,2-propanediol | 99% | 33, 121, 168, 183, 198 |
| 105-57-7 | diethyl acetal | fruit | 2.00 | 1,2-propanediol | 99% | 41, 45, 49, ***63***, 70, ***71***, ***72***, 73, 90, 111, ***112***, ***127***, ***130***, 139, 148, 150, 153 |
| 106-32-1 | ethyl octanoate | fruit | 8.47 | 1,2-propanediol | ≥99% | 83, 117, 170, 185, 212 |
| 107-87-9 | 2-pentanone | fruit | 1.66 | 1,2-propanediol | 98% | 31 |
| 108-64-5 | ethyl 3-methylbutanoate | fruit | 0.127 | 1,2-propanediol | 98% | 74, 91, 155, 160, 161, 165, 180, 195 |
| 2305-05-7 | *γ*-dodecalactone | fruit | 22.4 | 1,2-propanediol | ≥97% | 23, 174, 184 |
| 539-82-2 | ethyl valerate | fruit | 7.18 | 1,2-propanediol | ≥98% | 2, 123, 155, 156, 162, 166, 181, 196 |
| 137-32-6 | 2-methyl-1-butanol | fusel oil | 7.20 | 1,2-propanediol | ≥99% | 43 |
| 66-25-1 | hexanal | grass | 1.26 | 1,2-propanediol | 98% | 10, 11, 38, 198, 204, ***207***, ***213*** |
| 928-96-1 | (*Z*)-3-hexenol | grass | 1.44 | 1,2-propanediol | 98% | 9, 37, 42, ***59***, ***145***, 161, 199, 204, 205 |
| 123-72-8 | butanal | green | 10.8 | 1,2-propanediol | ≥97% | 34 |
| 103-45-7 | *β*-phenethyl acetate | honey | 9.92 | 1,2-propanediol | 99% | ***15***, 79, 85, ***86***, ***175***, 190, ***211***, 216 |
| 122-78-1 | phenylethanal | honey | 2.32 | 1,2-propanediol | ≥90% | 88, 120, 151, 152 |
| 96-17-3 | 2-methylbutanal | malt | 3.57 | 1,2-propanediol | 95% | 76, 108 |
| 106-44-5 | *p*-cresol | medicine | 5.53 | 1,2-propanediol | ≥97% | 40, 154 |

| CAS. | Odorant | Odor | Cons. (mg/mL) | Solvent | Purity | Trial number* |
|---|---|---|---|---|---|---|
| 470-82-6 | 1,8-cineole | mint | 4.62 | 1,2-propanediol | ≥97% | 1, 7, 8, 9, 24, 68, ***75***, ***124***, 128, ***131***, 196, ***201***, 212 |
| 543-49-7 | 2-heptanol | mushroom | 1.74 | 1,2-propanediol | ≥97% | 39, 105, 110, 143 |
| 13327-56-5 | ethyl 3-(methylsulfanyl)propanoate | onion | 0.373 | 1,2-propanediol | >99% | 34, 42, 46, ***50***, ***54***, ***64***, ***69***, 73, 74, ***75***, ***113***, ***114***, 115, ***131***, ***132***, ***134***, 135, 137, 140, 154 |
| 111-11-5 | methyl octanoate | orange | 2.73 | 1,2-propanediol | 99% | 61 |
| 706-14-9 | *γ*-decalactone | peach | 7.36 | 1,2-propanediol | ≥98% | 78, 140, 173, 188, ***207***, ***213*** |
| 123-86-4 | butyl acetate | pear | 2.31 | 1,2-propanediol | 99% | 6, 158, 159, 167, 192 |
| 505-10-2 | methionol | potato | 0.593 | 1,2-propanediol | 98% | 55, 166, 191 |
| 110-62-3 | pentanal | pungent | 12.0 | 1,2-propanediol | ≥95% | 31, 66 |
| 693-95-8 | 4-methylthiazole | roasted meat | 0.385 | 1,2-propanediol | ≥98% | 98, 101 |
| 104-76-7 | 2-ethylhexanol | rose | 9.46 | 1,2-propanediol | ≥99% | 10, 78, 107, 141 |
| 105-87-3 | geranyl acetate | rose | 10.4 | 1,2-propanediol | 98% | 76, 138, 171, 186 |
| 106-22-9 | citronellol | rose | 3.65 | 1,2-propanediol | ≥97% | 48, 77, 139, 172, 187 |
| 106-24-1 | geraniol | rose | 4.79 | 1,2-propanediol | 98% | ***22***, 142, 164, 173, 183, 208, ***215*** |
| 4410-99-5 | phenylethylthiol | rubber | 0.279 | 1,2-propanediol | ≥97% | 6, 12, 17, 23, 66, 67, ***69***, 89, ***96***, 102, 103, ***112***, 122, ***127***, ***129***, ***132***, 147, 149, 152 |
| 3391-86-4 | 1-octen-3-ol | soap | 1.48 | 1,2-propanediol | 98% | 52, 102, 142 |
| 821-55-6 | 2-nonanone | soap | 3.05 | 1,2-propanediol | ≥97% | 48 |
| 99-48-9 | carveol | spearmint | 9.68 | 1,2-propanediol | 97% | 35, 80, ***96***, 104, 106, 109, 125, ***129*** |
| 2785-89-9 | 4-ethylguaiacol | spice | 3.58 | 1,2-propanediol | 98% | 99, 179, 189, 219 |
| 97-54-1 | isoeugenol | spice | 3.33 | 1,2-propanediol | ≥97% | 91, 115, 178, 179, 188, 194 |
| 88-15-3 | acetylthiophene | sulfur | 1.62 | 1,2-propanediol | ≥98% | 99, 111 |
| 503-74-2 | isovaleric acid | sweat | 79.0 | 1,2-propanediol | 98% | 3 |
| 112-44-7 | undecanaldehyde | sweet | 0.357 | 1,2-propanediol | ≥97% | 20 |
| 123-11-5 | *p*-anisaldehyde | sweet | 4.24 | 1,2-propanediol | ≥99% | 95 |
| 97-62-1 | ethyl isobutanoate | sweet | 4.49 | 1,2-propanediol | 99% | 1, 70, 159, 160, 168, 193, 200 |

| CAS. | Odorant | Odor | Cons. (mg/mL) | Solvent | Purity | Trial number* |
|---|---|---|---|---|---|---|
| 18640-74-9 | isobutyl thiazole | tomato leaf | 0.315 | 1,2-propanediol | 99% | 101, 138 |
| 51755-83-0 | 3-mercaptohexanol | tropical fruit | 0.593 | 1,2-propanediol | 98% | 220, 221, 222 |
| 121-33-5 | vanillin | vanilla | 0.786 | 1,2-propanediol | 98% | 65, 93, 122, 153 |
| 123-51-3 | 3-methyl-1-butanol | whisky | 2.06 | 1,2-propanediol | ≥99% | 47, 141, 157, 158, 164, 165, 169, 184, 190, 199, 221 |
| 123-25-1 | diethyl succinate | wine | 388 | 1,2-propanediol | 98% | 3, ***13***, 19, 24, ***25***, ***26***, 27, ***29***, 44, ***50***, 84, 92, 93, 97, ***113***, 117, 126, 143, 146, 147, 148 |

* The 24 trial numbers marked with bold fonts are duplicate trials

### 6.2.3 Sample Preparation

To prevent the formation of novel chemicals in the mixtures, odorants were not mixed in the liquid phase. For the unmixed odor samples, 200 μL of the diluted stimulus was poured onto a 0.1 g cotton ball and placed in a 20 mL brown glass bottle with a black screw cap. For the binary mixtures, 200 μL of each stimulus was poured onto separate sides of the 0.1 g cotton ball, such that the two odorants' vapors alone mixed in the glass bottle headspace. All of the stimuli were fully absorbed by the cotton ball. All samples were prepared one day before the sensory session and stored at room temperature (24°C)

### 6.2.4 General Procedures

Before the formal experiment, we began with two training sessions. The first session determined the standard odor references to be used in the experiment. Ethyl 2-methylbutanoate and linalool were selected as reference odor-active compounds because the majority of panelists did not object to sniff it frequently, and because their corresponding odors (fruity-green-apple and floral-citrus-lavender, respectively) were rather familiar to the participants, which might have helped them to memorize. To determine the standard intensity of these references, we gave participants ethyl 2-methylbutanoate (1.8 g/L) and linalool (10.7 g/L) and asked them to rate the intensity of these two samples. We asked them to evaluate ethyl 2-methylbutanoate first, and then, they need to evaluate the intensity of linalool by comparing the intensity of linalool with the intensity of ethyl 2-methylbutanoate. If the intensity of linalool smelled twice as strong as ethyl 2-methylbutanoate, its intensity was marked twice the distance from zero as the position of ethyl 2-methylbutanoate. The standard intensity was obtained by calculating the mean value of these ratings across all subjects. The intensities of standard I (ethyl 2-methylbutanoate) and standard II (linalool) were finally anchored as 3.0 and 7.0, respectively. The second session introduced the odor evaluation procedures. During this session, the two standards were provided to the subjects, and they were told that they needed to rate the perceived intensity of the samples presented during the formal sessions using the anchor intensities of the two standards.

In the formal sessions, a total of 222 trials, among which 24 were duplicated trials, were evaluated. Each session in the formal experiment comprised 14 to 15 trials, and each trial included three stimuli: two stimuli were single odorants, and the third stimulus was a binary mixture of these odorants. In each trial, all the unmixed odor samples were coded by three random digits, and the binary mixture sample was coded by its trial number. The binary mixture was always presented first and the order of presentation of the two unmixed odors was counterbalanced for each trial, and each trial was presented to the subjects in a random order. Subjects were given a rest of 45 seconds between each stimulus. Each trial was presented to subjects in a random order, and one trial was evaluated by a maximum of 30 subjects.

Each session included two parts (Figure 20). The first part consisted of a hedonic evaluation, and the other part consisted of intensity evaluations. During the hedonic evaluation, subjects had to mark off distance on a visual analog scale 100-mm in length (Figure 20).

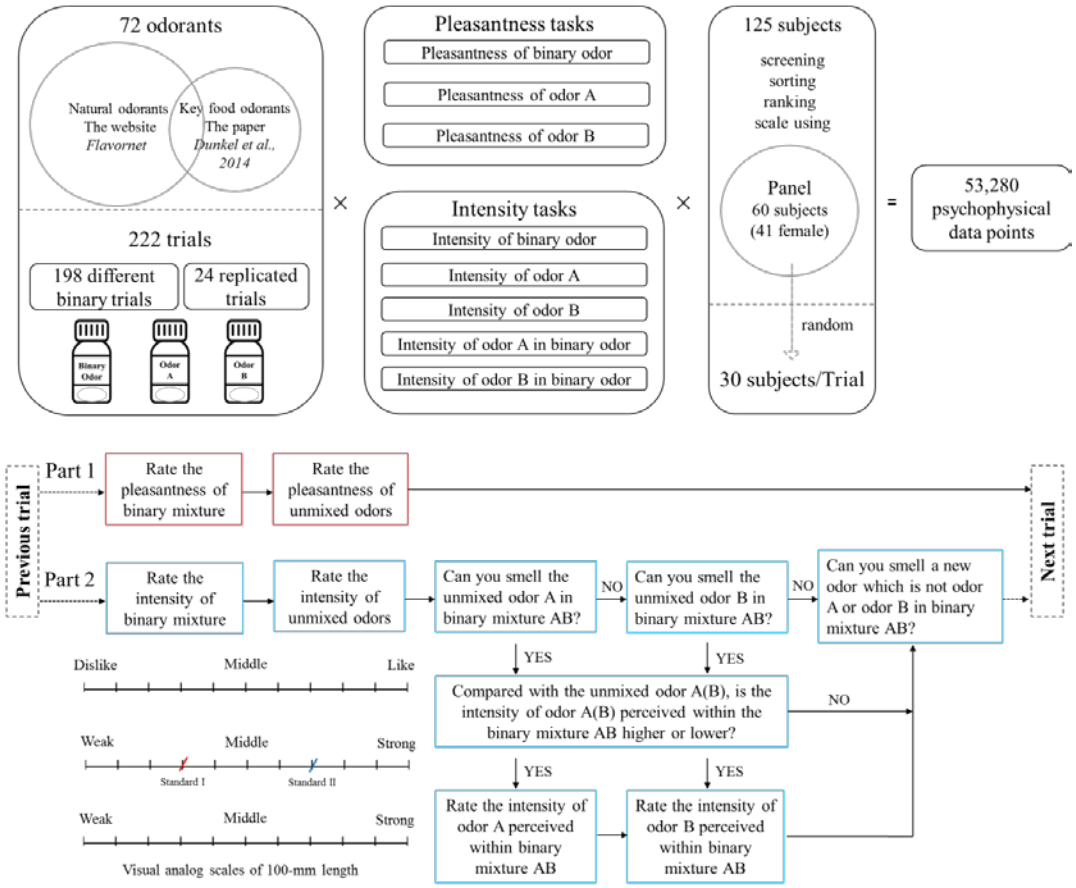


**Figure 20** Schematic of psychophysical experiment data collection. The upper scale shown in the figure presented two ticks labeled as standard 1 and standard 2. This scale was only provided in an instruction sheet during the training session along with the standard samples. This instruction sheet was also provided at the beginning of the first two sessions to remind subjects with the intensity scale. However, for all the samples' evaluation, we only used the bottom scale without the two ticks.

For the intensity evaluations, an adjusted explicit anchoring scale with markers of two standards that were determined in the training session was utilized in the odor intensity evaluation. This kind of anchoring scale, with the reference standards used in this study, was aimed at familiarizing the panelists with the scale in a similar way across the range of intensity. This scale has been employed in texture analysis and might generate more reliable sensory data by reducing the variability among the panelists (Muñoz, 1986). To rate the intensity, the subjects had to mark off a distance on the visual analog scale according to the two perceptual anchors. They were instructed that if the test stimulus smelled half as strong as the standard, its intensity should be marked half the distance, while if the stimulus smelled twice as strong as the standard, its intensity should be marked twice the distance from zero as the standard position. They should consider the two references to rate a given sample intensity (Figure 20). The two standards were presented in the first two sessions to help the subjects rate the odor intensity. To evaluate the intensity ($I_{Amix}$, $I_{Bmix}$) of the two components' odor perceived within the mixture, the subjects had to indicate if they perceived odor A and/or odor B in the mixture and then had to evaluate the intensity ($I_{Amix}$, $I_{Bmix}$) of the two components' odor perceived within the mixture in comparison to the intensity of the unmixed components. If the subjects perceived the intensity ($I_{Amix}$, $I_{Bmix}$) of the two components' odor in the mixture

to exceed the maximum of the scale, they were told to indicate it using a note. In this case, a maximum value of 11 for intensity was attributed instead of a maximum value of 10, which corresponds to the maximum value of the scale.

### 6.2.5 Data Processing

Psychophysical data obtained from the scales were transformed into numerical values. All the scores within the range of the scale were translated to a value between 0.0 and 10.0. Statistical analyses were performed with R software (version 3.5.3).

Principal component analysis (PCA) was applied to check the panel performance. A nonparametric mixed-effects analysis of variance (ANOVA) was applied to test the repeatability of 24 duplicated trials for each intensity variable using the *lmer* function and *glmer* functions from the *lme4* package (Bates, Mächler, Bolker, & Walker, 2014), and the subject effect and trial effect were set as random factors. Bonferroni correction was applied to account for multiple testing when necessary. Differences between trials were analyzed using an unpaired two-sample Wilcoxon test using the *wilcox.test* function from the *ddply* package (Wickham, 2011). The averages across subjects of the intensity or pleasantness of each odorant were compared to the mean values across odorants using unpaired Wilcoxon test from the *ggpubr* package (Kassambara, 2019). The difference between odor intensity (resp. pleasantness) of a binary mixture and its two components in each trial was analyzed using a paired Wilcoxon test (*wilcox.test* function), including: $I_A$~$I_B$, $P_A$~$P_B$, $I_A$~$I_{Amix}$, $I_B$~$I_{Bmix}$, $I_{AB}$~$I_A$, $I_{AB}$~$I_B$, $P_{AB}$~$P_A$, $P_{AB}$~$P_B$. Bonferroni correction was applied to account for multiple testing when necessary. Fisher's exact test was performed to test whether the different types of mixture-induced effects occurred dependently on the relative intensity, pleasantness and key food odorant (KFO) frequent value, which has been published by Dunkel (Dunkel et al., 2014). In all tests, results were considered to be statistically significant when $p< 0.05$.

The Tau-based model involving the $\tau$-value, which reflects the relative proportion of the perceived intensity of odor A or odor B to the sum of their intensities (Patte & Laffort, 1979), was applied to predict the binary mixture pleasantness.

$$P_{AB} = \tau_A P_A + \tau_B P_B$$

$$\tau_A = \frac{I_A}{I_A + I_B} \text{ or } \tau_B = \frac{I_B}{I_A + I_B}$$

This model was equivalent to the intensity weights model proposed by Lapid et al. (Lapid et al., 2008). The performance of this prediction model was tested by computing the prediction error Root Mean Squared Error (*RMSE*) and the R-square ($R^2$) between experimental and predicted values.

This model was applied to predict the mixture odor pleasantness at *panel* level as well as *individual* level. In the *panel* approach, a single pleasantness value was predicted for a given mixture, while in the *individual* approach, a pleasantness value was predicted for each subject for a given mixture. In addition, for the *panel* approach, predicted pleasantness was calculated using either the average pleasantness across trials and mean $\tau$-value of the 2 components (*mean* condition, Equation 7) or using the average pleasantness across subjects and $\tau$-values of each trial (*trial* condition, Equation 8).

$$P_{AB\_mean} = \tau_{A\_mean} P_{A\_mean} + \tau_{B\_mean} P_{B\_mean} \quad \text{(Equation 7)}$$

$$P_{AB\_trial} = \tau_{A\_trial} P_{A\_trial} + \tau_{B\_trial} P_{B\_trial} \quad \text{(Equation 8)}$$

For example, ethyl 2-methylbutanoate is an odorant that was used in 29 trials. In the *mean* condition of the *panel* approach, a single value of pleasantness and a single value of $\tau$ were calculated and used to predict the pleasantness of all the mixtures including this odorant. In contrast, in the *trial* condition of the *panel* approach, one average value of pleasantness and one $\tau$ value across subjects were calculated for each trial to predict one value of mixture pleasantness per trial.

In the *individual* approach, we also considered the two conditions *mean* and *trial*. Thus, predicted pleasantness was calculated for each subject using either the average pleasantness across trials and mean $\tau$-value of the 2 components (*mean* condition, Equation 9) or using the pleasantness value and $\tau$-value from a given subject on each trial (*trial* condition, Equation 10).

$$P_{AB\_subject} = \tau_{A\_mean/subject} P_{A\_mean/subject} + \tau_{B\_mean/subject} P_{B\_mean/subject} \quad \text{(Equation 9)}$$

$$P_{AB\_subject} = \tau_{A\_subject,\ value\ /\ trial} P_{A\_subject,\ value\ /\ trial} + \tau_{B\_subject,\ value\ /\ trial} P_{B\_subject,\ value\ /\ trial} \quad \text{(Equation 10)}$$

For example, for ethyl 2-methylbutanoate in the *mean* condition of the *individual* approach, an individual value of pleasantness and $\tau$ was calculated across trials and was used to predict the individual mean pleasantness of mixtures including this odorant, whereas in the *trial* condition of the *individual* approach, one value of pleasantness and $\tau$ was calculated per trial for each subject.

In addition to the Tau-based model (intensity weights model), the squared model and the sin model (Lapid et al., 2008) were applied to predict the binary mixture pleasantness. Using the *cor.test* function, the prediction performances of the three models were compared based on the Pearson's product moment correlation coefficient between predicted and experimental pleasantness and the 95 percent confidence interval on this correlation coefficient. The formula of each model and the correlation results were provided in Supplementary Table 11. The results showed that there was no significant difference between the three models since there is an overlap of the 95 percent confidence interval within each prediction approach/condition. Hereafter, only the simplest Tau-based model was considered.

## 6.3 Results and Discussions

### 6.3.1 Panel Performance and Repeatability

The subjects' overall performance and coherence were checked using PCA on the dataset I (for the intensity paper: $I_A$, $I_B$, $I_{Amix}$, $I_{Bmix}$, $I_{AB}$) and dataset II (for the pleasantness paper: $I_A$, $I_B$, $P_A$, $P_B$, $P_{AB}$). The PCA map is reported in Supplementary Figure 11 and Supplementary Figure 12. In dataset I, we discovered that one subject was a systematic outlier. Therefore, the data from this participant were discarded. In dataset II, we checked the individual results from the subjects outside of the central cloud for the different variables more in depth, and we did not identify any systematic outliers. Therefore, all the data were kept for further analyses.

Nonparametric Mixed Effects ANOVA was applied to test repeatability using the 24 duplicated trials for each attribute. Variables included the intensity of odor A ($I_A$) or odor B ($I_B$), the intensity of odor A ($I_{Amix}$) or odor B ($I_{Bmix}$) perceived within the mixture, the intensity of the binary mixture ($I_{AB}$), the pleasantness of odor A ($P_A$) or odor B ($P_B$), and the pleasantness of the binary mixture ($P_{AB}$). The results indicated no significant repetition effects ($p> 0.05$), except for the pleasantness of odor A ($P_A$, $p< 0.001$) and the intensity of odor A perceived within the mixture ($I_{Amix}$, $p<0.05$). An in-depth investigation of the repeatability of attributes $I_{Amix}$ for each repeated trial revealed no significant differences between the replicates (Wilcoxon test with Bonferroni correction for multiple testing). By checking the repeatability of attributes $P_A$ for each repeated trial, only the means of Trial 36 (ethyl 2-methylbutanoate, Supplementary Table 9) was found to be significantly different between the replicates (Wilcoxon-test with Bonferroni correction). Ethyl 2-methylbutanoate was used 29 times in the whole experiment (Table 12); thus, the pleasantness rating might have evolved as a result of increasing familiarity with the odor of this compound. Although the pleasantness of ethyl 2-methylbutanoate might have been overrated at the end of the pleasantness evaluation, the statistical assessment showed that the panel could rate odor intensity and pleasantness consistently and consensually in most cases and that the psychophysical data were statistically reliable.

### 6.3.2 Intensity and Pleasantness of the 72 Odorants (dataset II)

The mean intensity and pleasantness of each odorant were calculated across subjects in all trials (Figure 21). Uncorrected unpaired Wilcoxon test was used to test the difference between the intensity of each odorant and the mean intensity value across odorants. Although we tried to provide stimuli that had similar intensities (preliminary test with external panel of six subjects), the results showed that there were 19 out of 72 odorants whose odor intensity was significantly different from the mean value ($p< 0.001$). Among these odorants, ethyl octanoate, *o*-aminoacetophenone, ethyl valerate, *p*-cresol, *γ*-undecalactone, butanal, pentanal, phenylethylthiol and benzaldehyde had intensities that were significantly higher than the mean intensity ($p< 0.001$), with intensities ranging from 6.53 to 7.71, while the intensities of ethyl laurate, undecanaldehyde, 2-pentanone, vanillin, *γ*-butyrolactone, eugenol, ethyl 3-(methylsulfanyl)propanoate, nerol oxide, carveol, geraniol and isoeugenol were significantly lower than the mean intensity ($p< 0.001$), with intensities ranging from 3.68 to 5.15 (Supplementary Figure 14). Uncorrected unpaired Wilcoxon test was also used to test the difference between the pleasantness value of each odorant and the mean value, and there were 19 odorants whose odor pleasantness was significantly different from the mean value ($p< 0.001$) (Supplementary Figure 14).

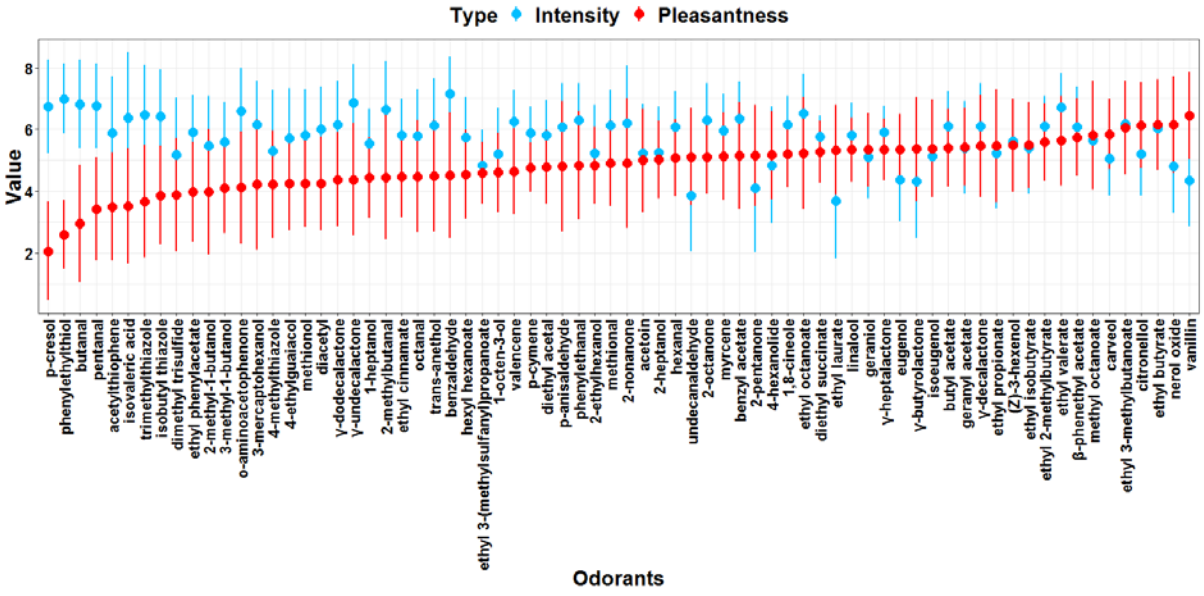


**Figure 21** Intensity and pleasantness of 72 odorants calculated across all the subjects in all the trials

### 6.3.3 Perception of Components' Odor within Mixtures (dataset I)

To highlight the mixture effect on the intensities of both components perceived in mixtures, we compared the intensities of the two components ($I_A$, $I_B$) out of mixture and those within mixture ($I_{Amix}$, $I_{Bmix}$). Because the choice of odor A and B is arbitrary, the notation is equivalent in the following and A can be replaced by B. We calculated the percent of the mixture-induced intensity change (IC) for an individual odorant's odor ($IC_A$, $IC_B$; Equation 2).

If IC is equal to 0, the intensity of the odor of the target odorant is not modified in the mixture, that is, *independence* occurs (Thomas-Danguin et al., 2014). If IC is less than 0, the odor intensity of the target odorant is reduced, that is, *masking* occurs; if IC is greater than 0, *synergy* occurs for the odor of the target odorant. The results are reported in Supplementary Figure 15, in which odorants in rows and columns were ordered by average intensity or pleasantness, but no specific pattern of interactions can be identified based on these perceptual dimensions. In most cases (90%), IC was less than 0 (*masking*), and this effect occurred for both components in most cases (82.8%). In other cases (10%), IC was higher than 0 (*synergy*), but it rarely occurred for both components in the mixture (3.6%). To check for statistical significance in these perceptual interaction effects, the difference between $I_{Amix}$ and $I_A$ was analyzed with a paired Wilcoxon test. These analyses confirmed that in most cases (54.3%), *independence* was significant, while significant *masking* occurred for 44.8% of trials and significant *synergy* occurred for only 0.9% of cases. The odorants for which synergy was significant were vanillin (mixed with diethyl acetal), ethyl isobutanoate (mixed with ethyl 3-methylbutanoate), ethyl 2-methylbutanoate (mixed with diethyl acetal), and methional (mixed with 3-methyl-1-butanol). Interestingly, the four compounds for which significant synergy occurred were also considered as key food odorants (KFO). The KFO status was obtained from (Dunkel et al., 2014); the higher the value, the more frequently the odorant appeared to be an important odorant for the flavors of different kinds of foods (Table 13).

**Table 13** Four Odorants that Showed Significant Synergistic Effects in Their Corresponding Mixtures

| Compounds | | KFO value (%)[a] | Odor quality[b] | Intensity | Pleasantness | Mixture-induced Effect[c] |
|---|---|---|---|---|---|---|
| Mixture 1 | **methional** | **53.7** | **cooked potato** | **5.98** | **4.53** | **synergy*** |
| | 3-methyl-1-butanol | 13.7 | whiskey | 5.49 | 3.97 | masking* |
| Mixture 2 | **vanillin** | **27.8** | **vanilla** | **4.03** | **6.48** | **synergy*** |
| | diethyl acetal | 5.7 | cream | 5.40 | 4.74 | independence |
| Mixture 3 | **ethyl 2-methylbutanoate** | **28.2** | **apple** | **5.16** | **5.43** | **synergy*** |
| | diethyl acetal | 5.7 | cream | 6.05 | 4.35 | independence |
| Mixture 4 | **ethyl isobutanoate** | **22.5** | **fruity** | **5.24** | **5.82** | **synergy*** |
| | ethyl 3-methylbutanoate | 0.0 | fruity | 5.79 | 6.34 | independence |

Note: [a] KFO value correspond to Key Food Odorant, which has been published by Dunkel (Dunkel et al., 2014), the higher the value, the more frequently the odorant appeared to be an important odorant for the flavors of different kinds of foods; [b] The odor quality was obtained from Flavornet (*http://www.flavornet.org/flavornet.html*); [c] Symbol '*' stands for $p < 0.05$

Considering the perception of the two components' odor within mixtures, we observed 5 different combinations: 1) masking for one component and independence for the other component (47.7%); 2) independence for the two components (29.7%); 3) masking for the two components (20.7%); 4) synergy for one component and independence for the other component (1.4%) and 5) synergy for one component and masking for the other component (0.5%). The only theoretical case that we did not observe experimentally is synergy for both components. To explore the distribution of these types of interactions as a function of both the intensity and the pleasantness, the 222 trials were classified into four groups based on the difference in intensity and pleasantness between the two components. Group E (55 trials) included mixtures for which no significant difference was observed in either intensity or pleasantness between components. Group I (60 trials) included mixtures for which a significant difference in intensity between components was observed. Group P (47 trials) included mixtures for which a significant difference in pleasantness between components was observed. Group IP (60 trials) included mixtures for which a significant difference in both intensity and pleasantness between components was observed. We calculated the relative proportion of each group within each type of mixture-induced interaction and showed the results graphically in Figure 22a. The proportions were compared using Fisher's exact test (Supplementary Table 10), which revealed that the type of mixture-induced interactions was significantly different only for mixtures in which the two components differed in terms of pleasantness (groups P and IP) (Figure 22b). More precisely, masking occurs more frequently ($p$= 0.016) when pleasantness is significantly different between the two components of the binary mixture.

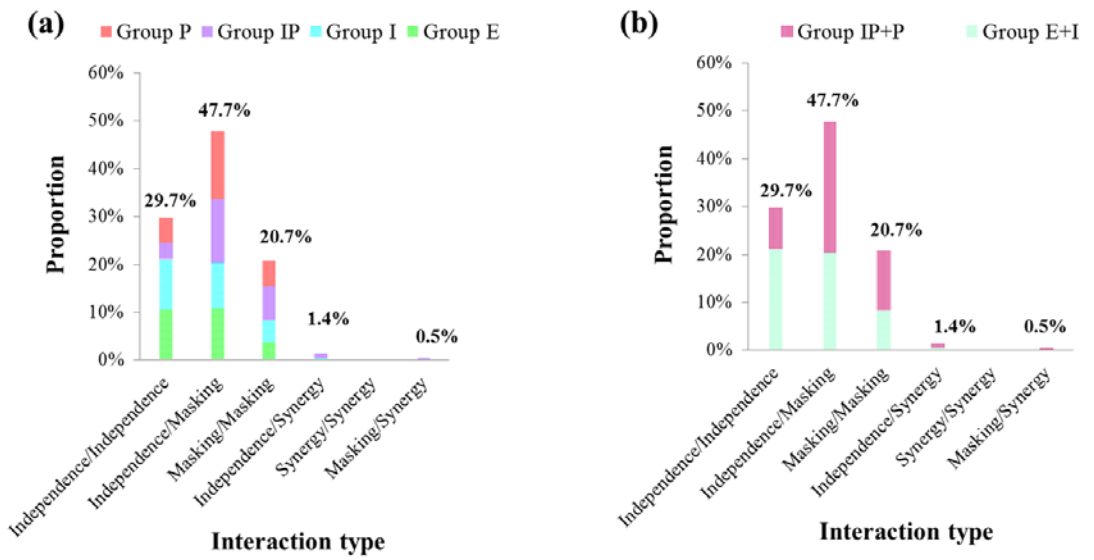


**Figure 22** Experimental observations of perceptual interactions within binary mixtures regarding odor intensity of components within the mixture. **Panel (a)** represents the frequency of mixture-induced effects on the intensity of components' odor within mixtures as a function of components' intensity and pleasantness: Group E included mixtures made with odorants that are not significantly different in their unmixed odor intensity and pleasantness; Group I included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness but significantly different in intensity; Group P included mixtures made with odorants that are not significantly different in their unmixed odor intensity but significantly different in pleasantness; and Group IP included mixtures for which a significant difference in both intensity and pleasantness. **Panel (b)** represents the frequency of mixture-induced effects on the intensity of components' odor within mixtures as a function of components' intensity and pleasantness: Group E+I (green color) included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness, and group P+IP (red color) included mixtures made with odorants that are significantly different in their unmixed odor pleasantness.

### 6.3.4 Overall Intensity of Binary Mixtures (dataset I)

The Sigma-Tau ($\sigma$-$\tau$) representation (Figure 4b), which was introduced by Patte and Laffort (Patte & Laffort, 1979), was employed to analyze the mixture-induced intensity effects when considering the overall intensity of the mixture ($I_{AB}$). The hypothesis behind this representation is that without perceptual interactions, the overall intensity of the mixture should be the sum of the (unmixed) intensities of the components (complete addition) (Thomas-Danguin et al., 2014). In this representation, Sigma ($\sigma$) accounts for the deviation from complete addition and is calculated as the intensity of the mixture divided by the sum of the (unmixed) intensities of the two components (Equation 3). Tau ($\tau$) reflects the perceptual proportion (i.e., in terms of intensity) of one component, which is chosen as a reference, and is calculated as the intensity of the reference divided by the sum of the (unmixed) intensities of the two components (Equation 4).

Considering the σ values, the intensity effects in the mixtures can be classified as complete addition ($I_{AB} = I_A + I_B$; $\sigma = 1$), hyper-addition ($I_{AB} > I_A + I_B$; $\sigma > 1$) and hypo-addition ($I_{AB} < I_A + I_B$; $\sigma < 1$). In the category of hypo-addition, there are three different subtypes: partial addition ($\max(\tau_A, \tau_B) < \sigma < 1$), compromise ($\min(\tau_A, \tau_B) < \sigma < \max(\tau_A, \tau_B)$) and subtraction ($\sigma < \min(\tau_A, \tau_B)$) (Berglund et al., 1976; V. Ferreira, 2012a; Thomas-Danguin et al., 2014). The

experimental observations of perceptual interactions within binary mixtures regarding overall mixture odor intensity were shown in Figure 23. A Sigma-Tau plot of the 222 binary mixtures (Figure 23a) showed that hypo-addition occurred in all cases. No significant case of hyper-addition was observed. The statistical significance of the mixture-induced intensity effects for each trial was tested by comparing the mixture intensity ($I_{AB}$) to the intensity of each (unmixed) component ($I_A$ or $I_B$) using paired samples Wilcoxon tests. In most cases (73.9%, Figure 23b), the mixture odor intensity was not significantly different from the intensity of the strongest (unmixed) component. Partial addition was observed in 21.7% of the mixtures, and compromise was observed for the remaining mixtures (4.4%). Statistically significant subtraction was never observed in our dataset. The mixture-induced intensity effects for the 198 different binary mixtures are reported in Supplementary Figure 16.

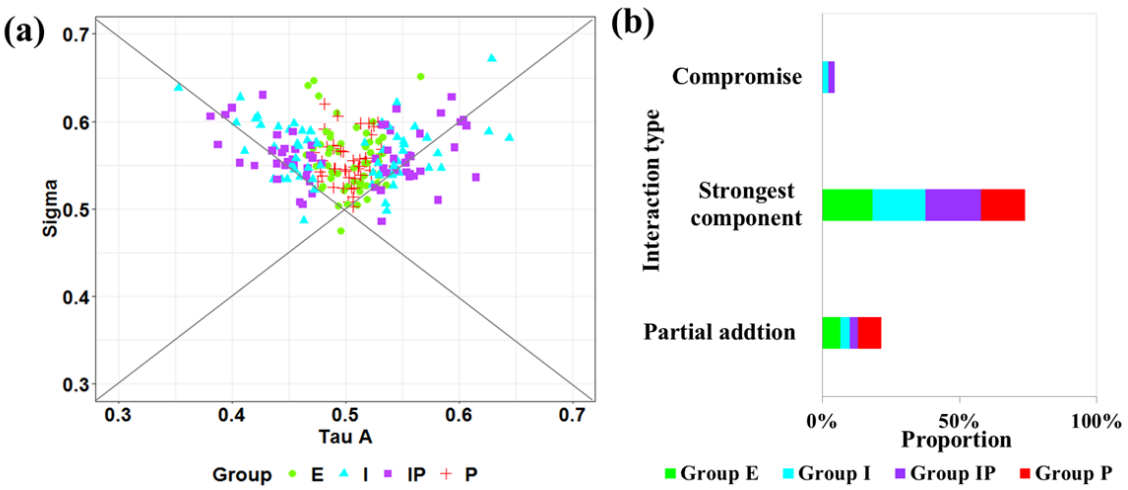


**Figure 23** Experimental observations of perceptual interactions within binary mixtures regarding overall mixture odor intensity. **Panel (a)** represents a plot of Sigma-Tau ($\sigma$-$\tau$) of the 222 binary mixtures classified into four different groups. **Panel (b)** represents the frequency of mixture-induced effects on the overall intensity of binary mixtures as a function of components' intensity and pleasantness: Group E included mixtures made with odorants that are not significantly different in their unmixed odor intensity and pleasantness; Group I included mixtures made with odorants that are not significantly different in their unmixed odor pleasantness but are significantly different in intensity; Group P included mixtures made with odorants that are not significantly different in their unmixed odor intensity but significantly different in pleasantness; and Group IP included mixtures for which there exists a significant difference in both intensity and pleasantness.

To explore the incidence of mixture-induced effects on the overall intensity of binary mixtures as a function of components' intensity and pleasantness, we calculated the proportion of each type of interaction observed for the groups that were previously employed to categorize mixtures based on their components' intensity and pleasantness (groups E, I, P and IP). The results are shown in Figure 23b for the 3 types of observed interactions. Partial addition occurred more frequently in group E and group P, while compromise only occurred in group I and group IP. However, the comparisons performed with Fisher's exact test showed that the distribution of the mixture effects was not significantly dependent on the four groups (Supplementary Table 10).

Nevertheless, to contrast for intensity or pleasantness of the components, mixtures were included according to a significant difference in the pleasantness of the (unmixed) components (Group IP+P) compared to no difference in the pleasantness of the (unmixed) components (Group E+I, Supplementary Figure 17a) and according to a significant difference in the intensity of the (unmixed) components (Group I+IP) compared to no difference in the intensity of the (unmixed) components (Group E+P, Supplementary Figure 17b). The comparisons performed with Fisher's exact test (Supplementary Table 10) showed that the distribution of these mixture effects was not dependent on pleasantness contrasts but was significantly dependent on the intensity difference between the components ($p< 0.05$). Partial addition is more likely to occur in mixtures in which components had similar odor intensities.

### 6.3.5 Binary OdorPleasantness Perception (dataset II)

Uncorrected paired Wilcoxon test was used to evaluate the difference in intensity and pleasantness between the two components of each of the 198 different binary odor samples (trials). As a result, four groups of trials were considered. First, group E, comprising 50 trials, showed no significant difference in either intensity or pleasantness ($p< 0.05$); group I, which included 52 trials, showed a significant difference in intensity only ($p< 0.05$); group P, comprising 39 trials, showed a significant difference in pleasantness only ($p< 0.05$); and finally, group IP, comprising the remaining 57 trials, showed a significant difference in both intensity and pleasantness ($p< 0.05$) was obtained (Supplementary Table 9).

The results of pleasantness rating of the 198 binary odor mixtures showed that, in most cases, mixture pleasantness was in-between pleasantness of the unmixed odorants (Figure 24), and that mixture pleasantness scores varied according to pleasantness and intensity scores of the unmixed odorants.

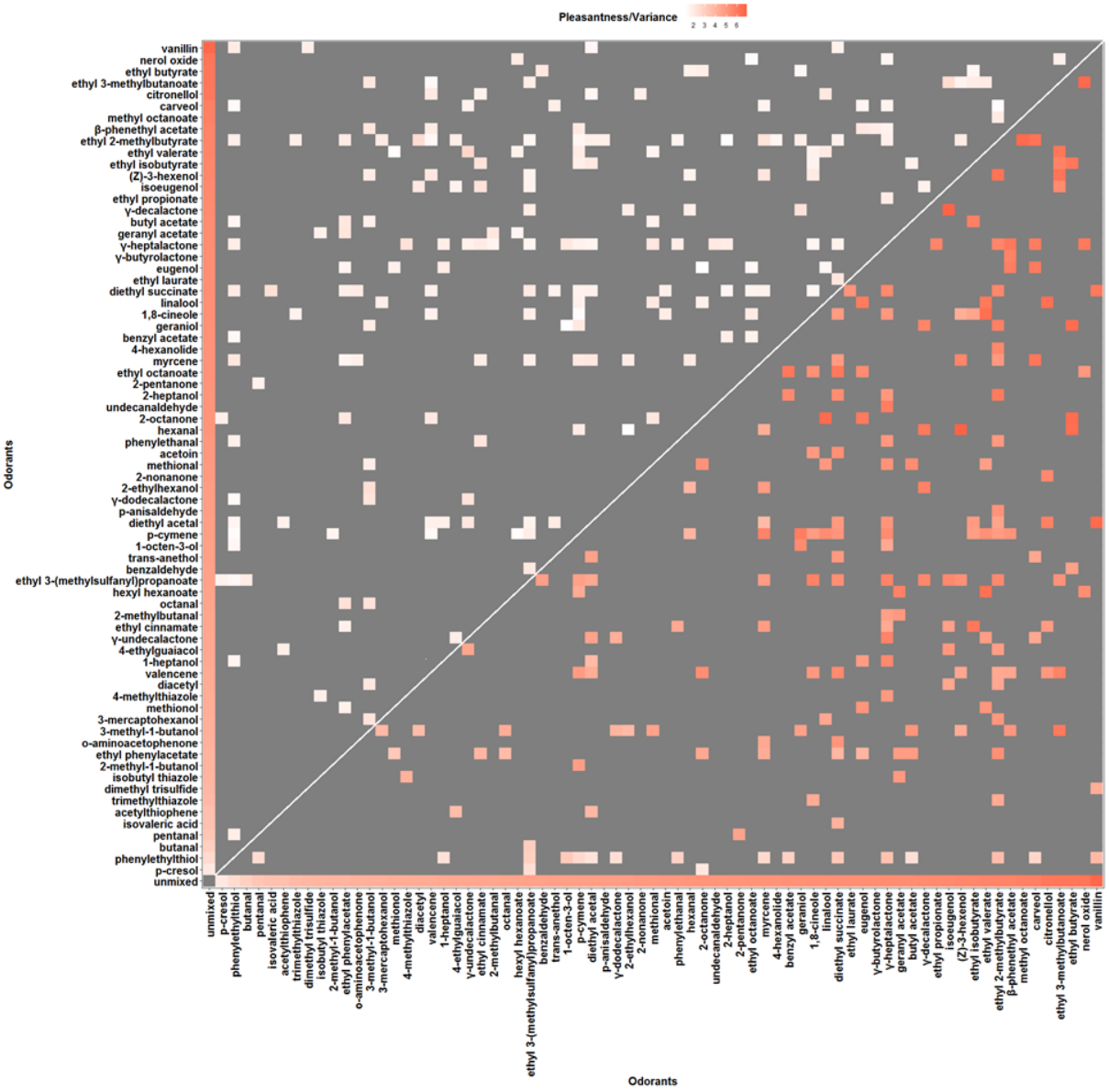


**Figure 24** Pleasantness of 198 different binary odor mixtures based on 72 different odorants. The top left triangle represents the value of the standard deviation; the bottom right triangle represents the value of the mean pleasantness. The pleasantness values of unmixed odorants are reported on the axes and correspond to the mean value shown in Figure 21. The data from the 24 duplicated trials were not included.

If the binary mixture consisted of two components with contrasted pleasantness and intensity (group IP), the pleasantness of the binary mixture was generally closer to that of the stronger odor component. For example, in the trial with ethyl valerate and *p*-cymene (Trial 2), the pleasantness (5.39) and intensity (7.19) of ethyl valerate were higher than the pleasantness (4.06) and intensity (5.21) of *p*-cymene, and the pleasantness of the binary odor (5.27) was closer to that of ethyl valerate. In the trial with 1-heptanol and phenylethylthiol (Trial 12), the pleasantness of 1-heptanol (4.58) was higher than that of phenylethylthiol (2.45), but its intensity (4.58) was weaker than that of phenylethylthiol (7.43). The pleasantness of the binary odor (2.34) was almost the same as that of phenylethylthiol. However, this pattern did not apply for all trials, such as that with γ-heptalactone and diethyl acetal (Trial 150), vanillin and diethyl acetal (Trial 153) or ethyl 3-methylbutanoate and ethyl isobutanoate (Trial 160). In the trial with vanillin and diethyl acetal, there were significant differences in both the

intensity and pleasantness of these two odorants; the intensity of vanillin (4.03) was weaker than that of diethyl acetal (5.40), but the pleasantness of the binary odor (6.52) was closer to that of vanillin (6.48) than diethyl acetal (4.74). This phenomenon might have resulted from perceptual interactions at the intensity level. For instance, a masking effect caused by vanillin could reduce the intensity of the odor of diethyl acetal in the mixture due to the perceptual dominance of the vanillin odor quality (Atanasova, Thomas-Danguin, Chabanet, et al., 2005), and therefore, the pleasantness of the mixture would be closer to that of vanillin alone.

If the binary odor mixture included two odorants with contrasted pleasantness but almost the same intensity (group P), the pleasantness of the binary mixture was generally near the mean pleasantness or was closer to the lower pleasantness value of the two odors. This phenomenon was observed in most trials, except for those with 2-octanone and ethyl butanoate (Trial 121), hexyl hexanoate and geranyl acetate (Trial 171), geraniol and ethyl butanoate (Trial 183), and ethyl butanoate and hexanal (Trial 198). In these four trials, the pleasantness of the binary mixture was close to the highest pleasantness value of the two odors. This specific case might result from perceptual interactions such as masking, synergy (V. Ferreira, 2012a) or perceptual dominance (Atanasova, Thomas-Danguin, Chabanet, et al., 2005), which may affect odor intensity and/or odor quality of the odor mixture and consequently its pleasantness.

If the binary mixture consisting of two components with similar pleasantness (group I and group E), the pleasantness of the binary mixture was, in most cases, the same as that of the components, but we also observed several cases indicating partial addition. A partial addition effect means that the pleasantness of the mixture is higher than the pleasantness of each component individually or that the pleasantness of the mixture is lower than that of each component individually. In the latter case, one can consider this effect as partial addition for unpleasantness. In our dataset, we observed that there were 52 trials (26%) showing partial additive pleasantness, meaning that the pleasantness of the binary mixtures was higher than either of its components, and 28 trials (14%) in which partial additive unpleasantness occurred (e.g., the pleasantness of the mixture was lower than that of either of its components). The statistical significance of the pleasantness partial addition effect for each trial was tested by uncorrected paired samples Wilcoxon tests. If there were significant differences between the pleasantness of each component ($P_A$ or $P_B$) and the pleasantness of the mixture ($P_{AB}$), and if the pleasantness of the mixture was lower than the sum of pleasantness score of each component ($P_A$+$P_B$), we considered that the pleasantness partial addition effect was significant. There were 6 trials with significant partial additive pleasantness: methyl octanoate and ethyl 2-methylbutanoate (Trial 61, $p< 0.05$), ethyl octanoate and benzyl acetate (Trial 83, $p< 0.01$), isoeugenol and $\gamma$-decalactone (Trial 188, $p< 0.05$), 1,8-cineole and ethyl valerate (Trial 196, $p< 0.05$), linalool and 2-octanone (Trial 206, $p< 0.01$), and eugenol and 2-octanone (Trial 217, $p< 0.05$) (Supplementary Table 9). Here, significant partial additive pleasantness was only observed in five binary mixtures consisting of two components with similar pleasantness and intensity and in one binary mixture consisting of two components with similar pleasantness but different intensity. Significant partial additive pleasantness was observed in a mixture of *L*-carvone and linalool at a 50–50% concentration ratio in Lapid's study (Lapid et al., 2008).

It is interesting to consider that at the 50–50% concentration ratio, the intensity and pleasantness of *L*-carvone and linalool were also similar in the abovementioned study. Therefore, it is reasonable to propose that pleasantness partial addition might tend to occur in mixtures with two components of similar pleasantness and similar intensity. One speculation that can explain partial additive pleasantness would consider an additive effect in the intensity of the mixture (Lapid et al., 2008), but the underlying principles of the effects need to be investigated more in depth through a systematic study of more binary mixtures of that kind.

There were 28 trials in which partial additive unpleasantness was observed, but none of them were found to reach a statistically significant level. A study (Laing et al., 1994) investigated the interactions between four sewage-related unpleasant odorants: hydrogen sulphide, isovaleric acid, butanethiol, and skatole. In this research, the pleasantness of a mixture was lower than the pleasantness of the individual (unmixed) components in most instances (Laing et al., 1994). Thus, based on this result, we assumed that the unpleasantness of a mixture might be stronger than that of the individual constituents if the binary mixture consists of two extremely unpleasant odorants. In our dataset, several binary mixtures included two components with extremely unpleasant odors (e.g., Trial 34, 40, 66, and 154). Nevertheless, the unpleasantness of these binary mixtures was not stronger than that of the individual constituents. Another example of partial additive unpleasantness was observed in the mixture of butanoic acid and phenylethyl alcohol, even though the effect was not significant (Lapid et al., 2008). The author speculated that partial additive unpleasantness might occur in cases in which at least one of the components shows a steep decline in pleasantness as a function of its intensity and an increase in the intensity of the mixture above the intensity of its constituents (Lapid et al., 2008).

### 6.3.6 Pleasantness Prediction (dataset II)

A model based on the $\tau$-value proposed by Patte and Laffort (Patte & Laffort, 1979) reflecting the relative proportion of the perceived intensity of odor A or odor B in a mixture was applied to predict the pleasantness of binary mixtures. This model was equivalent to the intensity weights model one used by Lapid et al. (Lapid et al., 2008). This model was applied to predict mixture odor pleasantness not only at the *panel* level but also at the *individual* level. In addition, for *panel* and *individual* approaches, predicted pleasantness was calculated as a *mean* condition and as a *trial* condition to check whether the differences in pleasantness and intensity that may arise for a given pair of odors (i.e., within a *trial*) have an impact on the mixture pleasantness rating or, in contrast, if pleasantness and intensity might be considered as properties of the compounds (i.e., *mean*) regardless of the odor pair. The model performance was evaluated by computing the prediction error *RMSE* and the $R^2$. *RMSE* represents the average difference between the perceptual pleasantness in trials and the predicted pleasantness by the model. The $R^2$ represents the correlation between the perceptual pleasantness and the predicted pleasantness. The lower the *RMSE* and the higher the $R^2$ are, the better the model.

The performance of the model for the *panel* approach obtained for the *mean* condition and the *trial* condition are shown in Figure 25a. In the *panel* approach, for all the trials in the *trial*

condition, the $R^2$ was 0.857, the prediction error *RMSE* was 0.428, whereas in the *mean* condition, the $R^2$ was 0.732, and the prediction error *RMSE* was 0.584, meaning that the prediction in the *trial* condition was better than that in the *mean* condition.

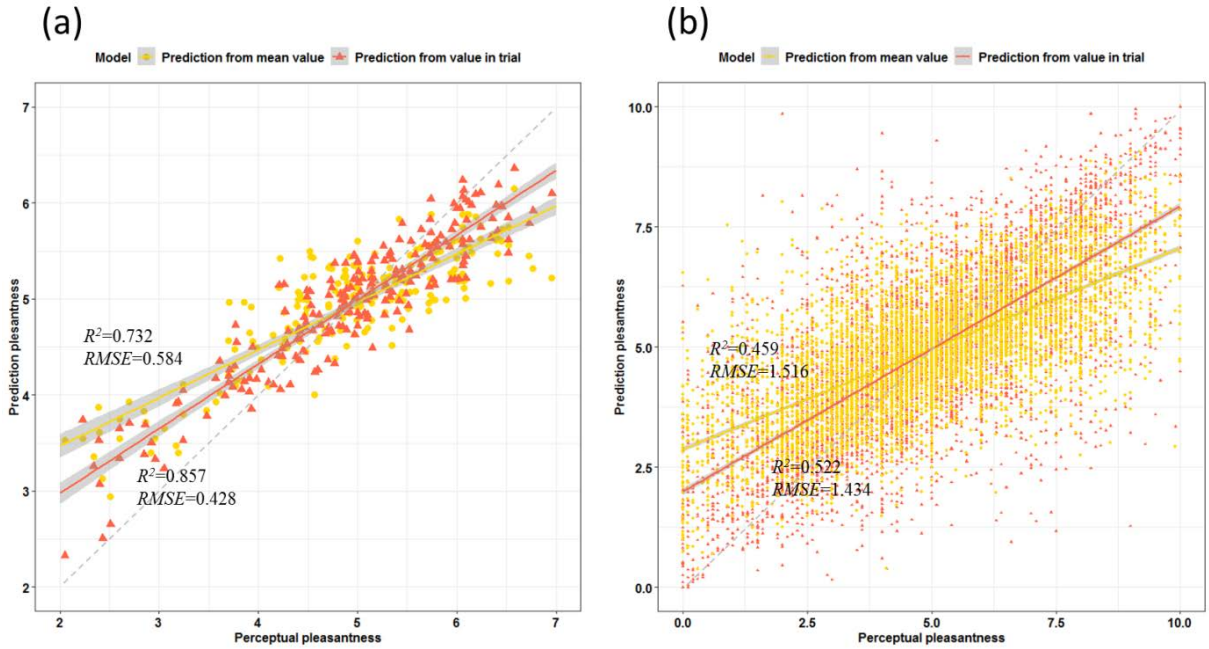


**Figure 25** $\tau$-value-based model prediction of binary mixtures in the **(a)** *panel* approach and **(b)** *individual* approach for the mean condition and the trial condition.

Then, the prediction model was used to predict the pleasantness of the four trial groups we defined above (group E, group I, group IP, group P). The results (Table 14) showed that in the *trial* condition, the prediction model performed quite well regardless of the group ($R^2$> 0.80). However, in the *mean* condition, only group IP obtained a high $R^2$ value. This result showed that the model performance in predicting group IP was high, meaning that the model based on the $\tau$-value predicting the pleasantness of a binary mixture consisting of two components with contrasted intensity and pleasantness performed quite well regardless of the odor pair. For group I, in which components had contrasted intensity, the $R^2$ value was low, but the prediction error *RMSE* was also low; in this case, it is likely that only a few instances of poor prediction might have been observed. For every group, especially for groups I, P and E, the model performance in the *mean* condition was worse than that in the *trial* condition. This result suggests that a context effect existed for specific combinations. The context effect, which implies that the perception of one odorant is influenced by the other odorant in the pair, might be an influential factor for pleasantness, especially for couples of odorants with similar odor pleasantness or intensity. In the future, at the *panel* level, an improved prediction model for the pleasantness of binary mixtures of two components with similar intensity or pleasantness must take into account the context effect, for instance, considering specific chemical features or specific odor quality features of the mixed odorants, to be able to account for additive effects.

**Table 14** Prediction Model Performances for Four Trial Groups Calculated with Different Approaches

| Prediction situation | | Groups | $R^2$ | *RMSE* |
|---|---|---|---|---|
| *Panel* approach | *Trial* condition | Group E | 0.868 | 0.368 |
| | | Group I | 0.803 | 0.318 |
| | | Group IP | 0.862 | 0.570 |
| | | Group P | 0.853 | 0.387 |
| | *Mean* condition | Group E | 0.565 | 0.606 |
| | | Group I | 0.704 | 0.434 |
| | | Group IP | 0.804 | 0.666 |
| | | Group P | 0.636 | 0.606 |
| *Individual* approach | *Trial* condition | Group E | 0.461 | 1.505 |
| | | Group I | 0.510 | 1.387 |
| | | Group IP | 0.548 | 1.433 |
| | | Group P | 0.540 | 1.407 |
| | *Mean* condition | Group E | 0.360 | 1.601 |
| | | Group I | 0.433 | 1.472 |
| | | Group IP | 0.513 | 1.495 |
| | | Group P | 0.478 | 1.496 |

This model was then used to determine whether the *individual* pleasantness of a given binary odor can be predicted (Figure 25b). Compared with the prediction in the *panel* approach, the predictions in the individual approach were relatively poor regardless of the condition (trial or the mean) and regardless of the trial group (Table 14). The significant variance and poor predictive performance of the model specified that predictions at an *individual* level are still a major challenge. This difficulty might be due to the high interindividual variability in odor pleasantness (Lindqvist et al., 2012), supported by individual genetic and cognitive differences. Indeed, previous research has shown that genetic variation across the human olfactory receptor repertoire alters odor perception in the intensity and pleasantness of a given odor (Keller et al., 2007; Trimmer et al., 2019), and stimulus intensity, repeated exposure, sex and hormonal status, aging, emotional status, and cultural background can all influence individual pleasantness ratings (Rouby et al., 2009).

### 6.3.7 General Discussion

In this work, we asked subjects to rate the intensity of each component and of the mixture within a large set of 222 binary odor mixtures. Our results showed that both components were still perceivable once in a mixture. This result was expected since the difference between the intensity ratios of the components never exceeded 1.82, meaning that components were present at approximately equal (unmixed) intensities (V. Ferreira, 2012b; Laing et al., 1984; Laing & Willcox, 1983). Although both components were perceived simultaneously in mixtures, their intensity perceived within the mixture could be modified. The first possible situation is that the intensity of the component remains identical to that in the unmixed situation (*independence*). From our data, this case is likely with a probability of 54.3% (Supplementary Figure 18). The second most likely situation would be that the intensity of the component decreases once in a mixture (*masking*); in this case, the probability would be 44.8%. The third situation would be

that the intensity of the component increases once in a mixture (*synergy*), with a probability of 0.9%.

Previous reports suggested that in an ideal mixture, the probability of identification of each component should be equal when the two odorants of the mixture are mixed at approximately equal (unmixed) intensities (Olsson, 1994). However, it was found that components did not mask each other equally in binary mixtures of equal intensities (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; Cain et al., 1995; Kurtz, Lawless, & Acree, 2009). Our results confirmed this trend to asymmetry since masking of only one component occurred in 47.7% of the total cases, while mutual masking was observed in only 20.7% (Figure 22a). Moreover, we also found that significant masking often occurs when the unmixed intensity of the components was not different. The question of masking ability was closely related to the concept of odor dominance. Several researchers have suggested that odor dominance could be explained by various features of mixed odorants, such as Steven's coefficients, hedonic tone, and polarity (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b). We observed that masking occurred more frequently when pleasantness significantly differed between the two components (groups P and IP), which is consistent with the influence of the hedonic tone. Nevertheless, our results did not show a higher propensity of unpleasant odors to mask pleasant ones, as has been previously suggested (V. Ferreira, 2012a). In our work, the pleasantness of compounds was not highly contrasted. Beyond intensity and pleasantness, masking could also involve the odor quality of the components, but in this case, it seems to be very difficult to predict why an odor would be more likely to mask another odor (Lindqvist et al., 2012).

We observed a trend for the synergy effect to be more frequent for the 'more' co-dominant mixtures and also for the components with equal and relatively high pleasantness or equal and relatively low intensity (Figure 26). Nevertheless, we only observed four binary mixtures in which odor synergy was statistically significant (Table 13). In one of these mixtures, the odor of vanillin was enhanced by the cream odor of diethyl acetal. In a study on beer flavor, vanillin was involved in synergy when mixed with monophenols such as salicylaldehyde, 4-vinylguaiacol or guaiacol (Sterckx et al., 2011). However, in that case, synergy likely resulted from peri-threshold additive effects. We found that diethyl acetal was also involved in the synergy of the fruity-apple odor of ethyl 2-methylbutanoate. It has been shown that ethyl 2-methylbutanoate can replace a mixture of 14 different esters that are commonly detected in wine without any discernible sensory change (De-La-Fuente-Blanco et al., 2020). Ethyl esters are known to be the backbone of wine fruity perception and to be involved in perceptual interactions with many other wine odorants (Lytra et al., 2016; San-Juan et al., 2011). Methional is known to be a key odorant in several food products, especially in icewine (Ma et al., 2017). In our study, methional's cooked-potato odor benefited from synergy when mixed with 3-methyl-1-butanol, which whiskey odor was significantly masked in return. Methional is known to produce an off-flavor reminiscent of cooked vegetables in several food products, and its detection was strongly dependent on the compound it was mixed with as well as its concentration (Burseg & de Jong, 2009). As a ubiquitous odorant, it is also found in lots of food products showing no such off-flavor. This can be explained because it can take part in blending effects (Romagny et al., 2018), for instance together with $\beta$-damascenone, methional is

involved in the dry-fruit and raisin character in Spanish Red Wines (San-Juan et al., 2011), which suggests that this compound may be prone to inducing perceptual interactions. The last compound for which we observed significant synergy was ethyl isobutanoate, whose involvement in synergy has been determined (Niu, Zhu, et al., 2020). Moreover, this compound, as methional, was also involved in blending effect (Barkat et al., 2012), which indicate that these odorants are more likely to appear as key odorants in flavor analysis procedures, especially during final checking using omission or recombination tests (Dunkel et al., 2014). The remaining question is why these compounds are involved in blending and synergistic effects. An initial hypothesis considers that key food odorants might be more important or ecologically relevant with regards to the origin and evolutionary history of the mammalian olfactory system and olfactory sensory neuron repertoire (Dunkel et al., 2014; Saraiva et al., 2019). Another hypothesis would be that these compounds might be involved in particular food rewards or avoidance cues. Interestingly, three of four of these odorants (vanilla, ethyl isobutanoate, and ethyl 2-methylbutanoate) have been determined to be associated with sweet taste (Guichard, Barba, Thomas-Danguin, & Tromelin, 2019), and thus, might be important cues in identifying sweet-containing food.

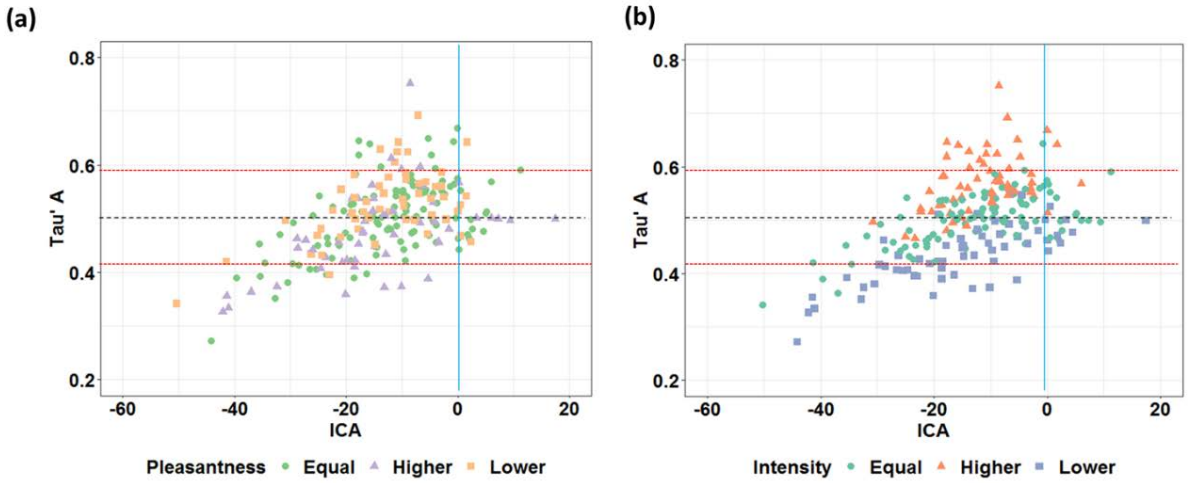


**Figure 26** Tau'=f(ICA) plots for one component. To keep the figure clear we only represented Tau and Tau' for one component. **Panel (a)** represents the experimental observations grouped by the component's relative pleasantness within binary mixtures. For a given binary mixture, if there is no significant difference ($p > 0.05$) between $P_A$ and $P_B$, odorant A and B were labelled as equal; if there is a significant difference between $P_A$ and $P_B$, the odorant with higher pleasantness was labelled as higher and the other one as lower. **Panel (b)** represents the experimental observations grouped by the component's relative intensity within binary mixtures. For a given binary mixture, if there is no significant difference ($p > 0.05$) between $I_A$ and $I_B$, odorant A and B were labelled as equal; if there is a significant difference between $I_A$ and $I_B$, the odorant with higher intensity was labelled as higher and the other one as lower.

Concerning the overall odor intensity of mixtures, only hypo-addition occurred in our dataset. Previous reports claimed that hypo-addition is the most frequently observed interaction in binary mixtures (Cain et al., 1995; Laffort & Dravnieks, 1982; Laing et al., 1984), but depending on the study, the most frequent subtype of the hypo-addition effect can differ from partial addition (Cain et al., 1995), compromise (V. Ferreira, 2012a) or the strongest component (Laffort & Dravnieks, 1982). Ferreira (V. Ferreira, 2012a) created a

general σ/τ plot with a pooled representation of 520 different binary mixtures from nearly 200 different pairs of odorants. This plot indicated that when two compounds were present in a mixture at dissimilar intensities, the overall odor intensity would be well estimated by the strongest component model (Laffort & Dravnieks, 1982). In contrast, when the two components are at similar intensities, the intensity of the mixture tends to be slightly higher than the maximum of the individual components' intensities (V. Ferreira, 2012a). Our results confirmed the latter, and showed that in more than 70% of cases, the intensity of a binary mixture was characterized by the stronger component. These previous reports also claimed that the subtraction effect never occurred (V. Ferreira, 2012a; Laing et al., 1984), while perfect addition and hyper-addition at real supra-threshold levels are infrequent. Our results support these claims since no significant subtraction effect and no significant hyper-addition effect were observed. It should be noted that hyper-addition was usually observed for mixtures in which at least one component had a very low intensity, or even less than the detection threshold (Berglund & Olsson, 1993b; Le Berre, Beno, et al., 2008), which could explain why we did not observe these interaction cases since we employed only odorants at a concentration that is much higher than their detection thresholds.

Perceptual interactions of odorant mixtures could arise from several biochemical or neurobiological interactions from the periphery to the brain (Mainland et al., 2014; Thomas-Danguin et al., 2014). Thus, several factors significantly influence different stages of olfactory information processing within the olfactory system and include odorants' ratio (Coureaud et al., 2011; Laing et al., 1984; Olsson, 1994, 1998; Romagny et al., 2018), odorants' chemical similarity (Linster et al., 2001), exponents of Stevens' law (Laffort et al., 1989), polarity (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b), odor intensity (Laing et al., 1984), odor quality (Barkat et al., 2012; Kay et al., 2005), odor familiarity (Rabin et al., 1989; Sinding et al., 2015), and odor pleasantness (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b). Compounds with higher exponents would be able to mask, to a large extent, the odor of odorants with low Stevens's law exponents (Laffort et al., 1989), and the unpleasant, and generally more polar, odor might be the odor that exerts masking (V. Ferreira, 2012a). In our data, it was not possible to check the effect of Stevens's law exponents because of a lack of available data. However, our results showed that the masking effect cannot be supported only by the hedonic character contrast between the components of the mixture.

For mixtures including a pleasant and a less pleasant component, we observed, in most of the cases, that the stronger constituent was more influential on the mixture's pleasantness than the weaker one. This rule is in accordance with previous observations (Laing et al., 1994; Lapid et al., 2008; Lawless, 1977; Moskowitz & Barbe, 1977; Spence & Guilford, 1933). Moreover, the weight of this influence was stronger for unpleasant components, as previously reported (Lawless, 1977). However, the special cases observed in group IP and group P also indicated that the pleasantness of binary mixtures is driven by the intensity of each component perceived within the mixture rather than by the intensity perceived out of the mixture. Indeed, mixing at least two odors can lead to several quantitative and qualitative effects on the mixture odor (Berglund & Olsson, 1993a) and/or quality effects (e.g., perceptual dominance

(Atanasova, Thomas-Danguin, Chabanet, et al., 2005)) that further influence the odor pleasantness of the mixture. These perceptual interactions can arise from several biochemical or neurobiological interactions during all stages of olfactory information processing within the olfactory system, from the periphery to the brain (Thomas-Danguin et al., 2014). As odor pleasantness is believed to be partially innate, but also strongly shaped by experience and learning (Prescott, Kim, & Kim, 2008), an odorant with higher recognition or carrying nutritious or poisonous information might capture more attention in a binary mixture (White, Thomas-Danguin, Olofsson, Zucco, & Prescott, 2020) and these factors might play an important role in the pleasantness judgement of the binary mixture. Indeed, the attentional capture effect has been highlighted in brain imaging studies using a binary odor mixture including a pleasant and an unpleasant component (Grabenhorst, Rolls, & Margot, 2011; Grabenhorst, Rolls, Margot, da Silva, & Velazco, 2007).

## 6.4 Conclusions

This study generalized several previous observations of the impact of perceptual interactions on odor intensity and pleasantness in binary mixtures on the basis of a sample set of 222 binary odor mixtures. We highlighted several rules: (1) the intensity of the components remains the same as that in the unmixed situation in most cases; otherwise, masking usually occurred as a second most likely case, especially when pleasantness is significantly different between the two components. (2) The overall intensity of the mixture generally follows the stronger component, but partial addition can be observed in mixtures that have no significant difference between the intensity of the two components. (3) Synergy was rarely observed, which suggests that synergy usually occurred when at least one component was barely detectable or at a low intensity. (4) If two odorants with significantly different intensity were mixed, in most cases, the pleasantness of the binary mixture was closer to that of the strongest odor component. (5) If two odorants with similar intensity but contrasted pleasantness were mixed, the pleasantness of binary mixture was generally near the mean pleasantness or was closer to that of the odor with the lower pleasantness value. (6) Partial additive pleasantness tended to occur in mixtures of two components with similar pleasantness and intensity ratings.

The Key Food Odorant concept was introduced for the first time as a variable to be considered in odor perceptual interactions, and the results showed that this variable might be related to supra-threshold synergy effects. Nevertheless, this hypothesis needs to be further investigated in dedicated experiments, including well-selected odor mixtures. We highlighted that a model based on the τ-value predicting the pleasantness of a binary mixture consisting of two components with contrasted or similar intensity and pleasantness performed quite well regardless of the odor pair, whereas prediction at the individual level was still a major challenge. In future studies, it would be interesting to use this model to predict the pleasantness of larger mixtures, while considering them as a series of binary mixtures.

# Chapter VII General Discussion and Perspective

It is not only key odorants in a product but also the interactions between different odorants that finally shape food aroma. Subtle changes in concentration of odorants in an odor mixture can considerably change the mixture's odor perception, and particular combination of several odorants also plays an important role in the overall perception of an aroma. However, perceptual interactions induced by odor mixtures are still a major lock in food flavor understanding and analysis. Among all categories of food, wine should be the most challenging and representative sample in odor mixture research because the interaction between wine odorants is likely more complex than in other foods due to the large number and the diversity of odor-active compounds and key odorants (Dunkel et al., 2014).

The aim of the thesis work was to explore the key odor elements that affected the perception of odor mixtures and the general laws behind olfactory perceptual interactions. In this thesis, icewine was used as the experimental object. Icewine is produced from grapes that have frozen naturally on vine under the temperature of –8°C or below. The grapes of icewine naturally undergo a dehydration process and freeze-thaw cycles and the must for icewine making has to be pressed from frozen grapes. This pressing process leaves water behind as ice crystals and allows the grape juice to be concentrated with more sugars, acids, and other dissolved solids, resulting in a slower-than-normal fermentation. These special procedures also lead to a unique aroma characteristic of icewine.

In our first experimental study (Chapter 3, Sensomic study), the numerous odor-active compounds which should contribute to the aroma characteristic of icewine were comprehensively studied through the Sensomics approach (Hofmann et al., 2018). The odor-active compounds were first screened from a volatile extract by GC-O analysis, and then identified by GC-MS. To verify the contribution of these aroma compounds, 28 odorants were further quantitated for aroma reconstitution and omission tests. Aroma recombination and omission experiments confirmed the contribution of these compounds to the global wine aroma of icewine. However, there were still subtle differences between aroma reconstruction based on identified key odorants and the original wine, which indicates some limitations in this study. One of the limitations are the neglecting of the contribution of some compounds with subthreshold or low FD or OAV that could be highly influential once embedded in the aroma buffer, that is through mixture-induced effects.

To investigate in depth this limitation, the second study (Chapter 4, Olfactoscan study) was conducted. This study is among the very first attempts to evaluate the contribution of odor-active compounds considering the mixture-induced perceptual effects on a complex aroma. In this study, we used the advanced Olfactoscan setup, which allowed us to consider the impact of a single odorant, identified by GC × GC-TOFMS, on the global wine aroma delivered as a background odor during GC-O analysis. To analyze the data, a new semiautomatic method was applied to allow the identification of odor zones in a similar way both in GC-O and Olfactoscan approaches based on the detection frequency method (19 trained judges). The results showed that considering a key odorant in the background odor of icewine could reveal mixture-induced effects such as masking or synergy resulting in a lower or higher detection

probability of the characteristic odor of this compound or in a modification of the overall aroma quality. In that sense, the Olfactoscan approach can lead to reconsider the impact of key odorants and to reveal specific compounds (discussed in section 7.1) that could be highly influential once embedded in the aroma buffer and not pointed in the standard Sensomics approach. This study also stressed the high complexity of perceptual odor interactions in real food and beverages, which advocates for the development of systematic research studies to better understand the impact of a compound, or a group of compounds, in complex aroma mixtures.

Wine has been described as a sensory buffer which is able to counterbalance the addition or omission of several odorants without any significant change in the overall aroma (V. Ferreira, 2010). Therefore, the odorants that can break the sensory buffer should play a key role in the perceptual interactions in the complex odor mixtures and thus in the wine overall aroma, such as methional, 1-octen-3-one and 3-methylbutanoic acid. Thus, the third study (Chapter 5, Olfactometer study) was designed to systematically explore the perceptual interactions between impact odorants of icewine. Inspired by a study which showed that sensory interactions between six common aroma vectors could explain four main red wine aroma nuances (V. Ferreira et al., 2016) and by another study which showed that the contribution of several ethyl esters can be mimicked by only one of them (De-La-Fuente-Blanco et al., 2020), the number of target impact odorants was reduced to 11 while still considering the potential interactions among main aroma nuances. These 11 odorants were selected from all of the odor-active compounds which were identified as highly impact odorants in two GC-O analysis and Olfactoscan analysis by discarding some compounds and keep other to get the best compromise and have the max number of possible odorants due to the olfactometer capacity. In this study, more than 150 odor mixtures related to the key odorants of icewine and wine aroma vectors, were designed considering the key odor elements (intensity, number and combination). All the odor mixtures samples were delivered by a 12-channels computer-controlled dynamic-dilution olfactometer, and 13 perceived odor attributes of the odor mixtures samples which related to the odor quality of the 11 odorants or related to the aroma of icewine were evaluated by RATA. The result gave a preliminary understanding of the influence of odor intensity, number of odorants in the mixture, and combinations of odorants on the overall perception of icewine, and these findings may provide original tools for food flavor design. Besides, perceptual interactions between different odorants or between common wine aroma vectors were highlighted, which could be verified or assessed in the future by psychophysical and/or neurobiological techniques. The result should be beneficial to understanding the interactions between different odorants in icewine, to promoting the quality control in the production of fast-moving consumer goods such as wine and to gaining knowledge of the information processing mechanism of the olfactory system.

Since food odor relies on mixtures of odor-active compounds, perceptual interactions in odor mixtures is of major relevance for food perception. To generalize the laws behind olfactory perceptual interactions, the fourth study (Chapter 6, Binary mixtures study) deeply explored binary odor mixtures in a large amount of food-related odor-active compounds. In this study, our first goal was to obtain more insight into the mixture-induced effect on the

intensity and pleasantness of odor mixtures. This topic has major relevance for the sensory aspects of food since the aroma of food is driven by mixture-induced quantitative effects, such as synergy or masking (V. Ferreira, 2012a). These mixture effects still constitute a major bottleneck to our understanding of the relationships between food VOCs and flavor perception. Only a few studies have focused on this topic, so the perceptual interaction effect on odor intensity among food odors remains almost unknown. The pleasantness of odor mixtures has been rarely investigated in the research on odor mixture perception, so that the mixture effects on this fundamental sensory dimension remains almost unknown. In this study, we conducted a systematic analysis of a large set of binary mixtures that are based on a set of 72 key odorants, which are especially detected in food to highlight and further generalize the rules and factors that are critical to the mixture-induced effects and their consequence for food flavor. The result highlighted the impact of perceptual interactions on odor intensity and pleasantness of binary mixtures whilst considering the intensity and pleasantness of the components. Besides, the large amount of data produced was especially modeled to highlight rules and predict the level of pleasantness of an odor mixture. The findings should provide solid knowledge about the perception of mixtures of key food odorants and provide a basis for predicting the expected outcome after mixing key compounds of food aroma.

In the following paragraphs, a general discussion of the innovation and limitations in experimental methods and results of this thesis work are addressed.

## 7.1 Odor-active Compounds in Icewine and Their Contribution to Icewine Aroma

### 7.1.1 Icewine Odor-active Compounds Identification

Major aroma characteristics of icewines have been described as caramel, honey, apricot, dried fruit, floral, raisin, tropical fruit (Table 2). In our research, the results showed that apricot, honey and tropical fruit were the most dominant attributes in Chinese Vidal icewine obtained by both descriptive analysis and RATA approaches. Yet, these two analyses were performed at different time, which could cause subtle differences in the composition of icewine samples. Moreover, one study was carried out in China and the other in France so that differences in the population could also account for subtle differences. The odor-active compounds of icewine were screened by AEDA in one study and by DF analysis in another study. The results are coherent between the two studies The results showed that 76% of odor-active compounds with a FD above or equal to 9 in AEDA were well detected by DF analysis. 3-methyl-1-butanol, *β*-damascenone, methional, 2,3-butanedione, ethyl isobutanoate, ethyl hexanoate and phenylethyl alcohol had relatively high FD (≥27) or NIF values (>60%) in both studies. Methional was not surprisingly detected and verified as impact odorants in Vidal icewine. This compound was proven to be a character impact odorant of some oxidized wines (Escudero et al., 2000). For icewine, the grape berries are harvested after freeze and thaw cycles, and the freeze and thaw cycles are subject to processes analogous to wine aging, which is an oxidative process (Boulton et al., 1996). It showed that the aging process can increase the monoterpene oxides, dimethyl sulfide, 3-(methylthio)-1-propanol, diethyl sulfide, and diethyl disulfide but decrease the monoterpene alcohols, 2-mercaptoethanol and ethyl

mercaptan (Bowen, 2011; Fedrizzi et al., 2007). In our research, we detected two oxides as odor-active compounds in icewine, namely linalool oxide and nerol oxide, which are not common as odor-active compounds in normal table wines. Although nut was not selected to describe the aroma characteristic of our icewine, in GC-O analysis, there were several odor zones were described as nut by the subjects. Some of the compounds corresponding to these odor zones were identified by GC−MS and GC × GC-TOFMS, but some of these odor zones fail to be identified by GC × GC-TOFMS. It is speculated that these compounds may be sulfur-containing and nitrogen-containing compounds, and their concentration should be very low. However, this part of the work has some limitations in the qualitative and quantitative analysis of the odor-active compounds. Due to the lack of chemical standards, some compounds corresponding to the odor zone have not been double-checked, and they were only matched with the RI and aroma description reported in databases or articles. In future research, studies focusing on the qualitative and quantitative of unknown compounds can be carried out.

### 7.1.2 The Contribution of Single Odorants to Icewine Aroma

The detected odor-active compounds can be divided into 1) genuine impact compounds or key odorants, 2) major contributors, 3) net contributors, 4) secondary or subtle contributors, and 5) aroma enhancers and aroma depressors based on their contribution in wines (V. Ferreira, 2010). The contribution of some odor-active compounds to the aroma characteristics of icewine was determined through odor recombination and omission test, and further evaluated by the advanced Olfactoscan setup considering the mixture-induced perceptual effect on icewine aromatic buffer.

Impact odorants or key odorants are normally present above their recognition threshold, and they provide the distinct aroma characteristic to the wine (V. Ferreira, 2010). In icewine, *β*-damascenone was shown to be a highly impact odorant in both AEDA and DF analysis. In AEDA, the flavor dilution factor of this peak was the highest, as large as 2187, but in the DF method, its NIF was not the highest. This difference might be explained by subjects' sensitivity, since the AEDA method is based on detection thresholds, and/or by suprathreshold sensitivity as reflected by Steven's power function slope. Indeed, *β*-damascenone has both a very low detection threshold (0.002 μg/L in water (Buttery et al., 1989) and a low Steven's power function slope (V. Ferreira, 2010). The recombination and omission test conducted in the Sensomic study showed that the reconstitution sample without *β*-damascenone could be pointed out by all of assessors, and it was evaluated with a very highly significant difference on every aroma characteristic, resulting in the decrease of every icewine main aroma nuances. The addition test conducted in the Olfactoscan study showed that the OZs related to *β*-damascenone were significantly masked (-31.6%) at low icewine background odor condition but not at high, which may reflect the effect of odor concentration on the olfactory perceptual interactions involving this compound. Test conducted in the Olfactometer study showed that the 'addition' or 'omission' of *β*-damascenone at medium intensity impacted the attribute apricot, which was significantly increased or decreased respectively. Thus, *β*-damascenone (honey and apricot) should act as an impact compound in icewine.

The contribution of methional was also evaluated individually, it was considered a high-impact odorant in GC-O analysis and its impact likely increased when embedded in icewine aroma buffer, especially when the compound was at the medium intensity. Besides, it is the only compound that was benefited from synergy with the icewine background odor at both low and high levels as shown in the Olfactoscan study. Although the dominant aroma attributes of icewine did not include the cooked potato-like odor of methional, the omission test in the Sensomic study showed that reconstitution sample without this compound could be distinguished from the completed reconstitution sample, while its addition in the Olfactoscan study and Olfactometer study showed that the cooked potato-like odor of methional seemed to be maintained in the icewine aroma buffer. These results suggested that methional should be involved in highly intricate interactions in the case of complex mixtures. Methional is known to produce an off-flavor reminiscent of cooked vegetables in several food products, and its detection was strongly dependent on the compound it was mixed with as well as its concentration (Burseg & de Jong, 2009). As a ubiquitous odorant, it is also found in lots of food products showing no such off-flavor. This can be explained because it can take part in blending effects (Romagny et al., 2018), for instance, together with $\beta$-damascenone, methional is involved in the dry-fruit and raisin character in Spanish Red Wines (San-Juan et al., 2011). Unfortunately, in the third study, we did not design complex mixtures related to methional to explore the perceptual interactions produced by this compound. Other odor-active compounds acting similarly to methional in icewine included 1-octen-3-one, guaiacol and 3-methylbutanoic acid. These compounds have recognizable aroma nuances that are quite different to the basic aroma profile of icewine, and they also showed the ability to transmit their specific aroma nuances to icewine when they were individually embedded in the icewine aroma buffer, but they lost their original odor qualities in the icewine, which advocates for perceptual interaction of these compounds with other compounds and the dependence of the interaction on their absolute and relative concentration. Thus, methional, 1-octen-3-one, guaiacol and 3-methylbutanoic acid in icewine should act as the major contributors.

The major contributors and net contributors are the compounds which can affect the aroma profile or the intensity of a primary generic descriptor in a given wine; however, it might provide an aroma characteristic that is not the same as the specific descriptor (V. Ferreira, 2010). In icewine, lots of esters were shown to be highly impact odorants in both AEDA and DF analysis, but they were masked once in the wine background odor and their impact would be much lowered. Omission test conducted in the Sensomic study showed that the reconstitution sample without a group of esters could be easily distinguished from the complete reconstitution sample, indicating their highly significant influence ($p \leq 0.001$). In the Olfactometer study, we tested the contribution of ethyl 2-methyl butanoate at three levels of intensity in the aroma of icewine. The results showed that the addition of ethyl 2-methyl butanoate significantly increased the attribute of apricot. Moreover, as the intensity of ethyl 2-methyl butanoate increased, its own tropical fruit odor could contribute to the aroma of icewine. Nevertheless, the omission of ethyl 2-methyl butanoate didn’t significantly decrease the attribute of apricot or tropical fruit. It has been shown that the contribution of several ethyl esters can be mimicked by only ethyl 2-methyl butanoate (De-La-Fuente-Blanco et al., 2020)

and synergy has also been reported to be able to occur between these ethyl esters (Lytra et al., 2013; Niu, Liu, et al., 2020), which reinforces the idea that they may have a similar odor quality contribution to the overall fruity/floral icewine odor. Other major contributors and net contributors, which could be highlighted in our studies included 3-methyl-1-butanol, 1-hexanol, phenylethyl alcohol, γ-decalactone. In the case of 3-methyl-1-butanol, we found that it remained a high impact odorant even in the icewine odor but that in the complex mixtures, the odor quality was different from its original odor. The odor quality associated with its OZ in Olfactoscan corresponded to a more fruity-sweet character and it hardly changed the aroma profile of icewine at medium intensity, that is, perceptual interaction occurred and caused the odorant to lose its original odor quality in the mixed system (Laing, 1991). This result is fully in line with previous reports demonstrating, in model solutions, that higher alcohols contribute to the fruity aroma expression of esters from red wine (Cameleyre et al., 2015).

Furaneol was identified, by omission test conducted in the Sensomic study, as a net contributor to icewine for its significant effect on the apricot, caramel, and tropical fruit characteristics. This is confusing because furaneol perceived as caramel and caramel is a descriptor of icewine, but the results obtained in Olfactoscan study and Olfactometer study showed that furaneol was masked once in the wine background odor and thus, should be unable to transmit its caramel odor to the icewine aroma even it was added at high intensity. The possible reason is that the subjects' definition of caramel in icewine is not consistent with the definition of caramel for furaneol. However, it can be considered to cooperate with ethyl 2-methyl butanoate to enhance the aroma attributes of caramel and tropical fruit.

Potential aroma enhancers in icewine, which were hard to detect by GC-O analysis, were actually revealed by Olfactoscan analysis and identified by GC × GC-TOFMS. They are 2-pentylfuran and/or 2-hexanol, γ-heptalactone and 9-decenoic acid and/or geranic acid and/or isophytol. These odorants were not considered in the GC-O analysis because their contribution was below the noise level. However, our results showed that they should benefit from synergy and were thus only considered impact odorants under mixture conditions. Nevertheless, these compounds have not yet been further identified by injecting their pure chemical standards into GC × GC-TOFMS nor verified by addition and omission test. In addition, based on our result, their contribution should highly depend on the intensity of the icewine background aroma and its own.

In our research of the contribution of single odorants to icewine aromas, we carried out the reconstruction and omission test according to the concentration of the compound in the icewine, but in the Olfactoscan study and Olfactometer study, we did not measure the concentration of the added compound, but rather used intensity levels. Therefore, this study cannot provide the precise concentration range for the effect of the aroma compound on the aroma attributes, which should be a limitation of this study. Further studies should include concentrations measurements to better reflect the observed effect in real icewine.

### 7.1.3 The Contribution of Different Odorant Combinations to Icewine Aroma

Correlations between aroma attributes and aroma compounds of icewine have been analyzed by partial least squares regression (PLSR) analyses (Lan et al., 2019) and partial least squares discriminant analysis (PLS-DA) (Huang et al., 2018). The result showed that higher alcohol acetates and ethyl esters of fatty acids were responsible for the fresh fruity aroma (Lan et al., 2019). Honey and nut had a close correlation with 1-hexanol, isoamyl acetate, phenethyl acetate and phenylethyl alcohol (Huang et al., 2018) and lactones (Lan et al., 2019). Smoky correlated better with volatile phenols (Lan et al., 2019) and caramel aroma was correlated with ethyl esters and lactones (Huang et al., 2018; Lan et al., 2019), while rose aroma was correlated with terpenes (Huang et al., 2018).

In our Olfactometer study, a series of samples in line with the complete block design of different odorant combinations which contained one to six odorants were evaluated to explore the contribution of different odorant combination to icewine aromas. The results showed that the dominant aroma attributes of icewine, namely apricot, honey and tropical fruit can be significantly changed by some odorants or some odorant combinations. We found that the 'addition' or 'omission' of some combinations containing ethyl 2-methyl butanoate or *β*-damascenone or both significantly enhanced or reduced the apricot and some combinations containing guaiacol and 3-methylbutanoic acid affected the apricot attribute. Unlike apricot, tropical fruit and honey attributes were not significantly enhanced by odorants, but 'omission' of some combinations containing ethyl 2-methyl butanoate, *β*-damascenone or furaneol could significantly reduce the intensity of these attributes. For the attribute caramel, it was rarely enhanced by adding ethyl 2-methyl butanoate and furaneol separately or by any combination under 'addition' or 'omission' situation except for the combination of ethyl 2-methyl butanoate and furaneol. This finding indicated that the two odorants can promote each other, or might even induce a blending-like effect, since the odor of strawberry and caramel, which match with the odor of ethyl 2-methyl butanoate and furaneol, have been showed to produce a pineapple-like odor (Barkat et al., 2012; Le Berre et al., 2010; Le Berre, Thomas-Danguin, et al., 2008). Further research work is required here to tackle this hypothesis and thus better understand the complex contribution of this combination to the overall icewine aroma.

Since the compounds related to the attribute rose and raisin were not involved in the study, it is impossible to explore the contribution of different odorant combination to these two attributes, which is also one of the limitations of this study. In addition, the intensity of the compounds involved in this study has been adjusted to approximately medium level, so it is impossible to study the effects of the odorant combinations at different concentration and ratios on the aroma of icewine. These studies can be carried out in the future.

## 7.2 The Perception of Odor Mixtures

### 7.2.1 Elemental Coding or Configural Coding

Due to the mechanism of binding and encoding of chemical signals by the olfactory system and the configural processing (or synthetic processing) (Livermore & Laing, 1998) or elemental processing (Berglund & Engen, 1993) of these signals, the odor mixtures could be

perceived as homogeneous or heterogeneous (Figure 3) (Thomas-Danguin et al., 2014).

For very complex odor mixtures, such as icewine, the configural processing (or synthetic processing) and elemental processing should coexist and the dominant transition between the two processing should depend on the odorants themselves and their intensity ratio regarding other odorants. The configural processing should be applicable to the interpretation of the sensory buffer effect, and the elemental processing should be applicable to the interpretation of the compounds which were able to break the sensory buffer effect. Our results obtained in the Olfactometer study can partially reflect the two processing. For instance, when 3-methylbutanoic acid or a binary mixture containing 3-methylbutanoic acid was embedded in icewine background odor, the cheese attribute of 3-methylbutanoic acid could be easily recognized. However, when most of the quinary combinations and senary combination containing 3-methylbutanoic acid were embedded in icewine background odor, the odorants combination didn't lead to a significant enhancement of the cheese attribute, which implied that the olfactory processing of 3-methylbutanoic acid might change from elemental coding to configural coding. Besides, our results showed that regardless of the number of odorants mixed, at most only two attributes related to the added odorants (especially cheese or smoky) were perceived. Nevertheless, in most cases, only one attribute specifically related to an added odorant could be distinguished. This result indirectly verifies the conclusions of previous research, which showed that humans were not able to identify more than three or four odorants in a mixture, and that the identification of odorants was significantly more accurate for the 'poor' blenders than for the 'good' ones (Laing & Francis, 1989; Livermore & Laing, 1998).

For binary mixtures which combined odorants with similar perceived intensities, our results obtained in the Binary mixtures study showed that the two components can be identified in all of our binary mixtures, which implied that for simple odor mixtures, elemental processing should be the rule the most often encountered. However, the results may be different for mixtures composed of odorants at different intensity ratios. The influence of the odor intensity or concentration of odorant, on the perception of odor mixtures has been studied before (Olsson, 1994). Configural perception has been shown in low complexity mixture when the compounds are at specific proportion of the components, such as a ternary blending mixture shown to produce the perception of a pineapple odor (Le Berre, Beno, et al., 2008). Moreover, a barely detectable modification of one component's concentration was enough to induce a significant alteration of the pineapple odor quality.

### 7.2.2 Separation Processing or Completion Processing

It has been hypothesized that the olfactory processing pathway followed other rules in the processing olfactory signals. These rules ought to balance two critical perceptual necessities: stability, for recognition despite missing elements, and discrimination for relevant choices (Barnes et al., 2008). These rules resulted from observations in animal studies showing that individual piriform cortex neurons easily 'refilled' one missing element in an odor mixture, especially for familiar odor, by processing a 'pattern completion'. In contrast, it was observed that animal can also clearly detected a novel element in a mixture by processing a 'pattern

separation' (Barnes et al., 2008; Wilson & Sullivan, 2011). Applied to our observations, we showed that for different odorants or different odor attributes, the weights of the two patterns may not be the same. For instance, the addition and omission of the combination containing guaiacol was able to significantly increase or reduce the intensity of its dominant smoky attribute in line with the idea of pattern separation. In contrast, the addition of 3-methylbutanoic acid was able to significantly enhance its dominant cheese attribute in most cases, but its omission was unable to significantly reduce the cheese attribute, suggesting a pattern completion in the case of omission. The finding might be challenged in neurophysiological studies and be very meaningful for the way we should understand the hierarchical relationships between odorants and for instance their contribution to it can be used as a useful criterion to classify odor space or aroma vectors in wines.

The addition situation reflected by the Olfactoscan study highlighted that the addition of large amount of odorants had almost no effect on the overall odor of the complex mixture of wine aroma. Moreover, the addition effect might not be the perception in the mixture of the added odorant, but a decrease in some of the basic attributes of the mixture (V. Ferreira, 2010). The olfactory discrimination sensitivity of human for missing element and adding element was found to be similar (Luckett et al., 2021). Our Olfactometer study showed that the olfactory discrimination ability of human was more sensitive for adding elements, especially for less than three elements, than missing elements, which proved an evidence of perceptual stability under certain restrictions. We observed that the 'omission' of certain odorants or combinations had less effect on the profile of the icewine odor than their 'addition'. The main influence of 'omission' was observed on attributes other than the ones related to the manipulated odorants. In contrast, the 'addition' of certain odorants leaded to the modification of not only the odorant-related attribute, but also on other attributes. This finding is partially in line with the conclusion before (V. Ferreira, 2010), which showed that the addition of huge amounts of some odorants had almost no effect, or even that the effect was not the perception in the mixture of the added odorant, but a decrease in some of the basic attributes of the mixture.

### 7.2.3 Olfactory Perceptual Interactions

The perceptual interactions among odorants of icewine were discussed throughout the successive studies. In the case of the very complex mixture of odorants that is the icewine headspace, it can be considered as an aromatic buffer formed by ethanol and by major fermentative aroma compounds present at levels close and above threshold (V. Ferreira, de-la-Fuente-Blanco, & Saenz-Navajas, 2021). This complex mixture can be compared to the 'olfactory white' proposed by Weiss et al. (Weiss et al., 2012), in analogy to visual and auditory 'whites'. In other words, this very complex mixture would provide a non-typical 'vinous' aroma, but the non-typical 'vinous' aroma enable some of the different odor objects to be recognized (V. Ferreira et al., 2021).

The perceptual impact of odorants could be enhanced or masked due to mixture effects and perceptual interactions. In the results of the Olfactoscan study, we observed that 57% of the odor-active compounds were not highly affected by mixture effects, while 30% were masked

and 13% benefited from synergy. Interestingly, when we tested more simple mixtures, namely a larger set of binary mixtures in Binary mixtures study, we observed very similar proportions of the different effects: 54.3% of the intensity of the component remains identical to that in the unmixed situation (independence), and 44.8% were masked but only 0.9% of the intensity of the component increases once in a mixture (synergy). Therefore, the mixture effects are likely independent of the mixture complexity, that is, regarding odor intensity, no matter of whether the mixture is chemically complex or only a binary mixture, the most common situation is independence, then masking, and rarely synergy.

For binary mixtures, it also showed that if components were present at approximately equal (unmixed) intensities, usually both components were still perceivable in mixture, although their intensity perceived within the mixture could be modified. Previous reports suggested that in an ideal mixture, the probability of identification of each component should be equal when the two odorants of the mixture are mixed at approximately equal (unmixed) intensities (Olsson, 1994), but it is also possible that one of the components induces a perceptual dominance over the other component (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b). Our results showed that components did not mask each other equally in binary mixtures of equal intensities, and masking occurred more frequently when pleasantness significantly differed between the two components. Several researchers have suggested that odor dominance could be explained by various features of mixed odorants, such as Steven's coefficients, hedonic tone, and polarity (Atanasova, Thomas-Danguin, Chabanet, et al., 2005; V. Ferreira, 2012b). Our results are thus consistent with the influence of the hedonic tone but do not help to check the influence of other factors, while suggesting that the masking effect cannot be supported only by the hedonic character contrast between the components.

In the Binary mixtures study, we observed a trend for the synergy effect to be more frequent for the 'more' co-dominant mixtures and also for the components with equal and relatively high pleasantness or equal and relatively low intensity (Figure 26). Nevertheless, we only observed four binary mixtures in which synergy were statistically significant for one of the components (Table 13). In the Olfactoscan study, we also observed only 13% of cases which could be indicative of a synergy effect and the results indicated that synergy appeared mostly in OZ which had a relatively low NIF ($\leq$ 31.6%), and when the icewine odor background was delivered at low level. These findings are consist with previous research based on binary mixture models which showed that synergy, or hyper-addition, is rare but may occur mostly at low intensity levels (V. Ferreira, 2012a). Back to the Binary mixtures study, we speculated that some food odorants, such as methional, may be more often involved in synergy, and we speculate that Key Food Odorant value (Dunkel et al., 2014) might be related to supra-threshold synergy effects, although the effect could strongly dependent on the compound with which it was mixed. It is worth mentioning that methional is the only compound benefited from synergy with the icewine background odor at both low and high levels and it benefited from synergy when mixed with 3-methyl-1-butanol, which also exist in abundance in icewine. However, in the Olfactometer study, we observed that the dominant attribute of methional didn't benefit from synergy effect. The rather divergent results showed the perceptual interactions are highly intricate and that perceptual interactions should strongly

depend on the compounds involved and their concentration and further investigations in dedicated experiments are needed to get a better understanding of this phenomenon likely driven by odor coding.

Concerning the overall odor intensity of mixtures, only hypo-addition occurred in our dataset of binary mixtures study and the overall odor intensity of mixtures generally follows the stronger component rule. Since elemental processing should be the rule the most often encountered for simple odor mixtures, both low-intensity and high-intensity odorants can be identified. Thus, it is reasonable that subject scores the overall intensity of a binary mixture according to the strongest component. However, no significant subtraction effect and no significant hyper-addition effect were observed. Since hyper-addition was usually observed for mixtures in which at least one component had a very low intensity, or even less than the detection threshold (Berglund & Olsson, 1993b; Le Berre, Beno, et al., 2008), it is reasonable that we did not observe such type of interactions since we employed only odorants at a concentration that is much higher than their detection thresholds. Concerning the overall odor pleasantness of mixtures, we observed that, in most of the mixtures including a pleasant and a less pleasant component, the stronger constituent was more influential on the mixture's pleasantness than the weaker one and the weight of this influence was stronger for unpleasant components. However, our results also indicated that the pleasantness of binary mixtures is driven by the intensity of each component perceived within the mixture rather than by the intensity perceived out of the mixture.

For binary mixtures, regarding odor intensity, perceptual interactions can be categorized depending on whether the mixture quality is homogeneous or heterogeneous (Thomas-Danguin et al., 2014). However, for more complex mixtures such as in real food, perceptual interactions among odorants become very complicated, and they are poorly defined and categorized. Classifications based on $\sigma$-$\tau$ plot (Patte & Laffort, 1979), dose addition or response addition approach (Lytra et al., 2013; Miyazawa et al., 2008b; Niu et al., 2019; Yu et al., 2020) and OAV approach (Niu, Zhu, et al., 2020) have been applied to assess the perceptual interactions among odorants in alcoholic beverage. However, these classifications refer mainly to effects on the odor intensity of the mixture of dissimilar non-blending odors and do not consider odor blending, which is one of the most relevant and influential perceptual interactions in complex mixtures (V. Ferreira et al., 2021). Recently, a new and systematic classification was proposed in which odor interactions are classified into four categories: competitive, cooperative, destructive and creative, but there is a lack of quantitative data to work with odor intensities reliably, and the author pointed that there is a pressing need to systematize the effects of creative interactions (V. Ferreira et al., 2021). Our results of the Olfactometer study showed that the addition or combined addition of key odorants can cause very complex changes in odor attributes of odor mixtures, but these changes in odor quality still can't be well defined by existing classifications. Thus, it is very necessary to modify the existing classifications or create new classifications for this type of research in the future.

## 7.3 Experimental Design and Methodologies in the Research on Olfactory

## Perceptual Interactions

The identification of key odorants in food has been abundantly explored by the Sensomics approach (Hofmann et al., 2018) which is based on GC-O and other separation and detection technology. The contribution of each odorant was first evaluated by comparing the FD and OAVs calculated from odor threshold data and quantification. All the compounds with OAVs above than 1 were confirmed by recombination and omission tests. However, this strategy, even if proven efficient in numerous cases, has also some drawbacks. One of them is that compounds with sub-threshold or low FD and OAVs are often ignored. Nevertheless, the actual impact of these minor odorants could be strengthened either by interactions with non-volatile compounds of the matrix, or by perceptual interactions with other odor-active compounds of the aromatic buffer. Although some studies investigated perceptual interactions among odorants (Cameleyre et al., 2015; Lytra et al., 2013; Miyazawa et al., 2008b; Niu et al., 2019; Niu, Zhu, et al., 2020; Yu et al., 2020), the effect of complex food aromatic buffer-induced interactions on odor-active compounds haven't yet been deeply studied. The advanced Olfactoscan setup that we used in the second study seems to be a good attempt to evaluate the contribution of odor-active compounds considering the mixture-induced perceptual effect of a complex aroma. In this study, we observed that considering the impact of a key odorant in the overall odor of icewine could reveal mixture-induced effects such as masking or synergy resulting in a lower or higher detection probability of the characteristic odor of a compound, or in a modification of the overall wine aroma supporting more qualitative perceptual interactions. In that sense, the Olfactoscan approach can be applied to other real food and beverages to carry out similar research, which can advocate for the development of systematic research studies to better understand the impact of a compound, or a group of compounds, in complex aroma mixtures.

Although perceptual interactions are usually mentioned in food flavor research, the strategy used in most cases to assess the perceptual interactions among odorants mainly focus on the odor intensity of the mixture such as in the $\sigma$-$\tau$ approach (Cameleyre et al., 2015; Niu et al., 2019), dose addition or response addition approach (Lytra et al., 2013; Miyazawa et al., 2008b; Niu et al., 2019; Yu et al., 2020) and OAV approach (Niu, Zhu, et al., 2020). However, these approaches seldom consider odor quality or blending effect, and little work has systematically assessed the mixture-induced effects on the aroma profile of real products. Inspired by the results obtained from other researches (De-La-Fuente-Blanco et al., 2020; V. Ferreira et al., 2021; V. Ferreira et al., 2016) and based on our studies and results, we propose the following strategies and procedures which may not be perfect but may be useful to explore the perceptual interactions in complex odor mixtures considering the aroma profile and odor quality of the complex odor mixtures:

1) Identification of odor-active compounds in a complex odor mixture;

2) Classification of compounds into the aromatic buffer and those that can break the aromatic buffer (genuine impact compounds or major contributors);

3) Classification of the impact odorants into different aroma vectors;

4) Determine the blending effects and other perceptual interactions induced by the aromatic

buffer;

5) Characterize the perceptual interactions among aroma vectors and with aromatic buffer by traditional or rapid, or even dynamic descriptive sensory analysis.

With regard to sensory methods, in our research, the aroma of icewine was evaluated by descriptive sensory analysis (DA) with 12 well trained subjects using a seven-point scale (Chapter 3) and by RATA with 36 semi-trained subjects using a five-point scales (Chapter 5). Comparison of discriminative ability and configuration similarity has been made between RATA and DA (Oppermann, de Graaf, Scholten, Stieger, & Piqueras-Fiszman, 2017). The results showed that naïve participants were able to provide similar results compared to those obtained by DA with trained panelists, even with samples belonging to an unfamiliar product category with subtle differences. In our studies, the results showed that apricot, honey and tropical fruit were the most dominant attributes in Chinese Vidal icewine in both DA and RATA, although the order of importance of these attributes in the two analyses was different. Besides, in DA, nut was not selected by the trained subjects; in RATA, the intensity values of the nut descriptor are very low, almost negligible. Therefore, these results suggested that RATA could provide a good alternative to the time- and resource-intensive DA for wine samples. Indeed, this method has been used to study of the effect of sulfur compounds on the sensory profile of wine model solutions (Franco-Luesma et al., 2016).

## 7.4 General Perspectives

The thesis explored the perception of odorant and odor mixtures in real wine by some original approaches. The Olfactoscan technology, Olfactometer equipment and RATA evaluation method used in the research provide new ideas and methods for carrying out accurate, controllable and efficient aroma-related flavor research as well as perceptual interactions research in the food and beverage field. The research ideas developed in this thesis, based on the perceptual effects of the interactions between aroma vectors can be used to screen and determine aroma vectors in other food systems, thereby simplifying the aroma combination in food, and optimizing the experimental design ideas to investigate interactions in more or less complex odor mixtures. The data obtained in the different experiments can be used in the research of odor mixtures related to big data modeling and prediction. Moreover, the odor combinations exhibiting perceptual interactions and unraveled in the thesis work can provide data support for quality control and the development of new products in the fast-moving consumer goods industry such as food, wine, cosmetic, flavors and fragrances. These odor combinations with perceptual interactions can also be used as candidates to explore the interactions mechanism within the olfactory system at different levels, so as to enhance our understanding of the olfactory system encoding and complex information processing. However, the selection and intensity of odorants studied in the thesis is obviously limited, and the conclusions are also likely affected by the odor mixture system, that is, the icewine system. Moreover, we did not carried out a systematic research on one influent factor in perceptual interactions, namely the intensity ratio between different aroma vector combinations. Therefore, in future experiments, this factor might be more systematically consider as well as aroma combinations involving components' intensity near detection threshold, in order to

bring complementary knowledge on perceptual interactions and the laws of odor mixtures processing.

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# Supplementary information

**Supplementary Table 1** Wine Samples and Reference for Data Used in Heatmaps Visualization in the Figure 6

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| Amontillado Sherry wines | Palomino Fino, Pedro Ximénez, and Moscatel | Sherry wines | (Collin, Nizet, Claeys Bouuaert, & Despatures, 2012) |
| Arbois Jura flor-sherry wines 1 | Savagnin | Sherry wines | (Collin et al., 2012) |
| Arbois Jura flor-sherry wines 2 | Savagnin | Sherry wines | (Collin et al., 2012) |
| Australia Cabernet Sauvignon rosé wine | Cabernet Sauvignon | Rosé wines | (Wang, Gambetta, & Jeffery, 2016) |
| Australia Shiraz rosé wine | Shiraz | Rosé wines | (Wang et al., 2016) |
| Australian Cabernet Sauvignon red wines | Cabernet Sauvignon | Red wines | (Gürbüz, Rouseff, & Rouseff, 2006) |
| Australian Merlot red wines | Merlot | Red wines | (Gürbüz et al., 2006) |
| Australian Semillon botrytis wines 1 | Semillon | Botrytis wines | (Siebert, Barter, de Barros Lopes, Herderich, & Francis, 2018) |
| Australian Semillon botrytis wines 2 | Semillon | Botrytis wines | (Siebert, Barter, et al., 2018) |
| Australian Shiraz red wines | Shiraz | Red wines | (Chin, Eyres, & Marriott, 2012) |
| Barossa Valley Shiraz red wines | Shiraz | Red wines | (Mayr et al., 2014) |
| Barsac botrytis wines | Semillon,Sauvignon blanc | Botrytis wines | (Sarrazin, Dubourdieu, et al., 2007) |
| Bierzo Mencía red wines | Mencía | Red wines | (San-Juan, Pet'Ka, Cacho, Ferreira, & Escudero, 2010) |
| Boal Madeira wines | Boal | Madeira wines | (E. Campo, Ferreira, Escudero, Marqués, & Cacho, 2006) |
| Boal white wines | Boal | White wines | (E. Campo et al., 2006) |
| Bordeaux Cabernet Sauvignon red wines | Cabernet Sauvignon | Red wines | (Kotseridis & Baumes, 2000) |
| Bordeaux Merlot red wines | Merlot | Red wines | (Kotseridis & Baumes, 2000) |
| Bordeaux musts | Cabernet Sauvignon | Musts | (Kotseridis & Baumes, 2000) |
| Calatayud rosé wines | Grenache | Rosé wines | (V. Ferreira et al., 2002) |
| California Cabernet Sauvignon red wines | Cabernet Sauvignon | Red wines | (Gürbüz et al., 2006) |
| California Merlot red wines | Merlot | Red wines | (Gürbüz et al., 2006) |
| Canary Islands white wines 1 | Gual | White wines | (López, Ortín, Pérez-Trujillo, Cacho, & Ferreira, 2003) |

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| Canary Islands white wines 2 | Verdello | White wines | (López et al., 2003) |
| Canary Islands white wines 3 | Marmajuelo | White wines | (López et al., 2003) |
| Canary Islands white wines 4 | Listan | White wines | (López et al., 2003) |
| Canary Islands white wines 5 | Malvas | White wines | (López et al., 2003) |
| Castro Iuvara rosé wines | Prieto Picudo | Rosé wines | (Álvarez-Pérez et al., 2012) |
| Chardonnay white wines 1 | Chardonnay | White wines | (Siebert, Barter, et al., 2018) |
| Chardonnay white wines 2 | Chardonnay | White wines | (Siebert, Barter, et al., 2018) |
| Chardonnay white wines 3 | Chardonnay | White wines | (Siebert, Barter, et al., 2018) |
| Chardonnay white wines 4 | Chardonnay | White wines | (Siebert, Barter, et al., 2018) |
| Château-Chalon Jura flor-sherry wines | Savagnin | Sherry wines | (Collin et al., 2012) |
| Chile aged sparkling wines | País | Sparkling wines | (Ubeda et al., 2019) |
| Chile sparkling wines | País | Sparkling wines | (Ubeda et al., 2019) |
| Chile white wines | País | White wines | (Ubeda et al., 2019) |
| Chinese icewines H3 | Beibinghong | Icewines | (Lan et al., 2019) |
| Chinese icewines H4 | Beibinghong | Icewines | (Lan et al., 2019) |
| Chinese Loess Plateau Region red wines 1 | Cabernet Sauvignon | Red wines | (Tang et al., 2019) |
| Chinese Loess Plateau Region red wines 2 | Cabernet Sauvignon | Red wines | (Tang et al., 2019) |
| Chinese Loess Plateau Region red wines 3 | Cabernet Sauvignon | Red wines | (Tang et al., 2019) |
| Chinese Loess Plateau Region red wines 4 | Cabernet Sauvignon | Red wines | (Tang et al., 2019) |
| Chinese Loess Plateau Region red wines 5 | Cabernet Sauvignon | Red wines | (Tang et al., 2019) |
| Chinese red wines 2014 | Marselan | Red wines | (Lyu et al., 2019) |
| Chinese red wines 2015 | Marselan | Red wines | (Lyu et al., 2019) |
| Chinese red wines H1 | Beibinghong | Red wines | (Lan et al., 2019) |
| Chinese red wines H2 | Beibinghong | Red wines | (Lan et al., 2019) |
| Coyotes Run Riesling icewine 1 | Riesling | Icewines | (Bowen & Reynolds, 2012) |
| Coyotes Run Riesling icewine 2 | Riesling | Icewines | (Bowen & Reynolds, 2012) |
| Coyotes Run Vidal icewine 1 | Vidal | Icewines | (Bowen & Reynolds, 2012) |

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| Coyotes Run Vidal icewine 2 | Vidal | Icewines | (Bowen & Reynolds, 2012) |
| Coyotes Run Vidal white wine 1 | Vidal | White wines | (Bowen & Reynolds, 2012) |
| Coyotes Run Vidal white wine 2 | Vidal | White wines | (Bowen & Reynolds, 2012) |
| Coyotes Run Riesling white wine 1 | Riesling | White wines | (Bowen & Reynolds, 2012) |
| Coyotes RunRiesling white wine 2 | Riesling | White wines | (Bowen & Reynolds, 2012) |
| Duero aged red wines | Unknown | Aged red wines | (V. Ferreira, Aznar, Lopez, & Cacho, 2001) |
| German Gewürztraminer botrytis musts | Gewürztraminer | Botrytis musts | (Pinar, Rauhut, Ruehl, & Buettner, 2016) |
| German Gewürztraminer musts | Gewürztraminer | Musts | (Pinar et al., 2016) |
| German red botrytis musts | Red Riesling | Botrytis musts | (Pinar et al., 2016) |
| German red Riesling musts | Red Riesling | Musts | (Pinar et al., 2016) |
| German white botrytis musts | White Riesling | Botrytis musts | (Pinar et al., 2016) |
| German white musts | White Riesling | Musts | (Pinar et al., 2016) |
| Huelva white wines | Zalema | White wines | (Gómez-Míguez, Cacho, Ferreira, Vicario, & Heredia, 2007) |
| Maccabeo white wines | Maccabeo | White wines | (Escudero et al., 2004) |
| Malvazia Madeira wines | Malvazia | Madeira wines | (E. Campo et al., 2006) |
| Malvazia white wines | Malvazia | White wines | (E. Campo et al., 2006) |
| Margaret River Shiraz red wines | Shiraz | Red wines | (Mayr et al., 2014) |
| Marlborough white wines 1 | Sauvignon Blanc | White wines | (Benkwitz et al., 2012) |
| Marlborough white wines 2 | Sauvignon Blanc | White wines | (Benkwitz et al., 2012) |
| Monterey white wines | Chardonnay | White wines | (Lee & Noble, 2003) |
| Montilla-Moriles PX wines | Pedro Ximénez | Pedro Ximenez wines | (E. Campo et al., 2006) |
| Ningxia Helan Mountain red wines | Syrah | Red wines | (Zhao, Gao, Qian, & Li, 2017) |
| Oregon Pinot Noir red wines 1 | Pinot Noir | Red wines | (Fang & Qian, 2005) |
| Oregon Pinot Noir red wines 2 | Pinot Noir | Red wines | (Fang & Qian, 2005) |
| Pardevalles rosé wines | Prieto Picudo | Rosé wines | (Álvarez-Pérez et al., 2012) |
| Penedès sparkling wines | Xarello, Macabeo, Chardonnay | Sparkling wines | (E. Campo, Cacho, & Ferreira, 2008) |

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| Portugal aged red wines 1 | Trincadeira | Aged red wines | (Barata et al., 2011) |
| Portugal aged red wines 2 | Trincadeira, Cabernet Sauvignon | Aged red wines | (Barata et al., 2011) |
| Portugal red wines 1 | Trincadeira | Red wines | (Barata et al., 2011) |
| Portugal red wines 2 | Trincadeira, Cabernet Sauvignon | Red wines | (Barata et al., 2011) |
| Premières Côtes de Blaye white wines | Semillon,Sauvignon blanc | White wines | (Sarrazin, Dubourdieu, et al., 2007) |
| Priorato aged red wines | Unknown | Aged red wines | (V. Ferreira et al., 2001) |
| Rheinhessen red wines | Dornfelder | Red wines | (Frank et al., 2011) |
| Ribera Duero aged Spanish red wines 1 | Unknown | Aged red wines | (Culleré, Escudero, Cacho, & Ferreira, 2004) |
| Ribera Duero aged Spanish red wines 2 | Unknown | Aged red wines | (Culleré et al., 2004) |
| Ribera Duero aged Spanish red wines 3 | Unknown | Aged red wines | (Culleré et al., 2004) |
| Rioja aged red wines 1 | Tempranillo, Grenache, and Graciano | Aged red wines | (Aznar, López, Cacho, & Ferreira, 2001) |
| Rioja aged red wines 2 | Tempranillo, Grenache, and Graciano | Aged red wines | (V. Ferreira et al., 2001) |
| Rioja aged Spanish red wines | Unknown | Aged red wines | (Culleré et al., 2004) |
| Rioja Graciano red wines | Graciano | Red wines | (San-Juan et al., 2010) |
| Riverside white wines | Chardonnay | White wines | (Lee & Noble, 2003) |
| San Joaquin white wines | Chardonnay | White wines | (Lee & Noble, 2003) |
| Sauternes 2002 aged botrytis wines 2 year | Semillon | Aged botrytis wines | (Bailly, Jerkovic, Meurée, Timmermans, & Collin, 2009) |
| Sauternes 2002 aged botrytis wines 3.5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2002 aged botrytis wines 5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2002 aged botrytis wines 6 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2002 aged botrytis wines 6.5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2003 aged botrytis wines 2 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2003 aged botrytis wines 3.5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2003 aged botrytis wines 5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes 2003 aged botrytis wines 5.5 year | Semillon | Aged botrytis wines | (Bailly et al., 2009) |
| Sauternes botrytis wines | Semillon, Sauvignon | Botrytis wines | (E. Campo et al., 2008) |

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| | blanc, Muscadelle | | |
| Sauternes botrytis wines 2002 | Sauvignon Blanc, Semillon, or Muscadelle | Botrytis wines | (Bailly, Jerkovic, Marchand-Brynaert, & Collin, 2006) |
| Sauternes botrytis wines 2003 | Sauvignon Blanc, Semillon, or Muscadelle | Botrytis wines | (Bailly et al., 2006) |
| Sercial Madeira wines | Sercial | Madeira wines | (E. Campo et al., 2006) |
| Slovak white wines | Devín | White wines | (Peťka, Ferreira, González-Viñas, & Cacho, 2006) |
| Somontano aged Spanish red wines | Unknown | Aged red wines | (Culleré et al., 2004) |
| Somotano red wines | Unknown | Aged red wines | (V. Ferreira et al., 2001) |
| Sonoma white wines | Chardonnay | White wines | (Lee & Noble, 2003) |
| Spainish Cabernet Sauvignon red wines | Cabernet Sauvignon | Red wines | (Lopez, Ferreira, Hernandez, & Cacho, 1999) |
| Spainish Grenache red wines | Grenache | Red wines | (Lopez et al., 1999) |
| Spainish Merlot red wines | Merlot | Red wines | (Lopez et al., 1999) |
| Spainish Sherry wines | Palomino Fino | Sherry wines | (E. Campo et al., 2008) |
| Spainish sparkling wines | Macabeu,Xarel·lo, Parellada | Sparkling wines | (Torrens, Riu-Aumatell, Vichi, Lopez-Tamames, & Buxaderas, 2010) |
| Spainish white wines | Macabeu,Xarel·lo, Parellada | White wines | (Torrens et al., 2010) |
| Spanish ABA aged red wines | Tempranillo, Cabernet Sauvignon,Merlot | Aged red wines | (Escudero et al., 2007) |
| Spanish MUR aged red wines | Garnacha Tinta, Garnó, Sausó, Mazuelo | Aged red wines | (Escudero et al., 2007) |
| Spanish POR aged red wines | Cariñena, Grenache | Aged red wines | (Escudero et al., 2007) |
| Tierra de Castilla-Leon aged Spanish red wines | Unknown | Aged red wines | (Culleré et al., 2004) |
| Turkish rosé wines | Çalkarası | Rosé wines | (Darici, Cabaroglu, Ferreira, & Lopez, 2014) |
| Uruguayan AMA aged red wines | Tannat | Aged red wines | (Escudero et al., 2007) |
| Uruguayan BOU aged red wines | Tannat | Aged red wines | (Escudero et al., 2007) |
| Verdelho Madeira wines | Verdelho | Madeira wines | (E. Campo et al., 2006) |
| Verdelho white wines | Verdelho | White wines | (E. Campo et al., 2006) |
| Viognier white wines 1 | Viognier | White wines | (Siebert, Barter, et al., 2018) |
| Viognier white wines 2 | Viognier | White wines | (Siebert, Barter, et al., 2018) |

| Wine samples | Grape | Wine type | Reference |
|---|---|---|---|
| Viognier white wines 3 | Viognier | White wines | (Siebert, Barter, et al., 2018) |
| Viognier white wines 4 | Viognier | White wines | (Siebert, Barter, et al., 2018) |
| Yunnan red wines | Syrah | Red wines | (Zhao et al., 2017) |
| Zhangyu Vidal icewine 1 | Vidal | Icewines | (Ma et al., 2017) |
| Zhangyu Vidal icewine 2 | Vidal | Icewines | (Ma et al., 2017) |
| Zhangyu Vidal icewine 3 | Vidal | Icewines | (Ma et al., 2017) |

**Supplementary Table 2** Odor Cocktail Solution and Its Corresponding Odor Zones (OZs) Defined in the GC-O Training Session

| No. | Retention indices [a] | Odorant | CAS. | Concentration (ug/L) | Retention indices (OZs) [b] | | | Frequency (N=19) | Odor descriptors given by subjects |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Start | End | Average | | |
| 1 | 973 | ethyl isobutanoate | 97-62-1 | 82800 | 970 | 1000 | 985 | 16 | fruity, red fruity, strawberry, sugar, sweet |
| 2 | 1067 | ethyl 2-methylbutanoate | 7452-79-1 | 55100 | 1055 | 1075 | 1065 | 17 | cassis, fruity, lemon, red fruity, strawberry |
| 3 | 1140 | isoamyl acetate | 123-92-2 | 82500 | 1125 | 1145 | 1135 | 14 | banana, lemon, plastic, solvent |
| 4 | 1153 | ethyl valerate | 539-82-2 | 116100 | 1145 | 1165 | 1155 | 14 | citrus, fruity, melon, orange, strawberry, wood |
| 5 | 1218 | 3-methyl-1-butanol | 123-51-3 | 870300 | 1205 | 1230 | 1218 | 19 | almond, caramel, cheese, chocolate, coffee, toast, plastic, sour butter |
| 6 | 1395 | (Z)-3-hexen-1-ol | 928-96-1 | 57400 | 1390 | 1405 | 1398 | 16 | apple, flowery, herb |
| 7 | 1477 | methional | 3268-49-3 | 18500 | 1470 | 1500 | 1485 | 18 | bread, potato, sugar |
| 8 | 1556 | $\beta$-Linalool | 78-70-6 | 33400 | 1555 | 1565 | 1560 | 17 | flowery, lavender, lemon, plant, vanilla |

[a] Retention indices calculated from a chromatographic peak of a given compound on capillary column DB-Wax (30 m length, 0.25 i.d., 0.50 μm phase thickness); [b] Retention indices calculated from detection time of the same compound on the same capillary column.

**Supplementary Table 3** Information on the 11 Odorants and the Parameters of Olfactometer Set up for the 11 Odorants and the Icewine

| Module [a] | Volume [b] (mL) | Odorants | Flow (mL/min) [c] low | medium | high | Solution [d] (wt. %) | Solvent | Odor description | CAS. | Involved block [e] |
|---|---|---|---|---|---|---|---|---|---|---|
| Control L/R | 40 | ultra-pure water | 1500 | | | | | | | |
| Dilution L/R | 40 | ultra-pure water | Odorants + Dilution = 1500 | | | | | | | |
| L1 | 10 | 3-methyl-1-butanol | 30 | 50 | 70 | 8.89 | propylene glycol | alcohol | 123-51-3 | A; B; C; D |
| L2 | 10 | ethyl 2-methyl butanoate | 35 | 48 | 55 | 0.798 | propylene glycol | tropical fruit | 7452-79-1 | A; B; C; D; Warm up |
| L3 | 5 | *β*-damascenone | 40 | 55 | 65 | 0.677 | propylene glycol | honey | 23696-85-7 | A; B; C; D |
| L4 | 5 | 3-methylbutanoic acid | 100 | 130 | 180 | 0.223 | ultra-pure water | cheese | 503-74-2 | A; B; D |
| L5 | 5 | guaiacol | 40 | 60 | 80 | 0.899 | propylene glycol | smoky | 90-05-1 | A; B; C; D; Warm up |
| L6 | 5 | furaneol | 30 | 60 | 80 | 0.336 | propylene glycol | caramel | 3658-77-3 | A; B; C; D |
| R1 | 5 | 1-hexanol | 35 | 55 | 75 | pure | | green | 111-27-3 | A; C; D; Warm up |
| R2 | 10 | phenylethyl alcohol | 40 | 90 | 160 | pure | | rose | 60-12-8 | A; C; D |
| R3 | 5 | *γ*-decalactone | 30 | 80 | 150 | pure | | apricot | 706-14-9 | A; C; D |
| R4 | 5 | methional | 35 | 50 | 70 | 0.0786 | propylene glycol | cooked potato | 3268-49-3 | A; C; D |
| R5 | 5 | 1-octen-3-one | 15 | 35 | 50 | 0.0757 | propylene glycol | mushroom | 4312-99-6 | A; C; D |
| R6 | 15 | icewine | 100 | | | original | | | | A; B; Warm up Control |

[a] Flow module controlled by olfactometer, L represents the left channel and R represents the right channel; [b] The volume of odor solution injected in each olfactometer chamber; [c] The air flow went through the odorants in each olfactometer chamber under different intensity level; [d] Odor solution was used in the formal experiment delivered by Olfactometer; [e] The label of 4 experiment blocks listed in Supplementary Table 4

**Supplementary Table 4** Information of Odor Stimuli in the Different Blocks

| Session and Blocks | Involved odorants | Experimental design description | Combination | Number of stimuli |
|---|---|---|---|---|
| Training (52) | All the 11 odorants in Supplementary Table 3 and real icewine at medium intensity | To train subject to identify 11 odorants and to be familiar with different odor descriptors; determining the perceived intensity of different odorants' aroma attributes at low and high concentrations. | The 11 odorants at medium intensity (mark) | 11 |
| | | | The 11 odorants at medium intensity (feedback) | 11 |
| | | | Real icewine odor at medium intensity (2); The 11 odorants at low and high intensity | 24 |
| | | | Real icewine + 6 single odorants at medium intensity | 6 |
| Warm up | Ethyl 2-methyl butanoate, guaiacol, 1-hexanol and real icewine at medium intensity | To activate subjects' performance | One single odorants (3); real icewine (3) | |
| | | | One single odorant and one icewine for each session | 6 |
| Control (3) | Real icewine at medium intensity | control; repeatability and stability assessment | real icewine (3), and one for each session | 3 |
| A (33) | All the 11 odorants in Supplementary Table 3 at low, medium and high intensity | Exploring the influence of the intensity of key odorants | Real icewine + 11 odorants at 3 intensity levels | 33 |
| B (57) | 3-methyl-1-butanol (vector L)<br>ethyl 2-methyl butanoate (vector E)<br>*β*-damascenone (vector N)<br>3-methylbutanoic acid (vector A)<br>guaiacol (vector M)<br>furaneol (vector F) | Exploring the influence of different combinations, different numbers of Ferreira's aroma vectors (V. Ferreira et al., 2016) | Real icewine + binary combinations | 15 |
| | | | Real icewine + ternary combinations | 20 |
| | | | Real icewine + quaternary combinations | 15 |
| | | | Real icewine + quinary combinations | 6 |
| | | | Real icewine + senary combination | 1 |
| C (11) | all the 11 odorants in Supplementary Table 3 except for 3-methylbutanoic acid | Omission test (result was not shown) | A mixture of 10 odorants | 1 |
| | | | A mixture of 9 odorants (1 odor omission) | 10 |
| D (55) | All the 11 odorants in Supplementary Table 3 | Interaction of binary odor mixtures of key odorants in icewine | All binary combinations | 55 |

**Supplementary Table 5** Sample Information for Panelists Selection

| Sample | Compounds | Solution [a] (wt. %) | Odor description | CAS. |
|---|---|---|---|---|
| 1 | 3-methyl-1-butanol | 0.47 | alcoholic | 123-51-3 |
| 2 | ethyl 2-methyl butanoate | 0.12 | tropical fruit | 7452-79-1 |
| 3 | β-damascenone | 0.98 | honey | 23696-85-7 |
| 4 | 3-methylbutanoic acid | 0.74 | cheese | 503-74-2 |
| 5 | guaiacol | 0.027 | smoky | 90-05-1 |
| 6 | furaneol | 0.072 | caramel | 3658-77-3 |
| 7 | 1-hexanol | 0.85 | green | 111-27-3 |
| 8 | phenylethyl alcohol | 5.4 | rose | 60-12-8 |
| 9 | γ-decalactone | 0.41 | apricot | 706-14-9 |
| 10 | methional | 0.0025 | cooked potato | 3268-49-3 |
| 11 | 1-octen-3-one | 0.0074 | mushroom | 4312-99-6 |
| 12, 13 | icewine | | | |
| 14 | icewine | | | |
| | 1-octen-3-one | 0.0020 | | 4312-99-6 |
| 15 | icewine | | | |
| | furaneol | 0.22 | | 3658-77-3 |
| | ethyl 2-methyl butanoate | 0.013 | | 7452-79-1 |

[a] Odor solution used in panel selection which was diluted by propylene glycol or ultra-pure water (only for 3-methylbutanoic acid)

**Supplementary Table 6** Panel Performance in Evaluating the Same Icewine Odor Sample in 3 Different Sessions.

| Aroma attributes | Sample | Sample × subject | Sample × session |
|---|---|---|---|
| tropical fruit | 0.187 | 0.190 | 0.097 |
| nut | 0.261 | 0.089 | 0.412 |
| cooked potato | 0.291 | 0.117 | 0.982 |
| mushroom | 0.358 | 0.438 | 0.445 |
| honey | 0.625 | **0.027** | 0.138 |
| rose | 0.655 | **0.006** | **0.026** |
| smoky | 0.710 | 0.117 | 0.349 |
| green | 0.810 | 0.663 | 0.347 |
| apricot | 0.823 | 0.360 | 0.161 |
| caramel | 0.878 | 0.214 | 0.402 |
| cheese | 0.953 | 0.973 | 0.808 |
| raisin | 0.971 | 0.497 | 0.916 |
| alcoholic | 0.989 | 0.520 | 0.531 |

Note: Figures are *p*-values obtained in ANOVA including the 3 factors (columns); statistically significant values ($p < 0.05$) appeared in bold.

**Supplementary Table 7** Panel Performance in Evaluating Different Icewine Odor Mixture Samples in 2 Sessions.

| Aroma attributes | Sample | Sample × subject | Sample × session |
|---|---|---|---|
| cheese | **0.000** | **0.000** | 0.694 |
| smoky | **0.000** | **0.011** | 0.748 |
| apricot | **0.000** | 0.103 | 0.538 |
| caramel | **0.000** | 0.453 | 0.292 |
| tropical fruit | **0.000** | 0.897 | 0.840 |
| honey | **0.002** | 0.774 | 0.230 |
| alcoholic | **0.005** | 0.288 | 0.092 |
| cooked potato | 0.119 | **0.000** | 0.989 |
| rose | 0.216 | 0.213 | 0.436 |
| mushroom | 0.360 | 0.180 | 0.881 |
| green | 0.443 | 0.846 | 0.150 |
| nut | 0.810 | 0.089 | 0.232 |
| raisin | 0.904 | **0.037** | **0.022** |

Figures are *p*-values obtained in ANOVA including the 3 factors (columns); statistically significant values ($p < 0.05$) appeared in bold.

**Supplementary Table 8** The Pairs of Samples with the Closest Odor Profile in Figure 17

| Two stimuli (S1+Icewine, S2+Icewine) with the closest odor profile | | shared odorants | The difference between the two stimuli | |
|---|---|---|---|---|
| S1 | S2 | | replace | addition or omission |
| **L2L3L4L5L6** | L1**L2L3L4L5L6** | L2L3L4L5L6 | | L1 |
| **L2L3L5** | L1**L2L3L5** | L2L3L5 | | L1 |
| **L4L5L6** | L1**L4L5L6** | L4L5L6 | | L1 |
| L1**L4L6** | **L4L6** | L4L6 | | L1 |
| L1**L5L6** | **L5L6** | L5L6 | | L1 |
| L1 | | | | L1 |
| **L3** | L1L2**L3** | L3 | | L1L2 |
| **L4** | L1L3**L4** | L4 | | L1L3 |
| L1**L3L5**L6 | **L3L5** | L3L5 | | L1L6 |
| L1L3L6 | **L1**L2**L3**L5**L6** | L1L3L6 | | L2L5 |
| **L2**L3 | **L2** | L2 | | L3 |
| **L2**L3L4**L5** | **L2L5** | L2L5 | | L3L4 |
| L3L4**L5** | **L5** | L5 | | L3L4 |
| **L1L3L4L5** | **L1L3L4L5**L6 | L1L3L4L5 | | L6 |
| **L2L4L5** | **L2L4L5**L6 | L2L4L5 | | L6 |
| **L3L4**L6 | **L3L4** | L3L4 | | L6 |
| **L1**L2L5 | **L1**L6 | L1 | L2L5 | L6 |
| **L1**L3 | **L1**L2L6 | L1 | L3 | L2L6 |
| **L1**L3L5 | **L1**L2L4 | L1 | L3L5 | L2L4 |
| **L1**L2**L4**L5 | **L1**L3**L4**L6 | L1L4 | L2L5 | L3L6 |
| L1**L2** | **L2**L6 | L2 | L1 | L6 |
| L1**L2L3L4** | **L2L3L4**L6 | L2L3L4 | L1 | L6 |
| L1L2**L4L6** | L3**L4**L5**L6** | L4L6 | L1L2 | L3L5 |

**Supplementary Table 9** Intensity and Pleasantness of the 198 Different Trials

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | E | ethyl isobutanoate | 1,8-cineole | 4.94±1.72 | 5.62±2.12 | 4.90±1.78 | 4.46±1.60 | 4.55±1.80 | | | | |
| 2 | IP | *p*-cymene | ethyl valerate | 5.21±1.76 | 7.19±1.63 | 4.06±1.42 | 5.39±1.72 | 5.27±1.78 | *** | ** | ** | |
| 3 | P | diethyl succinate | isovaleric acid | 6.40±2.01 | 6.36±2.14 | 4.99±2.23 | 3.52±1.86 | 4.03±2.34 | | * | | |
| 4 | P | *γ*-heptalactone | methional | 5.81±1.41 | 6.05±1.23 | 5.49±1.70 | 4.61±1.74 | 5.16±2.11 | | * | | |
| 5 | E | diacetyl | ethyl 2-methylbutanoate | 5.44±1.83 | 6.24±1.55 | 4.04±1.68 | 4.82±1.52 | 4.45±2.48 | | | | |
| 6 | IP | butyl acetate | phenylethylthiol | 6.11±1.76 | 7.57±1.64 | 5.37±1.70 | 2.03±1.63 | 2.39±1.52 | ** | *** | *** | |
| 7 | E | 1,8-cineole | *p*-cymene | 5.41±1.77 | 4.75±1.69 | 4.77±1.54 | 4.57±1.07 | 4.89±1.33 | | | | |
| 8 | E | 1,8-cineole | *γ*-heptalactone | 5.00±1.90 | 5.71±1.87 | 5.18±1.88 | 5.17±1.70 | 5.01±1.55 | | | | |
| 9 | E | (*Z*)-3-hexenol | 1,8-cineole | 6.41±1.50 | 5.80±1.82 | 3.66±2.36 | 4.50±1.91 | 4.22±1.96 | | | | |
| 10 | E | hexanal | 2-ethylhexanol | 6.54±1.54 | 6.22±1.63 | 3.83±1.63 | 4.47±1.19 | 3.86±1.22 | | | | * |
| 11 | IP | hexanal | *p*-cymene | 6.69±1.49 | 5.31±1.49 | 3.46±2.07 | 4.35±1.74 | 3.93±1.92 | ** | * | | |
| 12 | IP | 1-heptanol | phenylethylthiol | 4.58±2.07 | 7.43±1.64 | 4.58±1.79 | 2.45±1.49 | 2.34±1.61 | *** | *** | *** | |
| 13 | E | diethyl succinate | *p*-cymene | 5.77±1.89 | 5.04±1.97 | 5.06±1.77 | 4.90±1.33 | 5.09±1.70 | | | | |
| 14 | I | *p*-cymene | *γ*-heptalactone | 5.05±1.84 | 6.17±1.87 | 4.94±1.13 | 5.61±1.93 | 5.45±1.71 | * | | | |
| 15 | I | *β*-phenethyl acetate | *p*-cymene | 6.65±1.71 | 5.26±1.58 | 5.27±1.83 | 4.95±1.40 | 5.13±2.02 | ** | | | |
| 16 | P | *p*-cymene | ethyl 2-methylbutanoate | 6.19±1.66 | 5.58±1.64 | 4.53±1.56 | 5.63±1.56 | 4.86±1.53 | | * | | |
| 17 | IP | *p*-cymene | phenylethylthiol | 5.87±1.54 | 7.19±1.19 | 4.51±2.04 | 2.39±1.35 | 2.60±1.33 | ** | *** | *** | |
| 18 | E | *p*-cymene | hexyl hexanoate | 5.74±1.72 | 5.07±1.97 | 4.36±1.41 | 3.82±1.78 | 4.32±1.31 | | | | |
| 19 | E | diethyl succinate | ethyl laurate | 4.80±1.78 | 3.68±1.85 | 5.03±1.67 | 5.33±1.46 | 5.14±1.78 | | | | |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | I | *γ*-heptalactone | undecanaldehyde | 6.50±1.48 | 3.86±1.82 | 5.25±1.86 | 5.10±1.58 | 5.94±2.01 | *** | | | * |
| 21 | E | linalool | eugenol | 5.23±1.59 | 4.69±1.91 | 4.82±1.31 | 5.63±1.87 | 5.98±1.52 | | | ** | |
| 22 | I | geraniol | ethyl 2-methylbutanoate | 4.90±1.0 | 6.34±1.35 | 5.38±1.62 | 5.87±2.08 | 5.84±1.83 | ** | | | |
| 23 | IP | *γ*-dodecalactone | phenylethylthiol | 6.17±1.46 | 7.35±1.34 | 4.53±1.97 | 1.84±1.25 | 2.40±1.40 | ** | *** | *** | |
| 24 | E | diethyl succinate | 1,8-cineole | 5.83±2.14 | 6.38±2.24 | 4.90±1.69 | 5.15±1.69 | 4.87±1.55 | | | | |
| 26 | E | *γ*-heptalactone | diethyl succinate | 6.60±1.62 | 5.86±1.69 | 5.70±1.73 | 5.50±1.71 | 5.54±1.55 | | | | |
| 27 | P | diethyl succinate | ethyl phenylacetate | 5.37±1.33 | 5.73±1.43 | 4.92±1.91 | 3.46±1.77 | 3.91±1.83 | | ** | | |
| 30 | P | *γ*-heptalactone | ethyl cinnamate | 5.58±1.53 | 5.23±1.69 | 5.16±1.60 | 3.97±1.57 | 4.39±1.93 | | * | | |
| 31 | IP | 2-pentanone | pentanal | 4.09±2.08 | 6.70±1.29 | 5.15±1.64 | 3.69±1.50 | 4.57±1.71 | *** | *** | | *** |
| 33 | P | ethyl butanoate | benzaldehyde | 6.36±1.46 | 7.06±1.19 | 5.56±2.01 | 4.57±2.06 | 4.71±2.07 | | * | ** | |
| 34 | IP | butanal | ethyl 3-(methylsulfanyl)propanoate | 6.83±1.42 | 4.94±1.93 | 2.96±1.91 | 3.61±1.66 | 3.05±1.89 | *** | * | | |
| 35 | IP | 1-methoxy-4-[(*E*)-prop-1-en-1-yl]benzene(trans-anethol) | carveol | 6.58±1.38 | 4.73±1.92 | 4.19±1.96 | 5.66±1.70 | 4.46±1.87 | *** | *** | | *** |
| 36 | E | ethyl 2-methylbutanoate | valencene | 6.38±1.70 | 6.01±1.60 | 4.41±2.18 | 4.34±1.57 | 4.40±2.03 | | | | |
| 37 | I | valencene | (*Z*)-3-hexenol | 6.62±1.40 | 5.47±1.80 | 4.86±1.99 | 5.22±2.17 | 4.49±2.09 | ** | | | * |
| 38 | P | myrcene | hexanal | 6.47±1.95 | 7.09±1.35 | 4.98±2.38 | 3.88±2.20 | 4.20±1.97 | | * | | |
| 39 | P | benzyl acetate | 2-heptanol | 6.41±1.13 | 6.12±1.41 | 5.78±2.17 | 4.28±1.58 | 5.49±1.86 | | *** | | ** |
| 40 | P | *p*-cresol | 2-octanone | 6.60±1.80 | 7.18±1.36 | 1.97±1.84 | 2.67±1.86 | 2.05±1.79 | | * | | * |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | I | 1-heptanol | diethyl acetal | 4.77±1.94 | 6.57±1.73 | 4.57±1.90 | 3.93±2.10 | 3.70±1.75 | *** | | * | |
| 42 | E | ethyl 3-(methylsulfanyl)propanoate | (*Z*)-3-hexenol | 5.19±1.76 | 5.47±1.44 | 4.73±1.23 | 5.21±1.99 | 5.28±1.96 | | | | |
| 43 | E | 2-methyl-1-butanol | *p*-cymene | 5.48±1.61 | 6.08±1.70 | 3.98±2.03 | 4.64±1.69 | 4.77±1.61 | | | * | |
| 44 | E | myrcene | diethyl succinate | 6.14±1.77 | 5.70±1.79 | 5.09±2.20 | 4.76±1.84 | 4.87±1.71 | | | | |
| 45 | E | myrcene | diethyl acetal | 6.05±1.62 | 6.24±1.78 | 4.25±1.78 | 4.27±1.88 | 3.71±2.13 | | | | |
| 46 | I | *p*-cymene | ethyl 3-(methylsulfanyl)propanoate | 6.51±1.66 | 4.56±1.68 | 4.38±2.10 | 4.36±1.47 | 4.70±1.86 | *** | | | |
| 47 | E | octanal | 3-methyl-1-butanol | 5.79±1.77 | 5.56±1.58 | 4.57±2.08 | 4.23±2.08 | 4.24±2.27 | | | | |
| 48 | IP | 2-nonanone | citronellol | 6.20±1.85 | 5.33±1.66 | 4.92±2.10 | 5.72±1.74 | 5.53±1.77 | ** | * | | |
| 49 | E | γ-undecalactone | diethyl acetal | 6.71±1.72 | 6.83±1.17 | 4.91±2.01 | 4.76±2.22 | 4.61±2.09 | | | | |
| 50 | IP | diethyl succinate | ethyl 3-(methylsulfanyl)propanoate | 5.75±1.27 | 5.08±1.50 | 5.39±1.76 | 4.61±1.79 | 5.33±2.01 | * | * | | * |
| 51 | E | γ-heptalactone | ethyl propionate | 5.97±1.58 | 5.23±1.79 | 5.66±1.92 | 5.47±1.82 | 5.68±1.91 | | | | |
| 52 | P | γ-heptalactone | 1-octen-3-ol | 5.83±1.70 | 5.33±1.33 | 5.23±1.50 | 4.08±1.53 | 4.40±1.45 | | ** | * | |
| 53 | I | nerol oxide | γ-heptalactone | 4.70±1.68 | 5.67±1.07 | 6.35±1.60 | 6.16±1.66 | 6.06±1.72 | ** | | | |
| 54 | I | γ-heptalactone | ethyl 3-(methylsulfanyl)propanoate | 5.62±1.65 | 4.69±1.82 | 5.68±1.25 | 5.22±1.92 | 5.73±1.45 | ** | | | |
| 55 | E | methionol | ethyl phenylacetate | 5.71±1.67 | 6.41±1.30 | 3.69±1.71 | 3.38±2.32 | 3.24±1.98 | | | | |
| 56 | IP | eugenol | 1-heptanol | 5.12±2.09 | 6.66±1.45 | 5.31±1.67 | 4.24±1.99 | 4.85±1.90 | *** | ** | | * |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 57 | P | octanal | ethyl phenylacetate | 5.99±1.48 | 6.23±1.81 | 4.53±2.24 | 3.65±2.29 | 3.74±2.29 | | * | | |
| 58 | I | ethyl 2-methylbutanoate | 4-hexanolide | 6.42±1.73 | 4.84±1.89 | 5.35±1.88 | 5.17±1.43 | 5.54±1.68 | *** | | | |
| 59 | E | ethyl 2-methylbutanoate | (Z)-3-hexenol | 6.29±1.61 | 5.77±1.63 | 5.78±1.96 | 5.72±1.88 | 6.24±1.95 | | | | |
| 60 | E | ethyl 2-methylbutanoate | myrcene | 6.67±1.49 | 6.42±1.30 | 4.96±2.02 | 4.88±2.24 | 4.92±2.27 | | | | |
| 61 | E | methyl octanoate | ethyl 2-methylbutanoate | 5.64±1.28 | 5.80±1.41 | 5.81±1.76 | 5.89±1.61 | 6.52±1.52 | | | * | ** |
| 62 | E | ethyl 2-methylbutanoate | γ-heptalactone | 5.75±1.68 | 6.12±1.61 | 5.59±1.48 | 5.15±2.00 | 5.64±1.77 | | | | |
| 63 | IP | diethyl acetal | ethyl 2-methylbutanoate | 6.05±1.46 | 5.16±1.63 | 4.35±1.73 | 5.43±1.46 | 4.55±1.67 | ** | ** | | ** |
| 64 | IP | ethyl 2-methylbutanoate | ethyl 3-(methylsulfanyl)propanoate | 5.89±1.79 | 4.51±2.02 | 5.31±1.57 | 3.87±1.37 | 5.52±1.52 | ** | *** | | *** |
| 65 | P | dimethyl trisulfide | vanillin | 5.18±1.86 | 4.48±1.51 | 3.88±1.85 | 6.60±2.01 | 4.21±1.84 | | *** | | *** |
| 66 | E | phenylethylthiol | pentanal | 7.13±1.80 | 7.13±1.54 | 2.34±1.79 | 2.96±1.93 | 2.51±1.86 | | | | |
| 67 | P | phenylethylthiol | ethyl 2-methylbutanoate | 6.09±1.88 | 5.74±1.25 | 2.69±1.69 | 6.12±1.66 | 3.61±1.82 | | *** | ** | *** |
| 68 | I | 1,8-cineole | acetoin | 6.27±1.60 | 5.08±1.76 | 5.23±1.70 | 4.99±2.19 | 4.93±1.91 | *** | | | |
| 69 | IP | ethyl 3-(methylsulfanyl)propanoate | phenylethylthiol | 4.15±1.83 | 6.22±1.55 | 4.68±1.42 | 2.71±1.24 | 2.92±1.47 | *** | *** | *** | |
| 70 | P | ethyl isobutanoate | diethyl acetal | 5.37±1.91 | 5.59±1.85 | 5.19±1.71 | 4.55±1.84 | 4.90±2.06 | | * | | |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 71 | IP | valencene | diethyl acetal | 6.52±1.42 | 5.27±1.37 | 4.11±2.03 | 4.93±1.40 | 4.24±1.74 | *** | ** | | * |
| 73 | I | diethyl acetal | ethyl 3-(methylsulfanyl)propanoate | 5.26±1.76 | 4.60±1.89 | 4.40±1.81 | 4.30±1.77 | 4.42±1.69 | * | | | |
| 74 | IP | ethyl 3-methylbutanoate | ethyl 3-(methylsulfanyl)propanoate | 6.84±1.44 | 4.20±1.77 | 5.72±1.83 | 5.01±1.47 | 5.17±2.07 | *** | * | | |
| 75 | IP | 1,8-cineole | ethyl 3-(methylsulfanyl)propanoate | 6.11±1.53 | 3.96±1.95 | 5.48±2.13 | 4.61±1.56 | 5.78±1.83 | *** | * | | ** |
| 76 | I | 2-methylbutanal | geranyl acetate | 6.35±1.75 | 5.29±1.92 | 4.80±1.86 | 4.94±1.69 | 5.00±2.02 | * | | | |
| 77 | IP | citronellol | valencene | 5.17±1.17 | 5.91±1.39 | 6.21±1.77 | 4.68±1.67 | 5.00±2.07 | * | *** | ** | |
| 78 | I | 2-ethylhexanol | *γ*-decalactone | 4.67±2.08 | 6.18±1.63 | 5.11±1.49 | 5.66±1.82 | 5.76±1.85 | *** | | | |
| 79 | I | *γ*-butyrolactone | *β*-phenethyl acetate | 4.33±1.84 | 6.20±1.59 | 5.36±1.70 | 5.91±1.67 | 5.74±1.90 | *** | | | |
| 80 | I | carveol | eugenol | 4.86±1.87 | 4.10±1.82 | 6.01±1.62 | 5.86±1.56 | 6.03±1.54 | * | | | |
| 81 | P | 2-octanone | ethyl phenylacetate | 6.42±1.45 | 5.89±1.98 | 5.30±1.75 | 4.02±1.97 | 4.42±1.68 | | ** | * | |
| 82 | E | myrcene | ethyl cinnamate | 5.30±1.77 | 5.70±1.86 | 5.15±1.66 | 4.64±1.79 | 4.82±1.90 | | | | |
| 83 | E | ethyl octanoate | benzyl acetate | 6.80±1.59 | 6.44±1.46 | 5.16±2.33 | 5.28±1.97 | 6.09±2.22 | | | ** | ** |
| 84 | E | diethyl succinate | 1-methoxy-4-[(*E*)-prop-1-en-1-yl]benzene(trans-anethol) | 5.77±1.57 | 5.91±1.88 | 5.36±1.51 | 5.08±2.22 | 4.96±1.69 | | | | |
| 85 | I | *β*-phenethyl acetate | *γ*-heptalactone | 5.36±1.72 | 6.24±1.49 | 5.28±1.49 | 5.59±1.96 | 6.09±1.69 | ** | | * | |
| 86 | I | eugenol | *β*-phenethyl acetate | 3.86±2.12 | 5.95±1.74 | 5.31±1.84 | 5.70±1.95 | 5.93±2.05 | *** | | * | |
| 87 | IP | ethyl phenylacetate | ethyl 2-methylbutanoate | 5.69±1.95 | 6.79±1.22 | 4.26±1.86 | 5.85±1.92 | 5.28±1.70 | ** | ** | ** | * |
| 88 | E | phenylethanal | ethyl cinnamate | 6.26±1.71 | 5.63±1.75 | 4.56±1.94 | 4.22±1.95 | 4.38±2.14 | | | | |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | IP | phenylethylthiol | benzyl acetate | 7.99±1.33 | 6.31±1.48 | 2.41±1.70 | 4.62±1.92 | 2.85±1.47 | *** | *** | ** | *** |
| 90 | E | 1-methoxy-4-[(*E*)-prop-1-en-1-yl]benzene(trans-anethol) | diethyl acetal | 5.73±1.67 | 6.22±2.03 | 4.87±1.88 | 4.60±1.78 | 4.63±1.94 | | | | |
| 91 | I | isoeugenol | ethyl 3-methylbutanoate | 4.62±1.76 | 6.50±1.63 | 5.76±2.12 | 5.52±2.41 | 5.42±2.49 | *** | | | |
| 92 | I | ethyl 2-methylbutanoate | diethyl succinate | 6.33±1.42 | 5.76±1.44 | 5.49±1.84 | 4.97±1.74 | 5.59±1.75 | * | | | ** |
| 93 | IP | diethyl succinate | vanillin | 5.41±1.97 | 4.13±1.99 | 5.59±1.93 | 6.69±1.73 | 6.06±1.54 | ** | ** | | |
| 95 | E | ethyl 2-methylbutanoate | *p*-anisaldehyde | 6.27±1.60 | 6.08±1.43 | 5.64±2.12 | 4.80±2.11 | 5.14±2.09 | | | | |
| 96 | IP | phenylethylthiol | carveol | 7.14±1.39 | 4.68±1.45 | 2.47±1.21 | 5.61±1.50 | 2.70±1.40 | *** | *** | | *** |
| 97 | IP | *o*-aminoacetophenone | diethyl succinate | 6.61±1.52 | 5.80±2.01 | 4.11±2.11 | 5.68±2.12 | 5.07±1.96 | * | *** | *** | |
| 98 | P | *γ*-heptalactone | 4-methylthiazole | 6.01±1.40 | 5.48±1.89 | 5.57±1.79 | 4.10±1.64 | 4.92±2.31 | | *** | * | * |
| 99 | E | 4-ethylguaiacol | acetylthiophene | 5.65±1.95 | 5.49±1.63 | 3.99±1.94 | 3.99±1.89 | 3.66±1.85 | | | | |
| 100 | IP | ethyl 2-methylbutanoate | trimethylthiazole | 6.07±1.55 | 6.75±1.42 | 5.83±1.42 | 3.60±1.97 | 4.30±1.78 | * | *** | *** | * |
| 101 | I | 4-methylthiazole | isobutyl thiazole | 4.63±1.91 | 6.27±1.57 | 4.38±1.79 | 3.94±1.79 | 4.10±1.78 | ** | | | |
| 102 | IP | 1-octen-3-ol | phenylethylthiol | 5.49±1.53 | 6.86±1.37 | 4.84±1.46 | 3.16±1.51 | 3.17±1.50 | *** | *** | *** | |
| 103 | IP | phenylethylthiol | myrcene | 7.45±1.77 | 6.48±1.51 | 2.40±1.85 | 5.09±2.30 | 2.60±2.12 | ** | *** | | *** |
| 104 | I | carveol | ethyl 2-methylbutanoate | 4.99±1.42 | 6.27±1.38 | 6.08±1.44 | 6.13±1.41 | 6.24±1.29 | *** | | | |
| 105 | IP | 2-heptanol | ethyl 2-methylbutanoate | 5.07±2.22 | 6.10±1.65 | 5.59±1.89 | 6.23±1.76 | 6.00±1.23 | * | * | | |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 106 | E | carveol | myrcene | 5.25±1.84 | 5.53±1.68 | 6.15±1.43 | 5.82±1.57 | 6.15±1.62 | | | | |
| 107 | E | myrcene | 2-ethylhexanol | 5.74±1.10 | 5.16±1.59 | 5.28±1.67 | 4.60±1.94 | 4.87±1.89 | | | | |
| 108 | IP | *γ*-heptalactone | 2-methylbutanal | 5.63±1.56 | 6.78±1.81 | 5.29±1.76 | 4.21±2.24 | 4.71±2.23 | * | * | * | |
| 109 | I | *γ*-heptalactone | carveol | 6.28±1.35 | 4.58±1.47 | 5.94±1.63 | 6.06±1.42 | 5.97±1.62 | *** | | | |
| 110 | I | *γ*-heptalactone | 2-heptanol | 5.25±1.97 | 4.32±1.39 | 5.17±1.36 | 5.26±1.47 | 5.05±1.88 | ** | | | |
| 111 | P | acetylthiophene | diethyl acetal | 6.14±1.95 | 6.00±1.33 | 3.12±1.69 | 5.03±1.68 | 3.83±1.76 | | *** | ** | *** |
| 112 | IP | phenylethylthiol | diethyl acetal | 7.26±1.31 | 6.27±1.90 | 2.06±1.53 | 5.69±1.83 | 2.23±1.59 | ** | *** | | *** |
| 115 | I | isoeugenol | ethyl 3-(methylsulfanyl)propanoate | 5.98±1.74 | 5.13±1.62 | 5.53±2.20 | 4.77±1.43 | 5.53±1.66 | * | | | ** |
| 116 | I | myrcene | *p*-cymene | 5.72±1.52 | 6.55±1.56 | 5.68±1.68 | 5.43±2.14 | 5.72±1.72 | *** | | | |
| 117 | I | ethyl octanoate | diethyl succinate | 6.33±1.30 | 5.35±1.56 | 5.36±2.12 | 5.82±1.79 | 6.06±1.77 | *** | | * | |
| 118 | I | *γ*-heptalactone | *γ*-undecalactone | 5.67±1.60 | 6.69±1.52 | 5.23±1.64 | 5.05±2.02 | 5.72±2.00 | ** | | | * |
| 120 | I | *γ*-heptalactone | phenylethanal | 5.80±1.57 | 6.33±1.25 | 5.18±1.59 | 5.37±1.97 | 5.01±1.86 | * | | | |
| 121 | P | 2-octanone | ethyl butanoate | 6.29±1.28 | 6.60±1.36 | 5.63±1.73 | 6.29±1.77 | 6.45±1.83 | | ** | ** | |
| 122 | IP | phenylethylthiol | vanillin | 6.45±1.64 | 4.59±1.99 | 3.33±1.64 | 6.70±1.35 | 3.77±1.95 | *** | *** | | *** |
| 123 | E | linalool | ethyl valerate | 6.61±1.78 | 6.64±1.39 | 5.34±1.75 | 5.35±2.17 | 6.06±2.00 | | | | |
| 124 | P | valencene | 1,8-cineole | 6.64±1.56 | 6.21±1.53 | 4.60±2.01 | 5.84±1.46 | 4.99±1.73 | | *** | | *** |
| 125 | IP | carveol | *γ*-undecalactone | 5.58±1.36 | 7.02±1.54 | 5.68±1.87 | 3.71±1.83 | 4.42±2.09 | *** | *** | ** | ** |
| 126 | P | valencene | diethyl succinate | 6.00±1.28 | 5.83±1.31 | 5.21±1.70 | 6.39±1.66 | 5.45±1.79 | | *** | | * |
| 128 | P | 1,8-cineole | trimethylthiazole | 5.84±1.96 | 6.09±1.82 | 5.20±1.85 | 3.89±1.85 | 4.29±1.69 | | ** | * | |
| 133 | IP | nerol oxide | hexyl hexanoate | 5.31±1.46 | 6.20±1.85 | 6.55±1.94 | 4.51±1.88 | 5.32±1.95 | ** | *** | *** | ** |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 135 | I | benzaldehyde | ethyl 3-(methylsulfanyl)propanoate | 7.28±1.44 | 4.29±1.86 | 4.73±2.49 | 4.55±1.39 | 4.74±2.06 | *** | | | |
| 136 | IP | *o*-aminoacetophenone | myrcene | 6.61±1.21 | 5.57±1.63 | 4.07±1.79 | 5.35±1.57 | 4.41±1.79 | ** | ** | | * |
| 137 | I | myrcene | ethyl 3-(methylsulfanyl)propanoate | 6.29±1.57 | 5.52±1.63 | 5.05±1.75 | 4.80±1.68 | 4.87±1.80 | * | | | |
| 138 | IP | geranyl acetate | isobutyl thiazole | 5.72±1.67 | 6.43±1.39 | 5.36±1.55 | 3.99±1.56 | 4.97±1.72 | * | *** | | *** |
| 139 | E | diethyl acetal | citronellol | 5.77±1.90 | 5.24±1.80 | 5.27±1.81 | 5.85±1.61 | 5.80±1.75 | | | | |
| 140 | I | *γ*-decalactone | ethyl 3-(methylsulfanyl)propanoate | 6.01±1.72 | 5.62±1.27 | 5.58±2.00 | 5.33±1.68 | 5.58±1.90 | ** | | | |
| 141 | IP | 3-methyl-1-butanol | 2-ethylhexanol | 6.36±1.84 | 4.78±1.72 | 3.65±2.44 | 5.16±1.40 | 3.76±2.39 | *** | ** | | ** |
| 142 | E | 1-octen-3-ol | geraniol | 4.63±1.57 | 4.23±1.48 | 5.23±0.98 | 5.25±1.84 | 5.46±1.20 | | | | |
| 143 | I | 2-heptanol | diethyl succinate | 5.52±1.71 | 6.39±1.44 | 5.30±1.28 | 5.30±1.59 | 5.39±1.52 | * | | | |
| 144 | P | 1-heptanol | *γ*-heptalactone | 6.00±1.44 | 5.74±1.33 | 4.12±1.61 | 5.77±1.64 | 5.51±1.83 | | *** | *** | |
| 146 | IP | acetoin | diethyl succinate | 5.62±1.59 | 6.31±1.42 | 4.98±1.67 | 5.78±1.88 | 5.27±1.96 | * | * | | |
| 147 | IP | phenylethylthiol | diethyl succinate | 6.88±2.01 | 5.98±1.40 | 2.89±1.76 | 5.40±1.82 | 3.24±1.72 | ** | *** | | *** |
| 148 | I | diethyl succinate | diethyl acetal | 5.91±1.43 | 5.04±2.02 | 5.12±1.74 | 4.85±1.71 | 4.96±1.74 | * | | | |
| 149 | IP | *γ*-heptalactone | phenylethylthiol | 5.45±2.11 | 6.97±1.34 | 5.17±1.75 | 2.70±1.50 | 3.48±1.86 | *** | *** | *** | ** |
| 150 | IP | *γ*-heptalactone | diethyl acetal | 5.93±1.71 | 4.76±1.95 | 4.03±1.69 | 5.05±1.45 | 5.15±1.60 | ** | ** | ** | |
| 151 | P | phenylethanal | ethyl 2-methylbutanoate | 6.42±1.38 | 6.22±1.41 | 4.97±2.07 | 5.95±1.57 | 4.96±1.61 | | ** | | ** |
| 152 | IP | phenylethanal | phenylethylthiol | 6.19±1.46 | 7.05±1.48 | 5.15±1.86 | 2.42±1.35 | 2.86±1.77 | ** | *** | *** | |
| 153 | IP | vanillin | diethyl acetal | 4.03±1.84 | 5.40±1.70 | 6.48±1.64 | 4.74±1.38 | 6.52±1.73 | *** | *** | | *** |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 154 | P | *p*-cresol | ethyl 3-(methylsulfanyl)propanoate | 6.96±1.37 | 6.76±1.22 | 2.16±1.70 | 2.87±1.72 | 2.43±1.68 | | ** | | |
| 155 | I | ethyl 3-methylbutanoate | ethyl valerate | 5.86±1.65 | 6.50±1.47 | 6.01±1.77 | 6.00±2.14 | 6.12±1.96 | * | | | |
| 156 | P | ethyl valerate | methional | 6.53±1.76 | 6.56±1.71 | 5.79±1.98 | 4.45±1.89 | 4.74±1.59 | | ** | * | |
| 157 | E | methional | 3-methyl-1-butanol | 5.98±1.59 | 5.49±1.47 | 4.53±1.70 | 3.97±1.86 | 4.60±1.84 | | | | |
| 158 | P | 3-methyl-1-butanol | butyl acetate | 5.73±1.71 | 6.20±1.53 | 4.26±1.56 | 5.29±1.58 | 5.10±1.76 | | ** | ** | |
| 159 | I | butyl acetate | ethyl isobutanoate | 6.24±1.23 | 5.08±1.57 | 5.36±1.37 | 5.89±1.43 | 5.80±1.56 | *** | | | |
| 160 | IP | ethyl 3-methylbutanoate | ethyl isobutanoate | 5.83±1.46 | 5.19±1.76 | 6.27±1.66 | 5.79±1.78 | 5.74±1.90 | * | * | | |
| 161 | E | (*Z*)-3-hexenol | ethyl 3-methylbutanoate | 5.51±1.25 | 5.36±1.48 | 6.15±1.77 | 6.03±1.83 | 6.19±1.63 | | | | |
| 162 | P | *γ*-undecalactone | ethyl valerate | 6.99±1.30 | 6.67±1.47 | 4.13±2.36 | 5.34±2.20 | 4.81±2.71 | | ** | * | |
| 163 | I | methional | linalool | 5.80±1.40 | 6.44±1.27 | 4.79±1.83 | 5.21±1.87 | 4.99±2.05 | * | | | |
| 164 | IP | geraniol | 3-methyl-1-butanol | 5.02±1.51 | 5.98±1.55 | 5.38±1.68 | 4.02±1.81 | 4.67±1.91 | *** | *** | ** | ** |
| 165 | IP | ethyl 3-methylbutanoate | 3-methyl-1-butanol | 6.76±1.21 | 4.46±1.78 | 6.19±2.01 | 4.18±1.76 | 6.07±2.09 | *** | *** | | *** |
| 166 | IP | methionol | ethyl valerate | 5.71±1.79 | 6.64±1.55 | 4.30±1.27 | 5.67±1.71 | 5.12±1.27 | ** | *** | ** | |
| 167 | E | methional | butyl acetate | 5.70±1.29 | 5.83±1.64 | 5.04±1.59 | 5.46±1.51 | 5.38±1.72 | | | | |
| 168 | IP | ethyl isobutanoate | ethyl butanoate | 5.45±1.99 | 6.15±1.60 | 5.47±1.15 | 6.13±1.72 | 6.10±1.55 | * | * | * | |
| 169 | I | diacetyl | 3-methyl-1-butanol | 6.11±1.69 | 5.06±1.97 | 4.03±1.90 | 4.25±1.43 | 3.70±2.09 | * | | | |
| 170 | IP | nerol oxide | ethyl octanoate | 4.77±1.60 | 6.95±1.21 | 5.84±1.77 | 5.06±1.61 | 5.00±1.33 | *** | ** | * | |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 171 | P | hexyl hexanoate | geranyl acetate | 5.48±1.97 | 5.47±1.33 | 4.90±1.96 | 5.49±1.70 | 5.76±1.54 | | * | ** | |
| 172 | P | linalool | citronellol | 5.64±1.43 | 5.24±1.79 | 5.19±1.24 | 6.53±1.49 | 6.36±2.05 | | *** | ** | |
| 173 | I | geraniol | *γ*-decalactone | 5.02±1.76 | 6.16±1.57 | 5.23±1.28 | 5.37±2.26 | 5.65±2.26 | *** | | | * |
| 174 | I | *γ*-dodecalactone | *γ*-undecalactone | 5.77±1.74 | 6.83±1.38 | 4.11±1.69 | 4.11±2.29 | 4.15±2.23 | * | | | |
| 176 | IP | eugenol | ethyl phenylacetate | 4.56±1.84 | 5.79±1.85 | 4.99±1.49 | 4.11±1.29 | 3.94±1.58 | *** | *** | *** | |
| 177 | E | ethyl phenylacetate | ethyl cinnamate | 5.72±1.61 | 6.14±1.55 | 3.83±1.56 | 4.22±1.74 | 3.91±1.76 | | | | |
| 178 | IP | isoeugenol | ethyl cinnamate | 4.90±1.66 | 6.32±1.48 | 5.38±1.29 | 4.09±1.60 | 4.71±1.60 | *** | *** | * | |
| 179 | IP | 4-ethylguaiacol | isoeugenol | 6.01±1.56 | 4.23±2.08 | 4.32±1.21 | 5.28±1.67 | 5.00±1.66 | *** | * | ** | |
| 180 | I | nerol oxide | ethyl 3-methylbutanoate | 4.90±1.65 | 6.69±1.35 | 6.00±1.66 | 6.63±1.73 | 6.58±1.78 | *** | | | |
| 181 | IP | hexyl hexanoate | ethyl valerate | 5.81±1.32 | 6.58±1.34 | 5.11±1.76 | 6.09±1.80 | 6.34±1.71 | ** | * | *** | |
| 183 | P | geraniol | ethyl butanoate | 5.30±1.33 | 5.49±1.65 | 5.45±1.28 | 6.45±1.68 | 6.46±1.47 | | ** | *** | |
| 184 | I | *γ*-dodecalactone | 3-methyl-1-butanol | 6.50±1.57 | 5.31±1.78 | 4.21±1.96 | 4.09±1.99 | 3.98±2.19 | * | | | |
| 185 | I | ethyl octanoate | eugenol | 6.84±1.19 | 3.78±2.04 | 5.13±1.60 | 5.30±1.51 | 5.43±1.44 | *** | | | |
| 186 | P | ethyl phenylacetate | geranyl acetate | 6.27±1.73 | 5.52±1.8 | 4.38±2.24 | 5.81±1.35 | 4.83±2.17 | | *** | * | ** |
| 187 | IP | ethyl cinnamate | citronellol | 5.98±1.31 | 5.19±1.73 | 4.66±1.48 | 6.19±1.70 | 4.88±1.71 | * | ** | | ** |
| 188 | E | isoeugenol | *γ*-decalactone | 5.52±1.18 | 5.30±1.56 | 5.87±2.07 | 5.70±1.78 | 6.73±1.72 | | | ** | ** |
| 189 | I | *γ*-undecalactone | 4-ethylguaiacol | 6.69±1.29 | 5.24±1.89 | 4.81±2.06 | 4.69±1.90 | 4.48±1.86 | *** | | | |
| 190 | P | 3-methyl-1-butanol | *β*-phenethyl acetate | 5.94±1.61 | 5.66±1.69 | 3.80±1.93 | 5.90±2.00 | 5.02±2.17 | | *** | *** | * |
| 191 | I | eugenol | methionol | 3.27±1.83 | 6.00±1.80 | 5.10±1.22 | 4.69±1.83 | 5.10±1.75 | *** | | | |
| 192 | E | butyl acetate | ethyl phenylacetate | 5.91±1.73 | 5.48±1.90 | 5.52±1.83 | 4.45±2.01 | 4.84±2.08 | | | | |
| 193 | P | ethyl cinnamate | ethyl isobutanoate | 6.48±1.47 | 6.28±1.50 | 5.55±1.78 | 6.53±1.91 | 6.09±1.71 | | ** | * | * |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 194 | I | isoeugenol | diacetyl | 5.67±1.12 | 6.41±1.46 | 5.03±2.19 | 4.40±1.91 | 4.47±2.04 | * | | | |
| 195 | P | ethyl 3-methylbutanoate | valencene | 5.90±1.72 | 6.15±1.46 | 6.60±1.52 | 5.10±1.47 | 5.73±1.57 | | *** | ** | * |
| 196 | E | 1,8-cineole | ethyl valerate | 6.51±1.40 | 6.61±1.32 | 5.82±2.07 | 5.61±1.96 | 6.41±1.69 | | | ** | ** |
| 197 | E | 2-octanone | methional | 5.77±1.58 | 6.09±1.53 | 5.34±1.50 | 5.05±1.68 | 5.17±1.85 | | | | |
| 198 | P | ethyl butanoate | hexanal | 5.81±1.59 | 5.40±2.01 | 6.21±1.61 | 5.73±1.46 | 6.40±1.72 | | * | | ** |
| 199 | IP | (*Z*)-3-hexenol | 3-methyl-1-butanol | 4.78±1.62 | 5.53±1.56 | 6.16±1.41 | 4.28±2.34 | 4.26±1.91 | * | ** | *** | |
| 200 | IP | *p*-cymene | ethyl isobutanoate | 6.82±1.34 | 5.34±1.95 | 4.36±2.02 | 5.41±1.56 | 4.80±1.80 | ** | ** | * | |
| 203 | E | valencene | 2-octanone | 6.32±1.68 | 6.29±1.50 | 5.15±1.98 | 5.66±1.73 | 5.35±2.04 | | | | |
| 204 | E | hexanal | (*Z*)-3-hexenol | 5.40±1.64 | 5.87±1.96 | 6.14±1.52 | 5.72±2.08 | 6.77±1.68 | | | | ** |
| 205 | I | (*Z*)-3-hexenol | myrcene | 5.61±1.67 | 6.34±1.69 | 6.02±1.91 | 5.45±2.01 | 5.64±2.15 | * | | | |
| 206 | E | linalool | 2-octanone | 5.83±1.56 | 5.66±1.49 | 5.57±1.82 | 5.68±1.81 | 6.52±1.96 | | | ** | * |
| 207 | IP | *γ*-decalactone | hexanal | 6.61±1.68 | 5.95±1.60 | 5.20±1.65 | 6.08±1.74 | 6.06±1.64 | * | ** | ** | |
| 208 | I | geraniol | *p*-cymene | 4.86±1.9 | 5.87±1.31 | 5.85±1.47 | 5.37±1.96 | 5.89±2.05 | ** | | | |
| 209 | E | valencene | *p*-cymene | 6.30±1.27 | 5.85±1.20 | 5.04±1.59 | 5.17±1.41 | 4.89±1.43 | | | | |
| 212 | I | ethyl octanoate | 1,8-cineole | 5.73±1.46 | 6.38±1.24 | 5.12±1.96 | 5.94±2.05 | 5.26±1.95 | * | | | |
| 214 | I | *p*-cymene | linalool | 6.00±1.66 | 5.08±1.70 | 4.87±2.00 | 5.11±1.29 | 5.42±1.76 | ** | | * | |
| 216 | I | *β*-phenethyl acetate | valencene | 5.69±1.37 | 6.33±1.17 | 5.34±1.98 | 4.85±1.94 | 4.49±1.93 | ** | | * | |
| 217 | I | eugenol | 2-octanone | 5.83±1.61 | 6.69±1.15 | 5.01±1.25 | 5.39±1.17 | 6.02±1.18 | * | | ** | * |
| 218 | P | ethyl phenylacetate | myrcene | 5.19±2.01 | 5.53±1.55 | 4.33±1.47 | 5.38±1.84 | 4.27±1.60 | | *** | | ** |
| 219 | P | 4-ethylguaiacol | ethyl 2-methylbutanoate | 6.34±1.62 | 6.41±1.40 | 4.79±1.79 | 5.94±1.48 | 4.77±1.87 | | ** | | ** |

| Trials[a] | Groups[b] | odor A | odor B | $I_A$ | $I_B$ | $P_A$ | $P_B$ | $P_{AB}$ | $I_A$~$I_B$ | $P_A$~$P_B$ | $P_A$~$P_{AB}$ | $P_B$~$P_{AB}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 220 | P | 3-mercaptohexanol | linalool | 6.19±1.41 | 6.05±1.28 | 4.02±1.98 | 4.89±1.78 | 4.46±1.85 | | * | | |
| 221 | P | 3-methyl-1-butanol | 3-mercaptohexanol | 5.83±1.72 | 6.33±1.55 | 4.85±2.31 | 4.28±2.23 | 3.78±2.19 | | * | ** | |
| 222 | IP | 3-mercaptohexanol | ethyl 2-methylbutanoate | 5.58±1.56 | 6.27±1.45 | 4.18±2.00 | 5.87±1.91 | 5.03±1.98 | * | *** | ** | * |

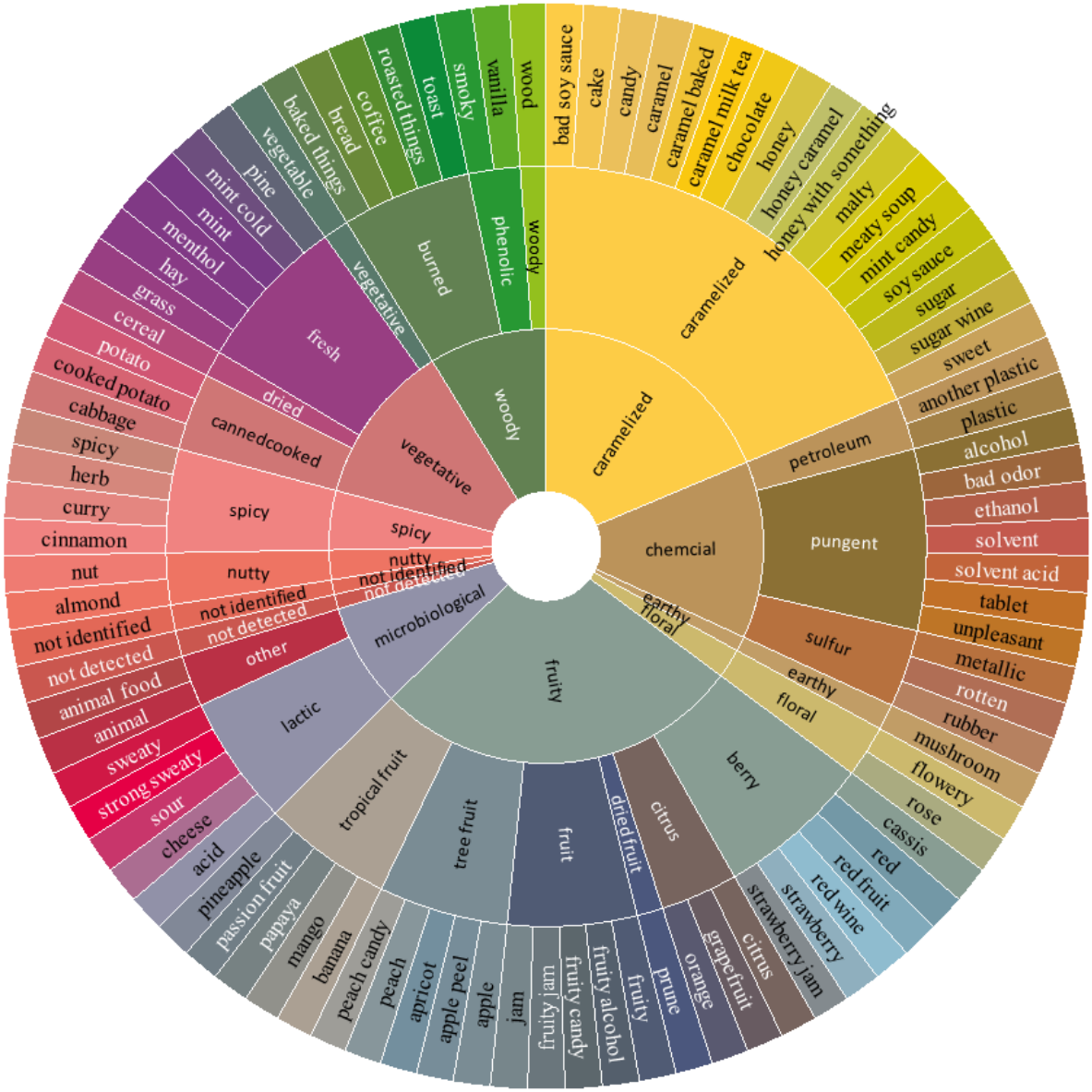
Note: Significant differences between odor intensity (resp. pleasantness) of a binary mixture and its two components in each trial are indicated (ns: not significant, *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$

**Supplementary Table 10** Fisher Extract Test

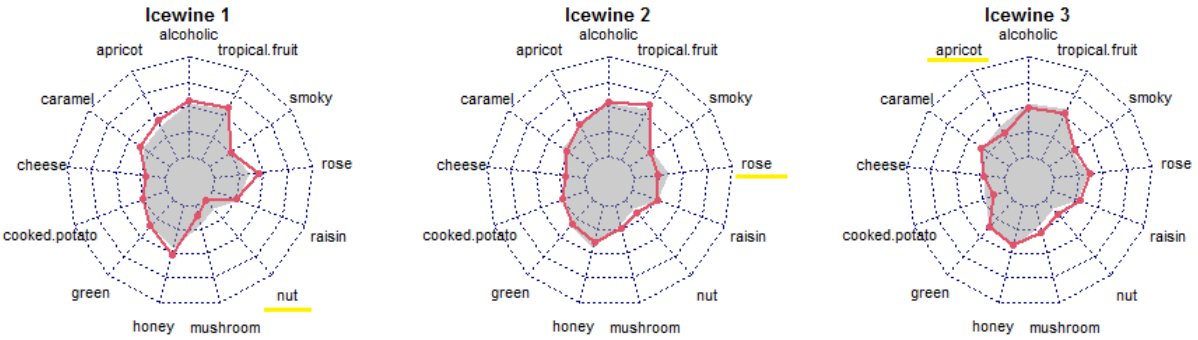
| Fisher extract test objects | | *p* value |
|---|---|---|
| **Mixture-induced interactions on perception of components' odor within mixtures** (Independence; masking; synergy) | Intensity level | 0.947 |
| | Pleasantness level | 0.381 |
| | Frequent value level | 0.487 |
| **Mixture-induced interactions on perception of components' odor within mixtures** (Independence/independence; masking /synergy; synergy/independence; masking/masking; masking/independence) | Four different groups (E, I, P, IP) | 0.306 |
| | Two odorants with significant pleasantness difference (P, IP) | 0.038* |
| | Two odorants with significant intensity difference (I, IP) | 0.753 |
| **Mixture-induced interactions on the overall intensity of binary mixtures** (strongest component; partial addition; compromise) | Four different groups (E, I, P, IP) | 0.216 |
| | Two odorants with significant pleasantness difference (P, IP) | 0.927 |
| | Two odorants with significant intensity difference (I, IP) | 0.006** |

**Supplementary Table 11** Comparison of Model Performance between Linear Model and Squared Model

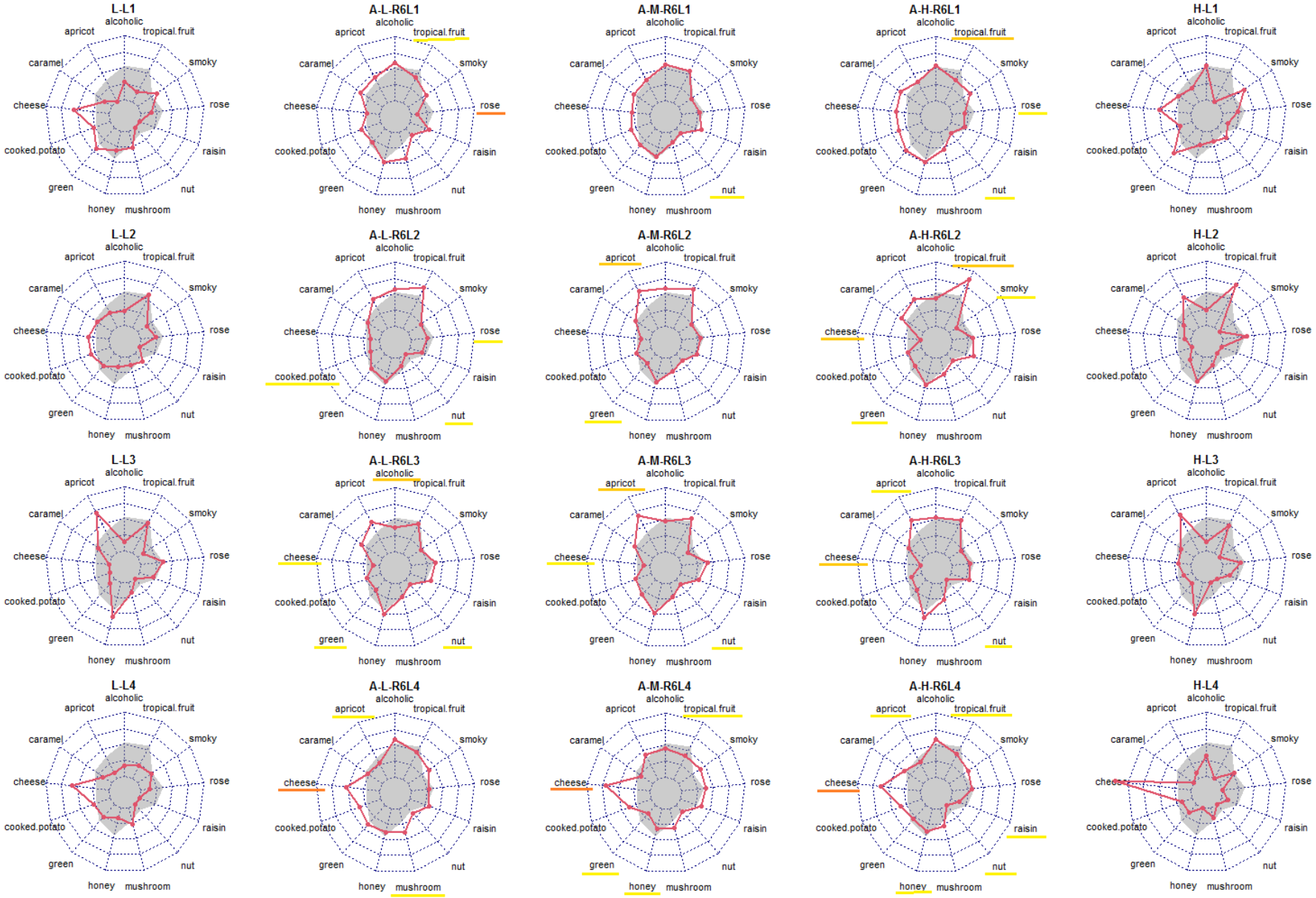
| Prediction situation | | Groups | Linear model | | Squared model | |
|---|---|---|---|---|---|---|
| | | | $R^2$ | *RMSE* | $R^2$ | *RMSE* |
| *Panel* approach | *Trial* condition | Group E | 0.868 | 0.368 | 0.867 | 0.369 |
| | | Group I | 0.803 | 0.318 | 0.809 | 0.313 |
| | | Group IP | 0.862 | 0.570 | 0.884 | 0.503 |
| | | Group P | 0.853 | 0.387 | 0.860 | 0.379 |
| | *Mean* condition | Group E | 0.565 | 0.606 | 0.564 | 0.606 |
| | | Group I | 0.704 | 0.434 | 0.698 | 0.434 |
| | | Group IP | 0.804 | 0.666 | 0.804 | 0.623 |
| | | Group P | 0.636 | 0.606 | 0.641 | 0.594 |
| *Individual* approach | *Trial* condition | Group E | 0.461 | 1.505 | 0.450 | 1.537 |
| | | Group I | 0.510 | 1.387 | 0.505 | 1.410 |
| | | Group IP | 0.548 | 1.433 | 0.553 | 1.430 |
| | | Group P | 0.540 | 1.407 | 0.531 | 1.427 |
| | *Mean* condition | Group E | 0.360 | 1.601 | 0.357 | 1.606 |
| | | Group I | 0.433 | 1.472 | 0.432 | 1.472 |
| | | Group IP | 0.513 | 1.495 | 0.522 | 1.469 |
| | | Group P | 0.478 | 1.496 | 0.469 | 1.502 |

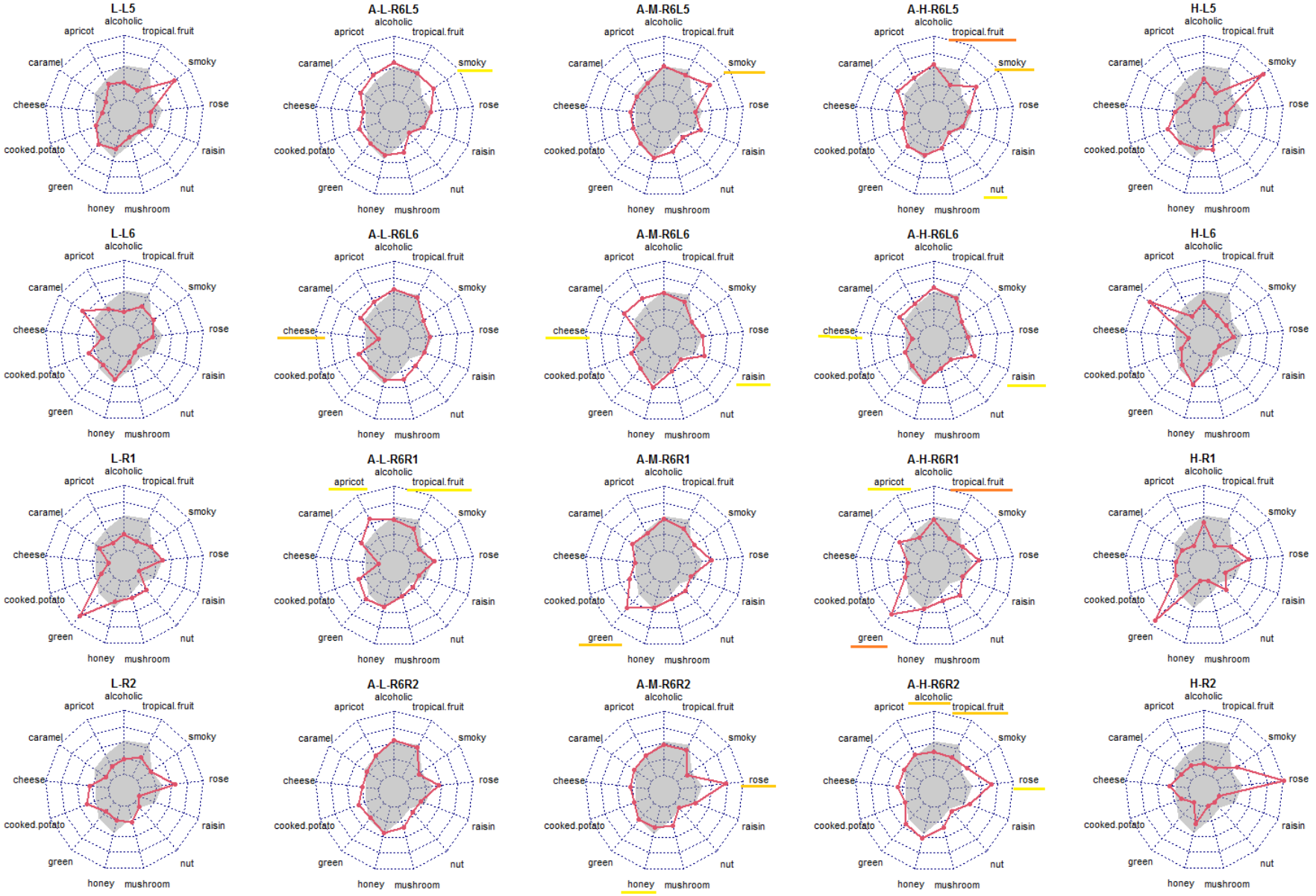


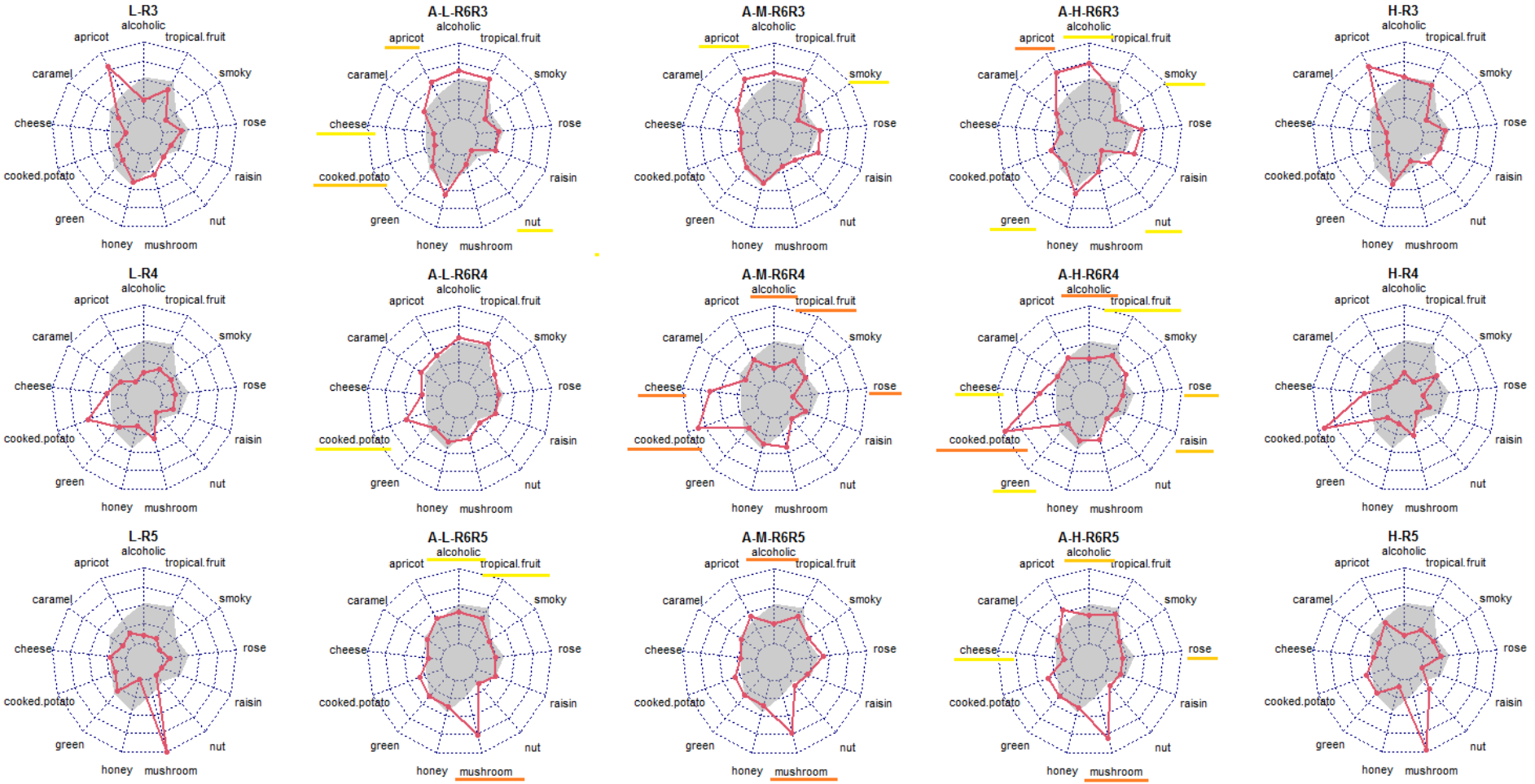

**Supplementary Figure 1** Aroma wheel for the categorization of the odor descriptors obtained in GC-O and Olfactoscan analysis. The aroma wheel was modified from the one proposed by Noble (Noble et al., 1987) and was adopted based on the terms that were actually used in GC-O and Olfactoscan analysis by the panelists. The aroma wheel was made by XLSTAT software (Version 2019.2, Addinsoft, France).




**Supplementary Figure 2** The odor profile of icewine. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$).

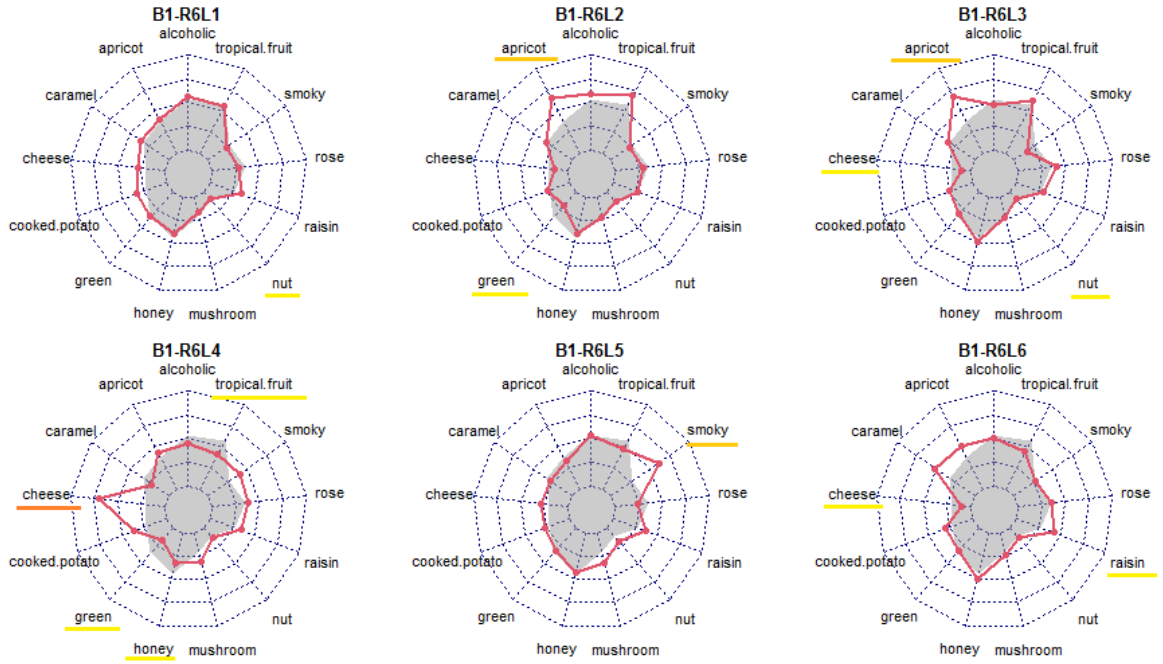

L-L1
A-L-R6L1
A-M-R6L1
A-H-R6L1
H-L1
L-L2
A-L-R6L2
A-M-R6L2
A-H-R6L2
H-L2
L-L3
A-L-R6L3
A-M-R6L3
A-H-R6L3
H-L3
L-L4
A-L-R6L4
A-M-R6L4
A-H-R6L4
H-L4
alcoholic
tropical.fruit
smoky
rose
raisin
nut
mushroom
honey
green
cooked.potato
cheese
caramel
apricot


L-L5
A-L-R6L5
A-M-R6L5
A-H-R6L5
H-L5
L-L6
A-L-R6L6
A-M-R6L6
A-H-R6L6
H-L6
L-R1
A-L-R6R1
A-M-R6R1
A-H-R6R1
H-R1
L-R2
A-L-R6R2
A-M-R6R2
A-H-R6R2
H-R2
alcoholic
tropical.fruit
smoky
rose
raisin
nut
mushroom
honey
green
cooked.potato
cheese
caramel
apricot

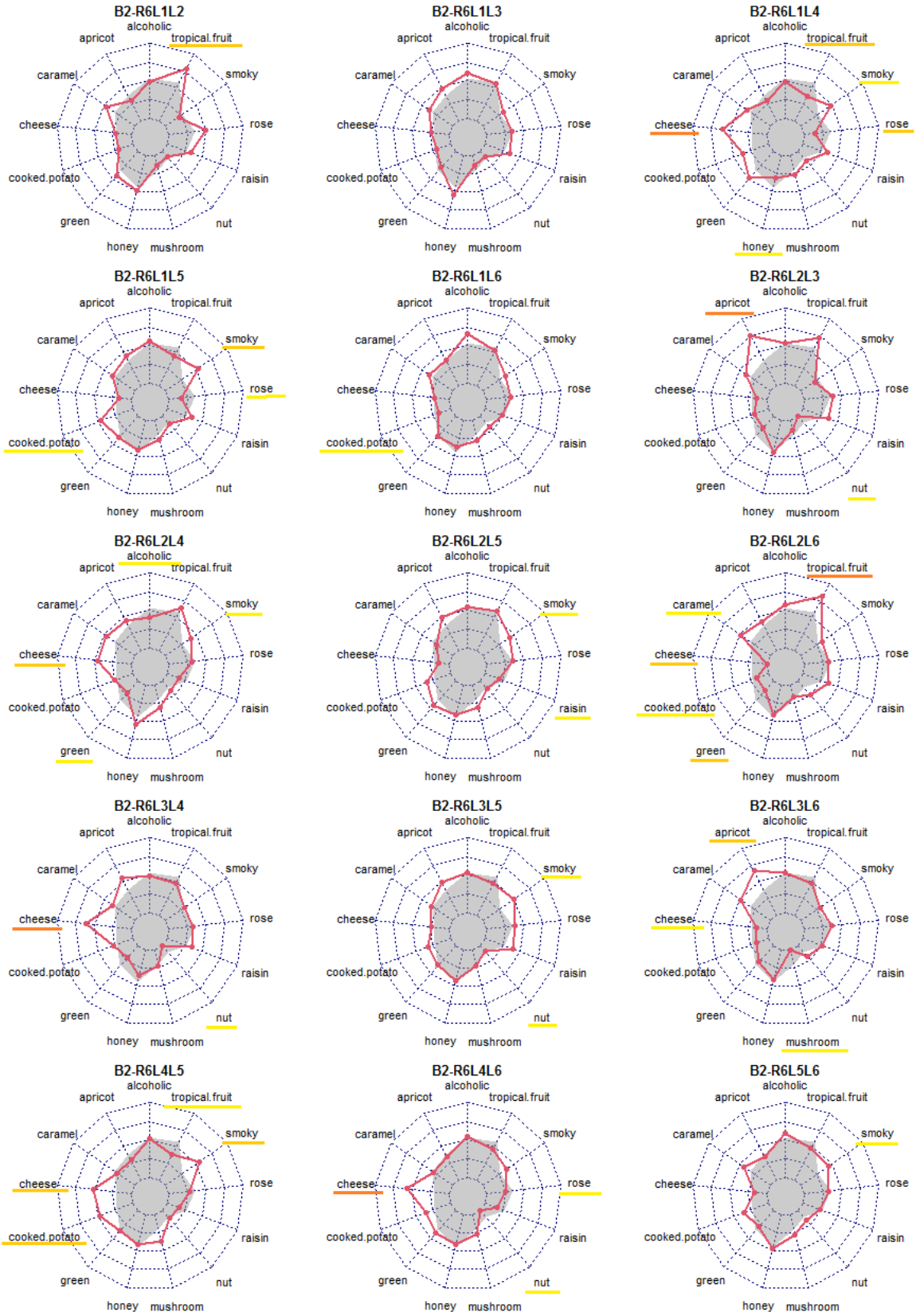



**Supplementary Figure 3** The odor profile of the 11 key odorants and the odor profile of the 11 key odorants within icewine odor buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code L-R5 represents the low-intensity odorant R5 (1-octen-3-one, see in Supplementary Table 3); the code H-R5 represents the high-intensity odorant R5; Code A-L-R6R5 represents the sample of a low-intensity odorant R5 mixed with R6 (icewine); code A-M-R6R5 represents the sample of a medium-intensity odorant R5 mixed with R6 (icewine); code A-H-R6R5 represents the sample of a high-intensity odorant R5 mixed with R6

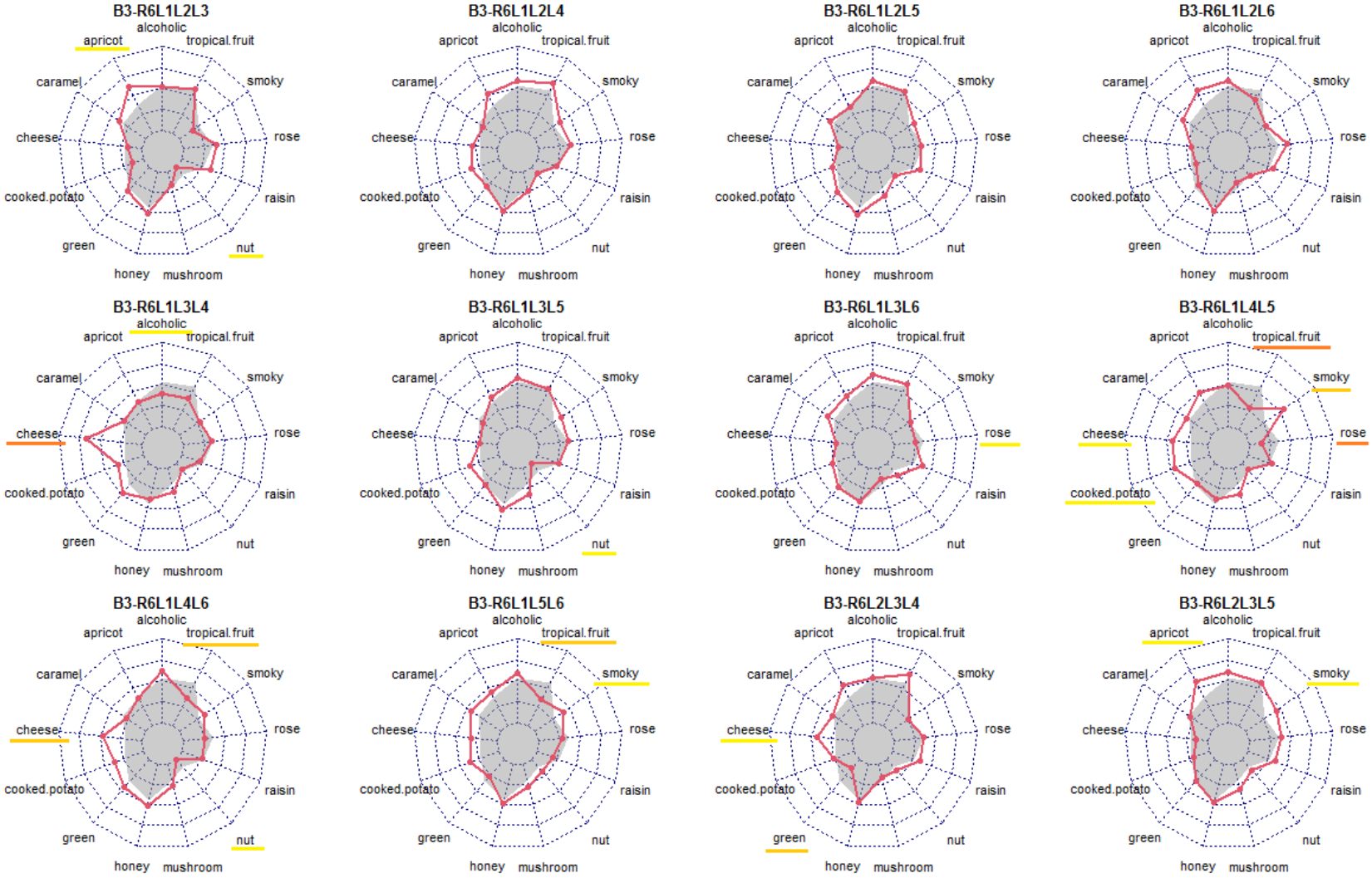
(icewine).

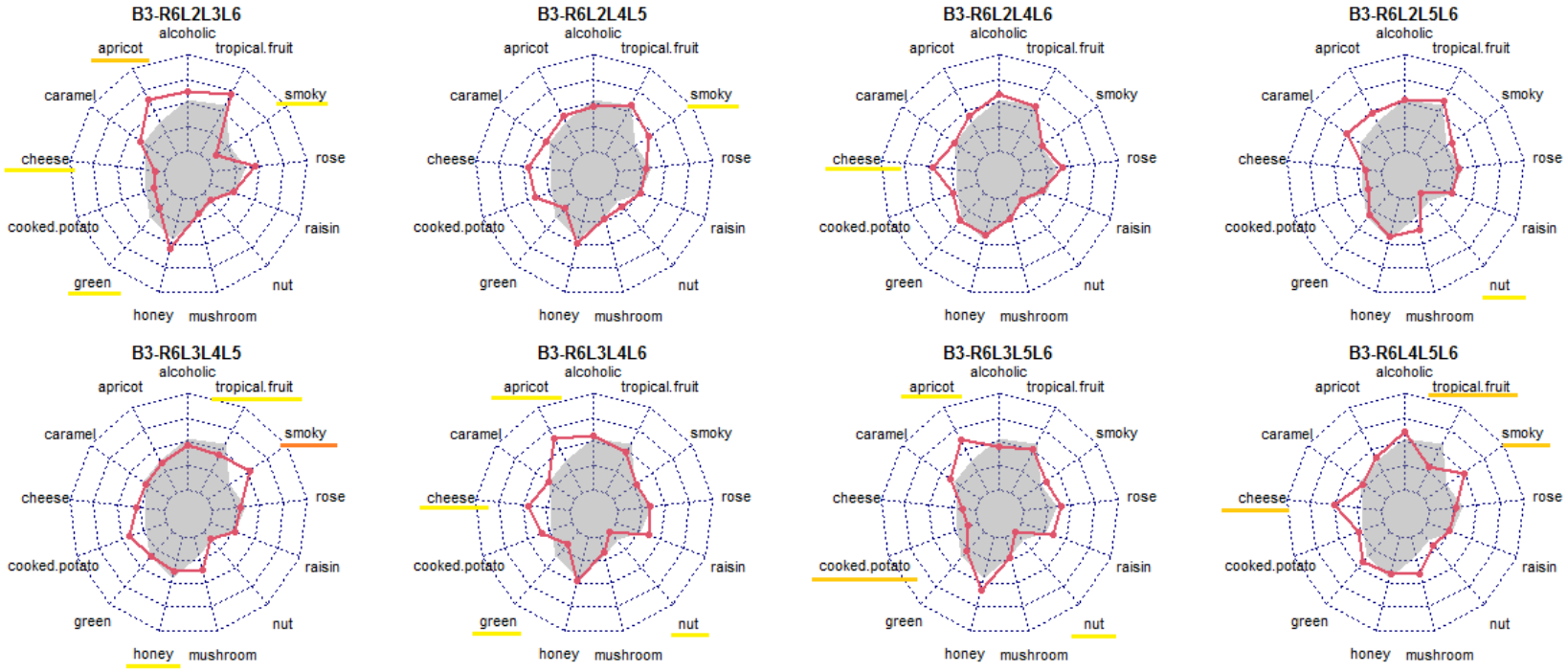



**Supplementary Figure 4** The odor profile of the 6 key odorants and the odor profile of the 11 key odorants within icewine aroma buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code L-R5 represents the low-intensity odorant R5 (1-octen-3-one, see in Supplementary Table 3); the code H-R5 represents the high-intensity odorant R5; Code A-L-R6R5 represents the sample of a low-intensity odorant R5 mixed with R6 (icewine); code A-M-R6R5 represents the sample of a medium-intensity odorant R5 mixed with R6 (icewine); code AH-R6R5 represents the sample of a high-intensity odorant R5 mixed with R6 (icewine).

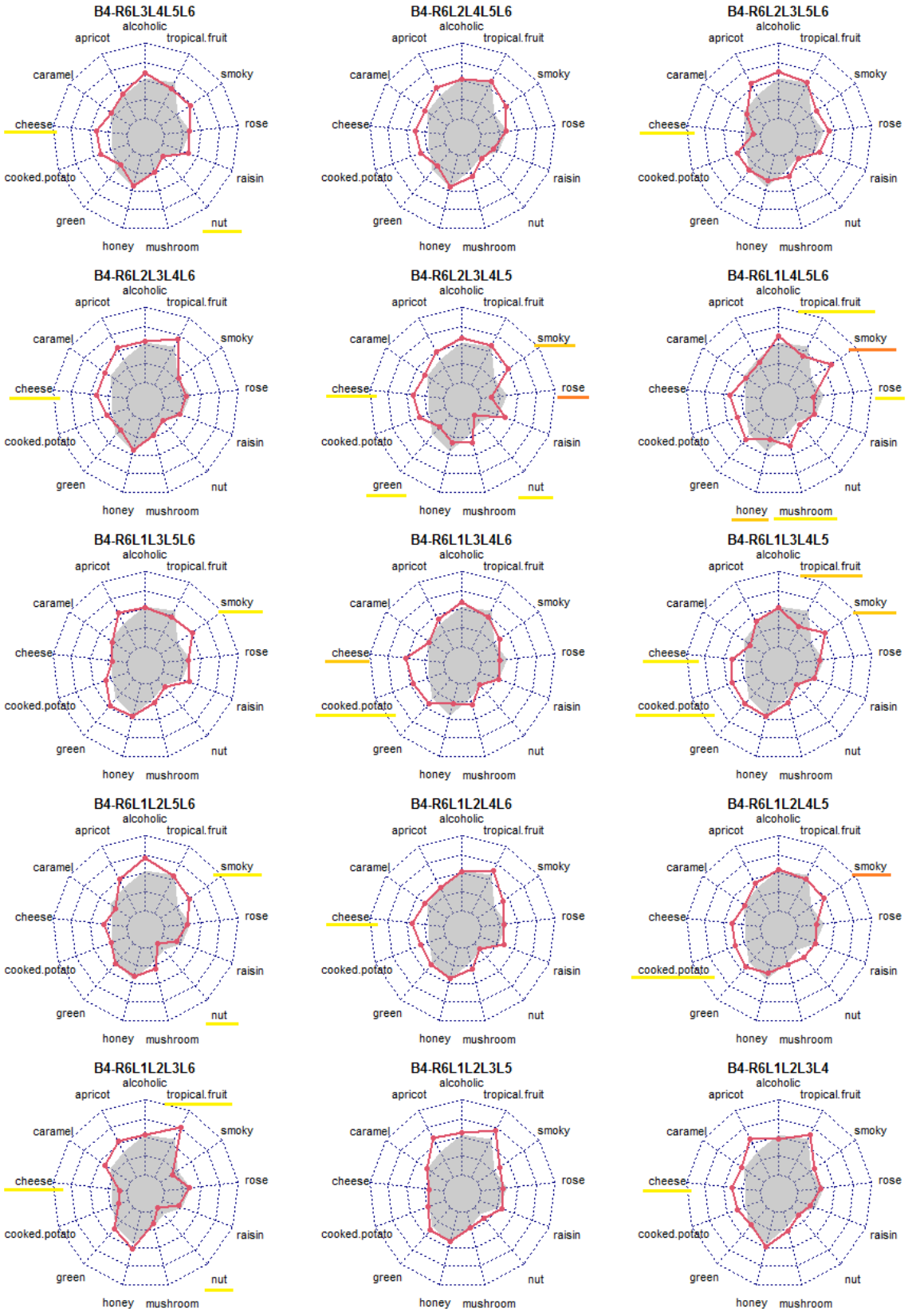



**Supplementary Figure 5** The odor profile of binary odor mixtures combined from the 6 key odorants within icewine aroma buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code B2-R6L5L6 represents odorant L5 (guaiacol) and L6 (furaneol) mixed with R6 (icewine).

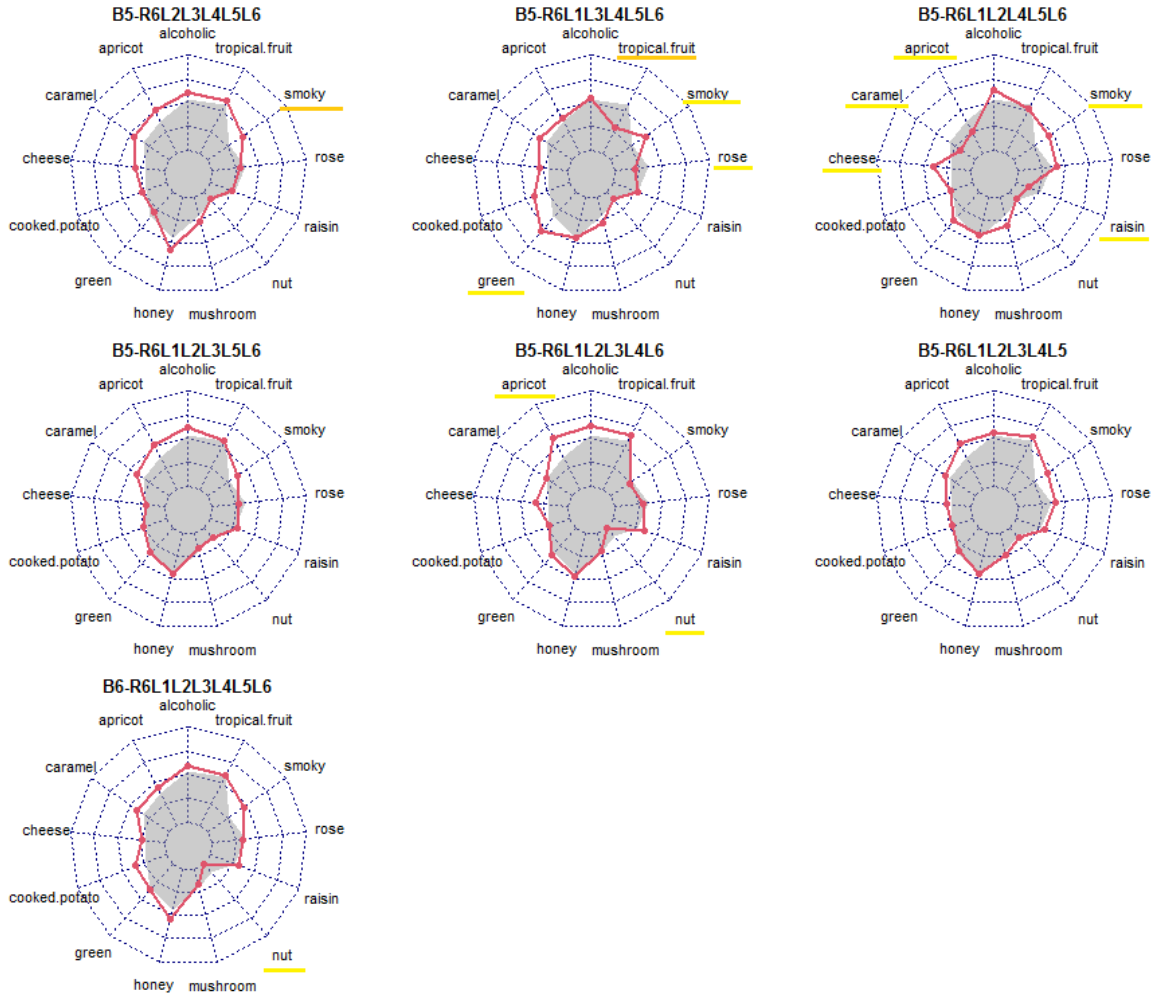

B3-R6L1L2L3
B3-R6L1L2L4
B3-R6L1L2L5
B3-R6L1L2L6
B3-R6L1L3L4
B3-R6L1L3L5
B3-R6L1L3L6
B3-R6L1L4L5
B3-R6L1L4L6
B3-R6L1L5L6
B3-R6L2L3L4
B3-R6L2L3L5
alcoholic
tropical.fruit
smoky
rose
raisin
nut
mushroom
honey
green
cooked.potato
cheese
caramel
apricot




**Supplementary Figure 6** The odor profile of ternary odor mixtures combined from the 6 key odorants within icewine aroma buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code B3-R6L4L5L6 represents odorant L4 (3-methylbutanoic acid), L5 (guaiacol) and L6 (furaneol) mixed with R6 (icewine).




**Supplementary Figure 7** The odor profile of quaternary odor mixtures combined from the 6 key odorants within icewine aroma buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code B4-R6L3L4L5L6 represents odorant L3 ($\beta$-damascenone), L4 (3-methylbutanoic acid), L5 (guaiacol) and L6 (furaneol) mixed with R6 (icewine).

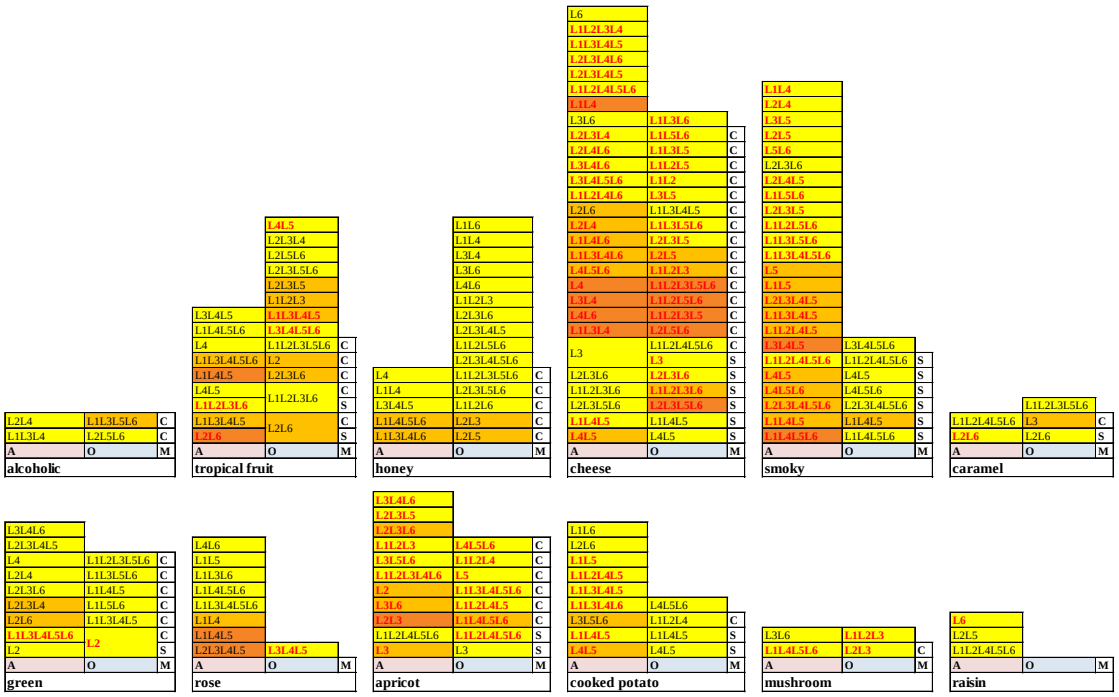



**Supplementary Figure 8** The odor profile of quinary and senary odor mixtures combined from the 6 key odorants within icewine aroma buffer. The red line represents the odor profile based on the sample's *IM* values, and the grey shading represents the odor profile of icewine based on the average *IM* value calculated from three sessions. The significance of the difference between odor attributes is marked by underlines in different colors (yellow: $p<0.05$; light orange: $p<0.01$; dark orange: $p<0.001$). The code of the sample is shown at the top of each small picture, where the code B6-R6L1L2L3L4L5L6 represents odorant L1 (3-methyl-1-butanol), L2 (ethyl 2-methyl butanoate), L3 (*β*-damascenone), L4 (3-methylbutanoic acid), L5 (guaiacol) and L6 (furaneol) mixed with R6 (icewine).

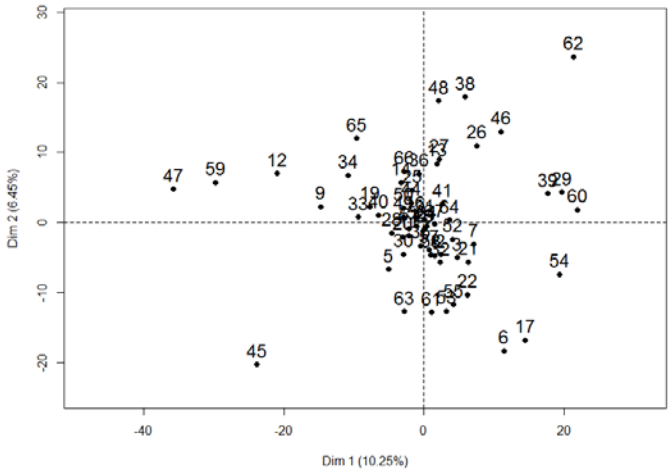
| No. | odorants | alcoholic | | tropical fruit | | honey | | apricot | | cheese | | smoky | | caramel | | cooked potato | | green | | mushroom | | nut | | raisin | | rose | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O |
| 1 | L1 | 6% | 2% | -5% | 3% | -10% | 3% | 4% | 13% | 37% | 29% | -13% | -4% | 8% | 6% | 47% | -19% | 2% | -19% | -17% | 52% | -18% | null | 23% | -23% | -21% | -9% |
| 2 | L2 | 9% | -7% | 20% | -50% | -10% | -18% | 73% | -5% | -30% | 22% | -13% | -4% | -1% | -3% | 10% | 16% | -44% | 50% | 6% | 62% | 16% | null | 0% | -11% | -16% | -33% |
| 3 | L3 | -12% | 7% | 6% | -11% | 10% | -26% | 76% | -41% | -50% | 63% | -38% | -3% | 4% | -54% | 18% | -29% | -10% | 8% | 6% | 83% | -18% | null | 6% | -55% | 19% | 25% |
| 4 | L4 | -16% | 4% | -29% | -5% | -26% | -18% | 13% | 20% | 244% | -20% | 47% | -17% | -34% | -3% | 66% | -24% | -51% | -3% | 51% | 0% | 16% | null | 23% | -6% | 9% | -15% |
| 5 | L5 | -1% | 7% | -18% | 8% | -2% | -14% | -15% | 37% | 37% | 41% | 121% | -47% | -16% | -23% | 26% | -36% | -7% | 5% | 57% | 14% | 60% | null | 32% | 13% | -31% | -9% |
| 6 | L6 | -6% | -5% | -22% | 4% | 14% | -18% | 32% | 21% | -50% | 7% | 0% | -6% | 56% | -8% | 34% | -36% | -7% | -11% | 17% | 41% | 16% | null | 51% | 0% | 1% | 22% |
| 7 | L1L2 | -8% | 2% | 36% | -19% | 10% | -22% | -22% | -11% | 8% | 73% | -21% | 3% | 40% | -25% | -12% | 16% | 24% | -34% | -31% | 41% | -18% | null | 17% | 17% | 42% | 5% |
| 8 | L1L3 | 14% | -14% | -4% | -2% | 25% | -19% | 27% | 11% | 19% | 59% | 17% | 0% | 17% | -13% | -12% | 3% | -15% | -29% | -31% | 72% | -18% | null | 28% | -38% | -3% | 5% |
| 9 | L1L4 | -8% | 3% | -37% | -5% | -29% | -35% | -22% | 25% | 194% | -59% | 70% | -21% | 17% | -34% | 66% | 3% | 39% | -3% | 26% | 72% | 40% | null | 23% | 4% | -60% | 33% |
| 10 | L1L5 | 6% | -7% | -22% | 14% | -4% | -22% | 18% | 25% | -16% | 73% | 94% | -36% | 8% | 0% | 109% | -14% | 10% | -29% | 26% | 28% | 16% | null | 23% | -23% | -51% | -9% |
| 11 | L1L6 | 22% | -1% | -10% | 0% | -17% | -41% | 1% | 11% | -4% | 68% | 32% | 9% | 17% | -15% | -22% | 7% | 5% | -40% | 34% | 90% | 60% | null | -13% | 17% | -8% | -56% |
| 12 | L2L3 | -1% | 3% | 23% | -28% | 1% | -49% | 99% | -27% | -30% | 68% | -21% | 32% | 20% | -30% | -12% | 3% | -37% | 18% | -31% | 114% | -100% | null | 32% | -17% | 9% | -33% |
| 13 | L2L4 | -24% | -13% | 12% | -19% | 22% | -16% | 18% | 21% | 121% | -20% | 47% | 12% | 40% | -30% | 18% | -9% | -37% | 29% | 51% | 52% | 40% | null | -38% | 23% | -11% | 0% |
| 14 | L2L5 | 4% | -2% | 2% | -19% | -4% | -49% | 29% | 1% | -30% | 112% | 55% | -21% | -21% | -30% | 47% | 31% | 27% | 18% | 51% | 72% | 16% | null | -30% | -11% | 1% | -20% |
| 15 | L2L6 | 7% | -13% | 41% | -46% | -4% | -16% | 13% | -8% | -100% | 59% | 23% | 9% | 47% | -48% | -22% | 22% | -51% | 21% | -17% | 52% | 98% | null | 32% | -17% | -8% | -9% |
| 16 | L3L4 | -8% | 18% | -16% | -7% | -19% | -28% | 45% | 13% | 203% | 29% | 13% | 0% | 8% | -42% | 26% | -29% | -37% | 0% | 6% | 72% | -100% | null | 23% | -38% | -8% | -4% |
| 17 | L3L5 | 0% | -13% | -18% | 7% | -2% | -22% | 29% | -13% | 19% | 78% | 81% | -9% | 4% | -13% | 42% | 0% | 2% | 5% | 6% | 72% | -18% | null | 43% | 9% | 9% | -4% |
| 18 | L3L6 | 0% | -7% | -16% | -14% | -4% | -35% | 74% | 0% | -30% | 59% | 13% | 5% | 44% | -25% | -22% | 12% | -15% | 15% | -100% | 41% | 60% | null | 0% | -11% | 4% | -20% |
| 19 | L4L5 | -3% | -11% | -33% | 27% | -4% | -6% | -22% | 21% | 153% | -59% | 100% | -58% | -10% | -3% | 114% | -59% | 5% | 5% | 77% | 0% | 16% | null | -38% | -30% | -21% | 5% |
| 20 | L4L6 | 4% | -5% | -20% | 18% | -7% | -24% | -7% | 32% | 183% | -20% | 38% | -21% | -5% | -23% | 61% | -29% | 14% | 11% | 26% | 28% | -100% | null | -38% | 0% | -26% | -9% |
| 21 | L5L6 | 11% | -19% | -18% | 8% | 8% | -12% | -7% | 29% | -16% | 59% | 62% | -29% | 31% | -13% | 61% | 3% | -15% | -15% | 34% | 52% | 40% | null | -13% | -38% | -8% | -4% |
| 22 | L4L5L6 | 14% | -13% | -49% | -2% | -10% | -6% | 7% | 39% | 148% | -29% | 77% | -58% | -10% | -8% | 26% | -59% | 20% | 5% | 91% | 0% | 60% | null | -13% | 17% | -21% | 22% |
| 23 | L3L5L6 | -16% | -2% | -13% | 11% | 29% | -12% | 62% | 20% | -30% | 22% | 23% | -21% | 17% | -38% | -54% | 0% | -20% | -15% | 6% | 41% | -100% | null | 28% | -23% | 12% | 18% |
| 24 | L3L4L6 | 6% | -1% | -18% | -7% | 8% | -3% | 69% | -23% | 118% | -29% | 6% | -26% | -1% | -8% | 47% | -19% | -51% | 8% | -17% | 72% | -100% | null | 32% | 13% | -3% | 0% |
| 25 | L3L4L5 | -14% | -2% | -22% | -25% | -17% | -12% | -7% | 28% | 46% | -20% | 94% | -36% | -10% | -3% | 85% | -50% | -2% | -19% | 71% | -17% | -18% | null | 0% | 0% | -16% | 42% |
| 26 | L2L5L6 | 0% | -34% | 6% | -30% | -7% | -30% | 27% | -17% | -16% | 178% | 23% | -36% | 53% | -25% | -22% | -9% | -10% | 29% | 66% | 72% | -100% | null | 0% | -30% | -11% | 5% |
| 27 | L2L4L6 | 10% | -4% | -5% | -10% | -10% | -5% | 18% | -3% | 121% | -10% | 6% | -17% | -1% | -38% | 26% | 7% | 10% | -6% | 6% | 90% | -18% | null | -21% | -11% | 19% | 9% |
| 28 | L2L4L5 | -14% | 3% | -1% | 2% | 10% | -26% | 18% | 0% | 118% | -20% | 62% | -36% | 11% | 0% | 85% | -19% | -37% | 5% | 6% | -17% | 60% | null | 0% | 23% | -16% | -20% |
| 29 | L2L3L6 | 16% | -18% | 22% | -53% | 22% | -30% | 69% | 11% | -50% | 78% | -64% | 19% | 8% | -15% | -36% | 29% | -37% | -11% | -17% | 90% | -18% | null | -6% | -6% | 31% | -56% |
| 30 | L2L3L5 | 14% | 4% | -1% | -39% | 8% | -6% | 55% | -17% | -30% | 95% | 60% | -23% | -5% | -34% | -12% | 7% | -2% | 21% | 51% | 52% | 40% | null | 23% | -17% | 9% | -20% |
| 31 | L2L3L4 | 0% | -2% | 20% | -43% | 8% | -11% | 41% | 1% | 113% | 29% | -6% | -9% | 4% | 3% | 18% | 7% | -59% | -24% | -17% | 62% | 40% | null | 23% | -38% | 1% | -15% |
| 32 | L1L5L6 | 9% | -10% | -41% | 16% | 12% | -14% | 18% | 21% | 54% | 78% | 51% | -44% | 31% | -18% | 66% | -24% | -20% | -61% | 34% | 0% | 60% | null | -30% | 9% | -21% | 9% |
| 33 | L1L4L6 | 16% | 2% | -37% | -4% | 17% | -14% | -4% | 33% | 133% | -41% | 28% | -4% | -16% | -25% | 66% | -43% | 27% | -6% | 26% | 83% | -100% | null | -6% | 9% | -26% | 18% |

| No. | odorants | alcoholic | | tropical fruit | | honey | | apricot | | cheese | | smoky | | caramel | | cooked potato | | green | | mushroom | | nut | | raisin | | rose | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O | A | O |
| 34 | L1L4L5 | -8% | 4% | -52% | 18% | -13% | -2% | 29% | 45% | 113% | -59% | 98% | -78% | 8% | -15% | 101% | -59% | -7% | -40% | 57% | 0% | -18% | null | 6% | -17% | -60% | 42% |
| 35 | L1L3L6 | 15% | -23% | 5% | -4% | -7% | -12% | 17% | 1% | -4% | 83% | 6% | -3% | 28% | -13% | 26% | 19% | 10% | -40% | -31% | 28% | 60% | null | 38% | -11% | -26% | -9% |
| 36 | L1L3L5 | 7% | -1% | -7% | -8% | 18% | -28% | 13% | 1% | 8% | 85% | 38% | -36% | -21% | -23% | 66% | -19% | -2% | 5% | 57% | 28% | -100% | null | 0% | -30% | 1% | 29% |
| 37 | L1L3L4 | -26% | -10% | -27% | 3% | -13% | -26% | -4% | 9% | 232% | -29% | 6% | -26% | -5% | 20% | 42% | -50% | 36% | -15% | 43% | 100% | -18% | null | -21% | -11% | -3% | -4% |
| 38 | L1L2L6 | 9% | -23% | -22% | -25% | 10% | -33% | 49% | -20% | -4% | 22% | 6% | 17% | 24% | -30% | -22% | 19% | -15% | -6% | -31% | 107% | 16% | null | 13% | -11% | 31% | -9% |
| 39 | L1L2L5 | 10% | -5% | -4% | -21% | 21% | -14% | -10% | 45% | -16% | 83% | 23% | -36% | 17% | -23% | 26% | -5% | 14% | -53% | 43% | 0% | 16% | null | 28% | 17% | -8% | 5% |
| 40 | L1L2L4 | 9% | -25% | 15% | -16% | 10% | 3% | 40% | 39% | 46% | -41% | 32% | -26% | -21% | -8% | 55% | -71% | -10% | -24% | 17% | 28% | -18% | null | -13% | 13% | 9% | 22% |
| 41 | L1L2L3 | -3% | 2% | 1% | -50% | 17% | -28% | 62% | -8% | -16% | 107% | -30% | 6% | 17% | -30% | -36% | -19% | 10% | 15% | -17% | 131% | -100% | null | 32% | -23% | 12% | -15% |
| 42 | L3L4L5L6 | 14% | -18% | -16% | 32% | -2% | -12% | 4% | -33% | 107% | -10% | 70% | -53% | -5% | 10% | 79% | -43% | -31% | 18% | 17% | -17% | -18% | null | 32% | 4% | -3% | 55% |
| 43 | L2L4L5L6 | -4% | 2% | 1% | -7% | 1% | 0% | 29% | 9% | 89% | 0% | 66% | -29% | 11% | -8% | 61% | -43% | -25% | -19% | 43% | -17% | 16% | null | -30% | 13% | -3% | 5% |
| 44 | L2L3L5L6 | 15% | -18% | -2% | -39% | -19% | -43% | 46% | -33% | -50% | 146% | 32% | 3% | -16% | -8% | 61% | 7% | 2% | 32% | 43% | 52% | 16% | null | 17% | 9% | 22% | -56% |
| 45 | L2L3L4L6 | 4% | -5% | 18% | -25% | -2% | -24% | 46% | 1% | 107% | -29% | 6% | 17% | 28% | -15% | 34% | 34% | -25% | 5% | 6% | 52% | -18% | null | -13% | 9% | -16% | -47% |
| 46 | L2L3L4L5 | 10% | 10% | 3% | -13% | -26% | -33% | 29% | -13% | 101% | -20% | 81% | -21% | 8% | -8% | 66% | -50% | -37% | 0% | 57% | 62% | -100% | null | 32% | -23% | -60% | 0% |
| 47 | L1L4L5L6 | 15% | -11% | -25% | 19% | -37% | -19% | -15% | 71% | 101% | -41% | 119% | -53% | -10% | -6% | 61% | -43% | 24% | -40% | 77% | -17% | 40% | null | -6% | 17% | -38% | 18% |
| 48 | L1L3L5L6 | -3% | -32% | -16% | 8% | 5% | -2% | 41% | 1% | -4% | 85% | 85% | -12% | -10% | 10% | 42% | -24% | 36% | -40% | 26% | 83% | 16% | null | 38% | -45% | -8% | -4% |
| 49 | L1L3L4L6 | 9% | -7% | -16% | -1% | -37% | -24% | 18% | 11% | 153% | -41% | 32% | -6% | -10% | -38% | 104% | -5% | 24% | 21% | 43% | 83% | -18% | null | 0% | -38% | -26% | 9% |
| 50 | L1L3L4L5 | -3% | -4% | -44% | 37% | 5% | -24% | 7% | -3% | 89% | -100% | 81% | -26% | -34% | 15% | 90% | -50% | 27% | -53% | 26% | 0% | -18% | null | -6% | 17% | -16% | 0% |
| 51 | L1L2L5L6 | 31% | -18% | -4% | -19% | -10% | -35% | 32% | 24% | 54% | 154% | 66% | -32% | -26% | -15% | 10% | -19% | 5% | -40% | 43% | 28% | -100% | null | -30% | 9% | -11% | 0% |
| 52 | L1L2L4L6 | -3% | -10% | 11% | -21% | -2% | -22% | 1% | 11% | 113% | 0% | 51% | 9% | 11% | -18% | 55% | -9% | 10% | -3% | 43% | 28% | -18% | null | 23% | 26% | -11% | 18% |
| 53 | L1L2L4L5 | 4% | -10% | -11% | -19% | -19% | -24% | 17% | 49% | 89% | -41% | 74% | -32% | -5% | 13% | 74% | -50% | 20% | -19% | 17% | -100% | 98% | null | 0% | -11% | -26% | 13% |
| 54 | L1L2L3L6 | -1% | -13% | 31% | -36% | 17% | -24% | 41% | -33% | -50% | 112% | -30% | 21% | 24% | -30% | -36% | 38% | 10% | 0% | -17% | 114% | -100% | null | -21% | -45% | -3% | -15% |
| 55 | L1L2L3L5 | 6% | -7% | 22% | -23% | -4% | -26% | 54% | -20% | -4% | 137% | 32% | -17% | -1% | -25% | 10% | 3% | 17% | 8% | 6% | 52% | 60% | null | 13% | -45% | -16% | -20% |
| 56 | L1L2L3L4 | -10% | 0% | 12% | -21% | 10% | -14% | 51% | -20% | 89% | -29% | 17% | -3% | 11% | 3% | 61% | 3% | -10% | -19% | 26% | 62% | 16% | null | -30% | -23% | -11% | 0% |
| 57 | L2L3L4L5L6 | 14% | -5% | 6% | -8% | 29% | -28% | 32% | -11% | 54% | 15% | 60% | -47% | 35% | -15% | 26% | -5% | -15% | -3% | 26% | 0% | -18% | null | -13% | 9% | -16% | -15% |
| 58 | L1L3L4L5L6 | 4% | -2% | -49% | 16% | 2% | -28% | 10% | 48% | 46% | -41% | 60% | -47% | 24% | -23% | 79% | -29% | 58% | -47% | 34% | 28% | -18% | null | 0% | -11% | -38% | -9% |
| 59 | L1L2L4L5L6 | 19% | -21% | -8% | 3% | -7% | -12% | -32% | 51% | 95% | -59% | 62% | -63% | -41% | -18% | 10% | -24% | 14% | -15% | 51% | 28% | -18% | null | -49% | -6% | 16% | 29% |
| 60 | L1L2L3L5L6 | 16% | -25% | -2% | -32% | 2% | -41% | 40% | -3% | -4% | 188% | 38% | -12% | 24% | -48% | 18% | 7% | 2% | -53% | -17% | 83% | 16% | null | 6% | 9% | -21% | 18% |
| 61 | L1L2L3L4L6 | 19% | -11% | 12% | -21% | 8% | -22% | 60% | -27% | 69% | 15% | -13% | 33% | -1% | -34% | -1% | -19% | 10% | -11% | -6% | 90% | -100% | null | 28% | 17% | -16% | -25% |
| 62 | L1L2L3L4L5 | 6% | -16% | 8% | -25% | 2% | -9% | 41% | 13% | 28% | -59% | 55% | -40% | 17% | 23% | -1% | -14% | -7% | -11% | 17% | 41% | 16% | null | 13% | 34% | 12% | 9% |
| 63 | L1L2L3L4L5L6 | 11% | -10% | 3% | -3% | 25% | -20% | 17% | -14% | 19% | -16% | 66% | -40% | 28% | -22% | 55% | -36% | 5% | -5% | -17% | 21% | -100% | null | 13% | -11% | -8% | 8% |

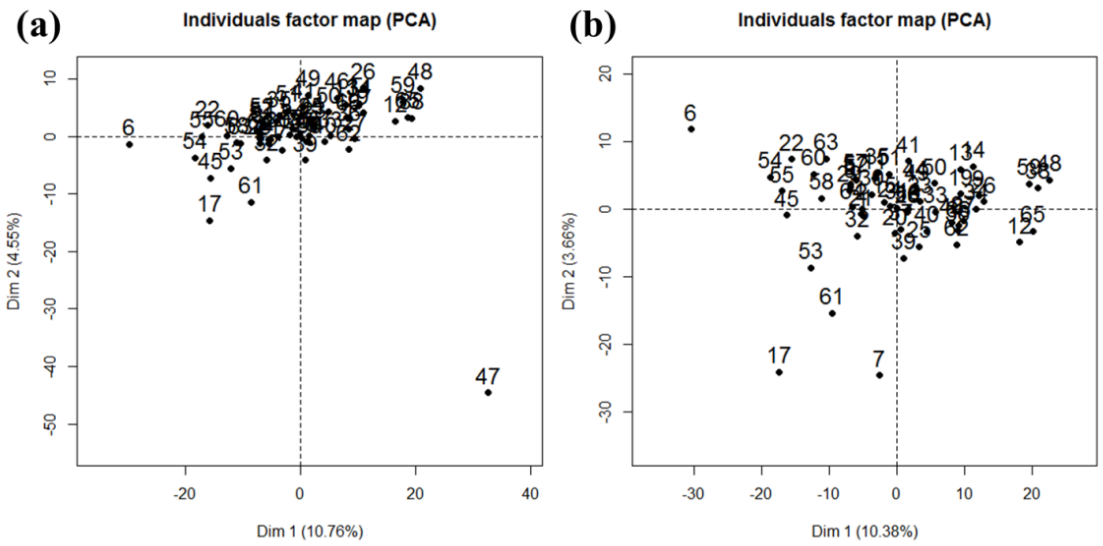
**Supplementary Figure 9** Analysis of the changes in odor attributes of mixed odor samples with different odorant combinations and the significance of differences. The column 'odorants' represents the odorants modified (added or omitted) in the sample. The descriptors marked in green cell were the dominant odors related to the different odorants (i.e. L1, alcoholic; L2, tropical fruit; L3, honey/ apricot; L4, cheese; L5, smoky; and L6, caramel). The column 'A' represents the percent change between the sample and the icewine odor sample; The column 'O' represents the percent change between the sample and the sample made of the icewine odor in which the 6 odorants were added (B6-R6L1L2L3L4L5L6). For example, for the sample L1L2L3L4L5L6, the value in column 'A' was calculated as following ($IM_{\text{B6-R6L1L2L3L4L5L6}}$-$IM_{\text{icewine}}$)/$IM_{\text{icewine}}$; the value in column 'O' was calculated as following ($IM_{\text{icewine}}$-$IM_{\text{B6-R6L1L2L3L4L5L6}}$)/$IM_{\text{B6-R6L1L2L3L4L5L6}}$. The significance of the difference between attributes is marked in each cell with different colors (yellow: $p$<0.05; light orange: $p$<0.01; dark orange: $p$<0.001); in column 'A', the difference was compared between $IM_{\text{sample}}$~ $IM_{\text{icewine}}$ and in column 'O', the difference was compared between $IM_{\text{sample}}$~ $IM_{\text{B6-R6L1L2L3L4L5L6}}$.



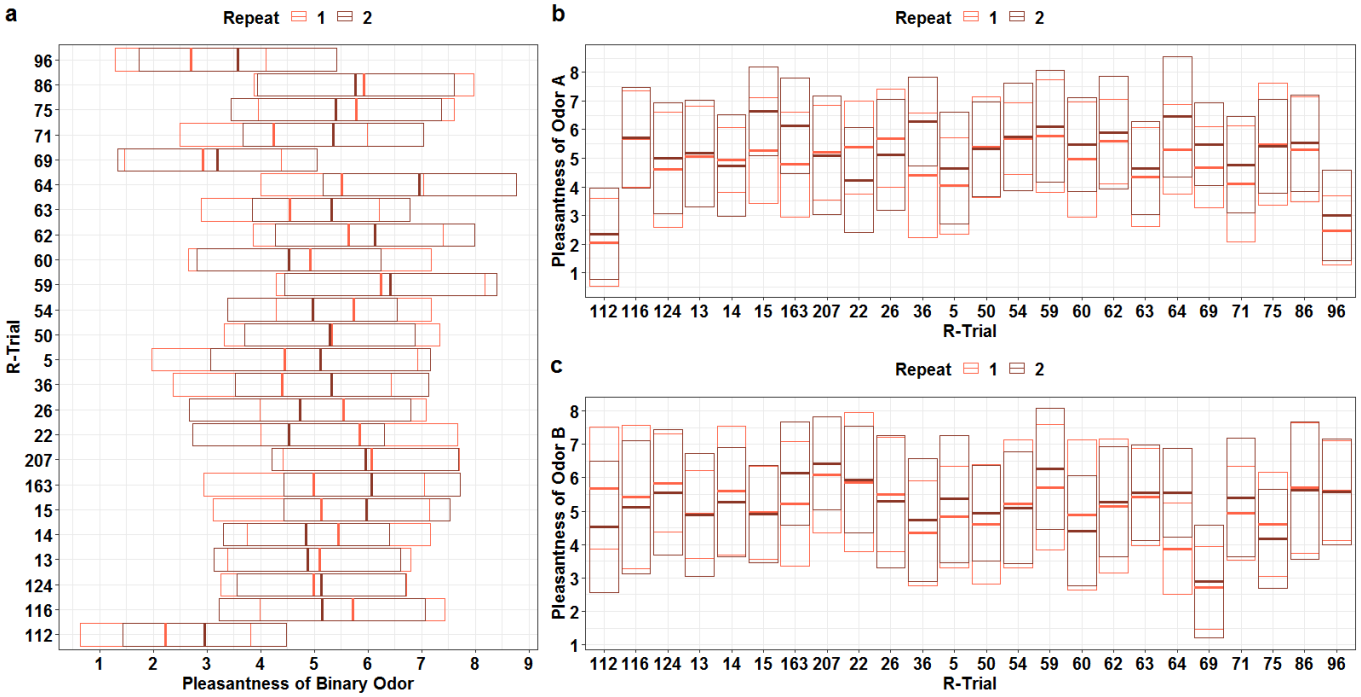

**Supplementary Figure 10** The influence of the different odorant combinations on the 13 odor attributes. The figure was drawn after collecting the odorant combinations which induced significant changes in the 13 attributes in Supplementary Figure 9. The column 'A' represents the influence in 'addition' test, the column 'O' represents the influence in 'Omission' test, and the column 'M' indicates whether the combination in 'A' and 'O' was the same (S) or complementary (C). In column 'A', the change was between $IM_{sample}$~ $IM_{icewine}$, and if $IM_{sample}$ > $IM_{icewine}$, the font color of the label is red, If $IM_{sample}$ < $IM_{icewine}$, the font color of the label is black; In 'O' column, the change was between $IM_{sample}$~ $IM_{B6\text{-}R6L1L2L3L4L5L6}$., and if $IM_{sample}$ > $IM_{B6\text{-}R6L1L2L3L4L5L6}$, the font color of the label is red, if $IM_{sample}$ < $IM_{B6\text{-}R6L1L2L3L4L5L6}$, the font color of the label is black. The difference between odor attributes could be found in Supplementary Figure 9, and the significance of the difference between odor attributes was marked in each cell with different colors (yellow: $p$<0.05; light orange: $p$<0.01; dark orange: $p$<0.001).



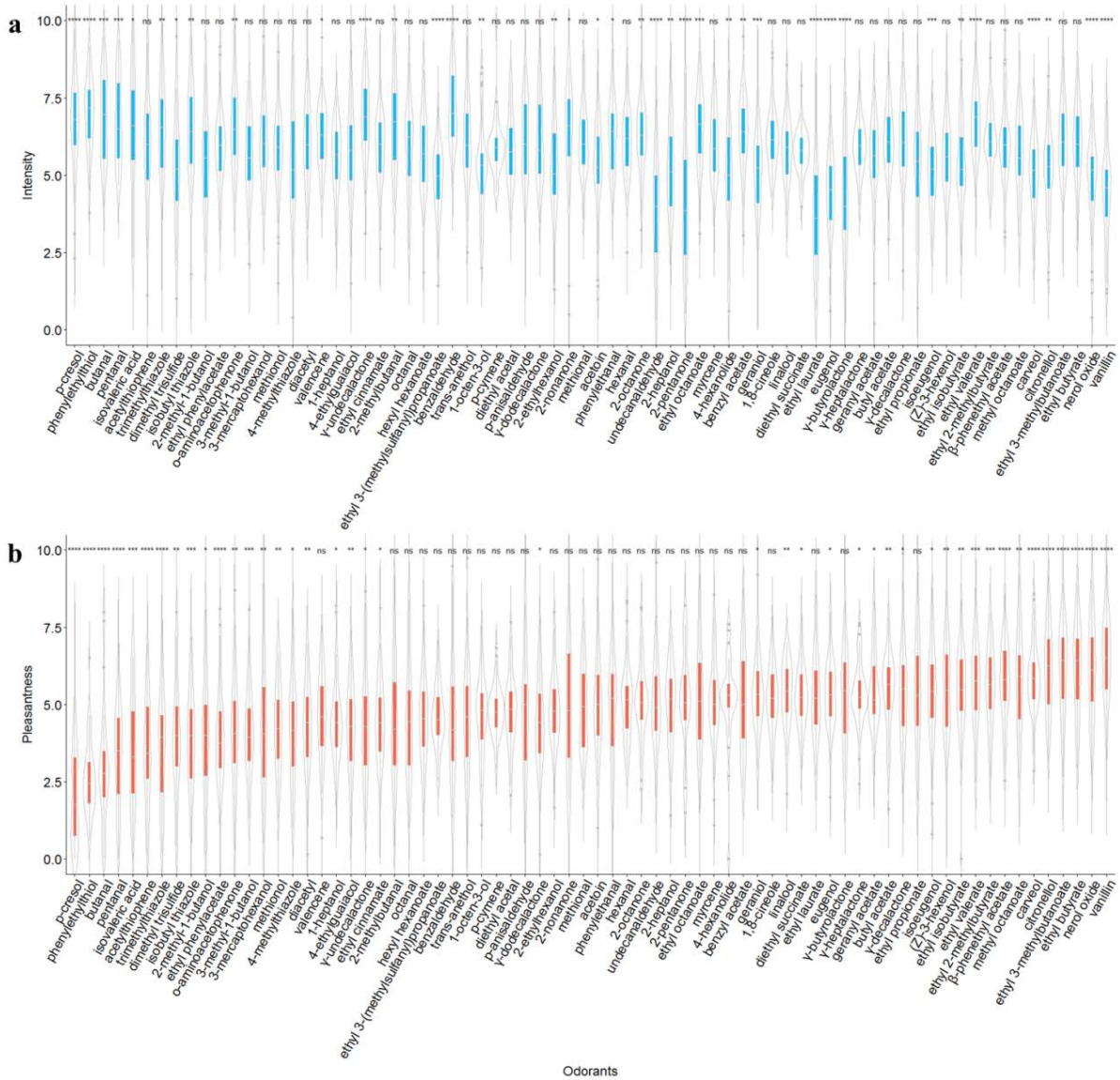

**Supplementary Figure 11** Subjects' overall performance and coherence checked by principal component analysis.



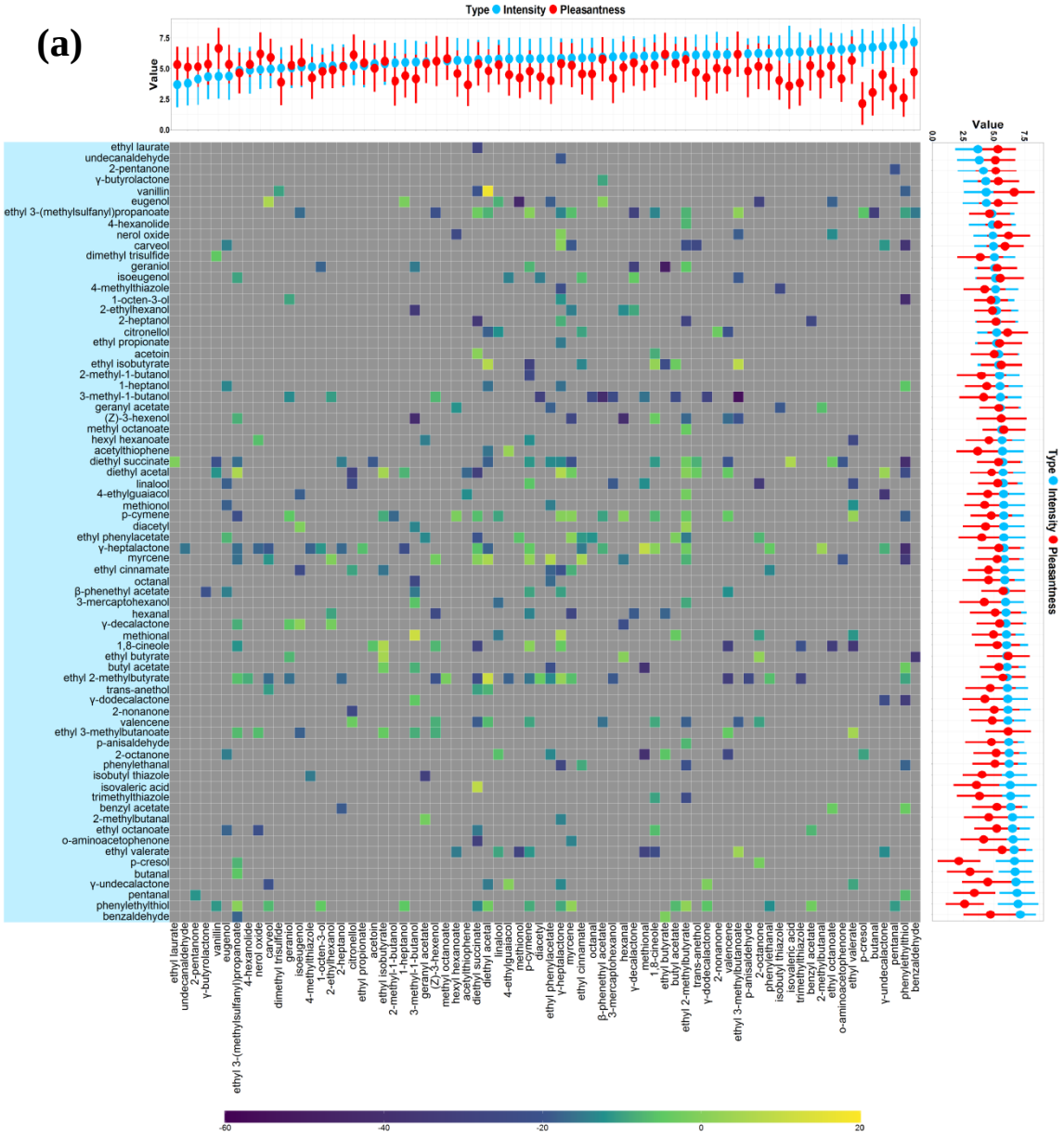

**Supplementary Figure 12** Subjects' overall performance and coherence checked by principal component analysis. **(a)** Results obtained from the original dataset. **(b)** Results obtained from the dataset without subject 47.

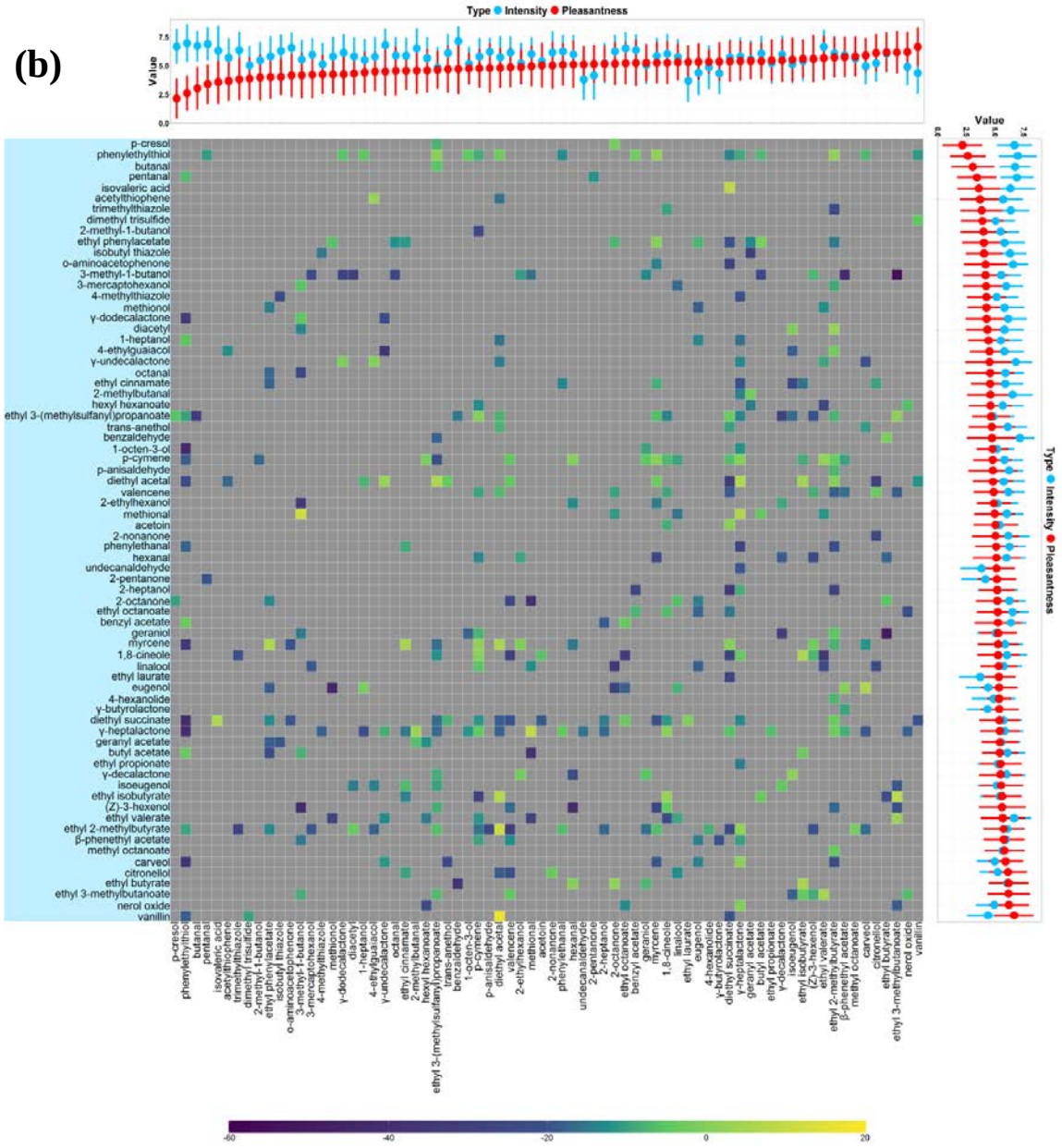



**Supplementary Figure 13** Repeatability of pleasantness. **(a)** Pleasantness of binary odor. **(b)** Pleasantness of odor A. **(c)** Pleasantness of odor B.

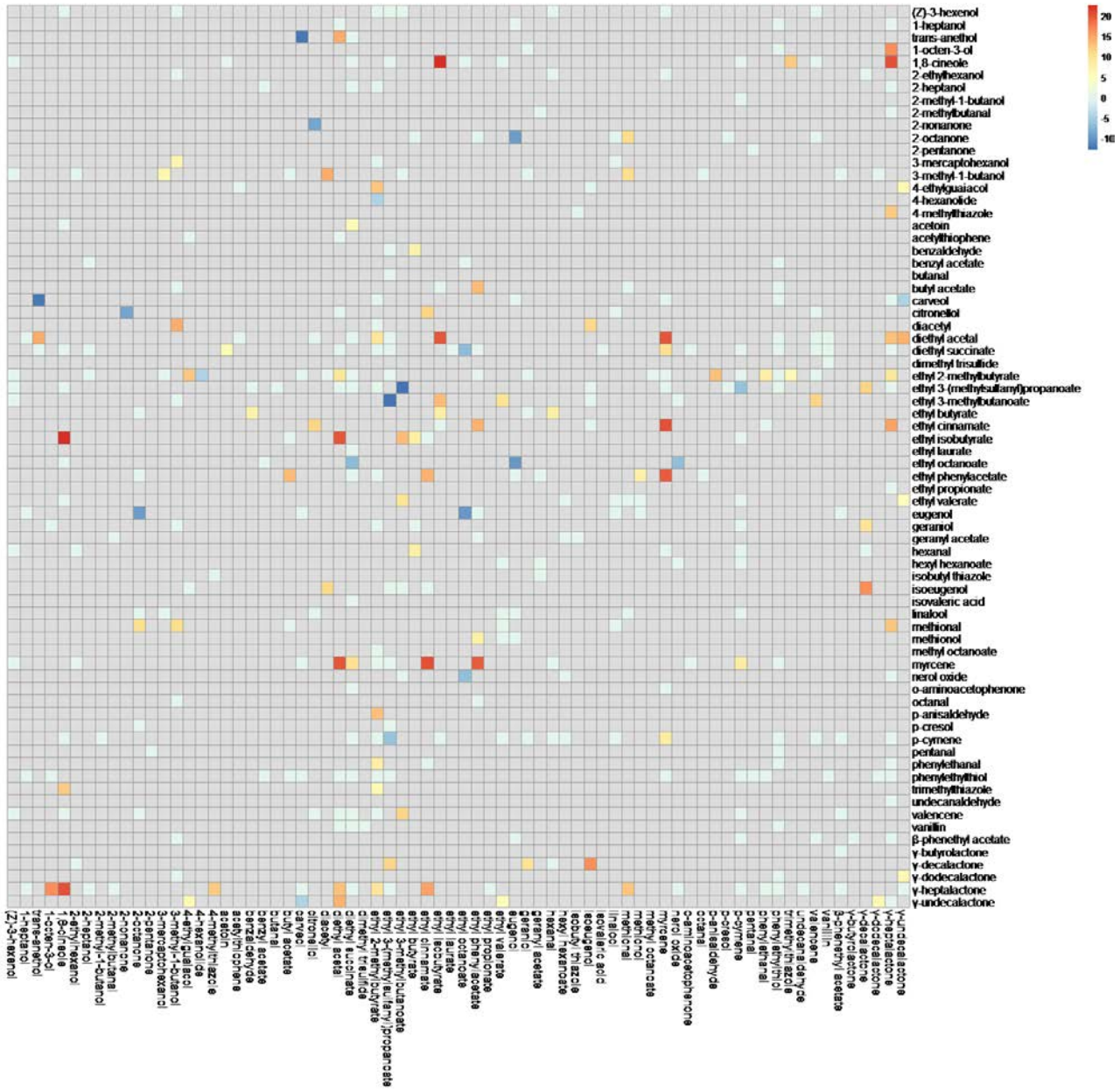



**Supplementary Figure 14** Distribution of intensity **(a)** and pleasantness **(b)** values for each odorant. Significant differences between the means of intensity or pleasantness compared to the mean value across odorants are indicated (ns: not significant, *: $p< 0.05$; **: $p< 0.01$; ***: $p< 0.001$; ****: $p< 0.0001$).


(a)
Type Intensity Pleasantness
Value
ethyl laurate
undecanaldehyde
2-pentanone
γ-butyrolactone
vanillin
eugenol
ethyl 3-(methylsulfanyl)propanoate
4-hexanolide
nerol oxide
carveol
dimethyl trisulfide
geraniol
isoeugenol
4-methylthiazole
1-octen-3-ol
2-ethylhexanol
2-heptanol
citronellol
ethyl propionate
acetoin
ethyl isobutyrate
2-methyl-1-butanol
1-heptanol
3-methyl-1-butanol
geranyl acetate
(Z)-3-hexenol
methyl octanoate
hexyl hexanoate
acetylthiophene
diethyl succinate
diethyl acetal
linalool
4-ethylguaiacol
methionol
p-cymene
diacetyl
ethyl phenylacetate
γ-heptalactone
myrcene
ethyl cinnamate
octanal
β-phenethyl acetate
3-mercaptohexanol
hexanal
γ-decalactone
methional
1,8-cineole
ethyl butyrate
butyl acetate
ethyl 2-methylbutyrate
trans-anethol
γ-dodecalactone
2-nonanone
valencene
ethyl 3-methylbutanoate
p-anisaldehyde
2-octanone
phenylethanal
isobutyl thiazole
isovaleric acid
trimethylthiazole
benzyl acetate
2-methylbutanal
ethyl octanoate
o-aminoacetophenone
ethyl valerate
p-cresol
butanal
γ-undecalactone
pentanal
phenylethylthiol
benzaldehyde
-60
-40
-20
0
20

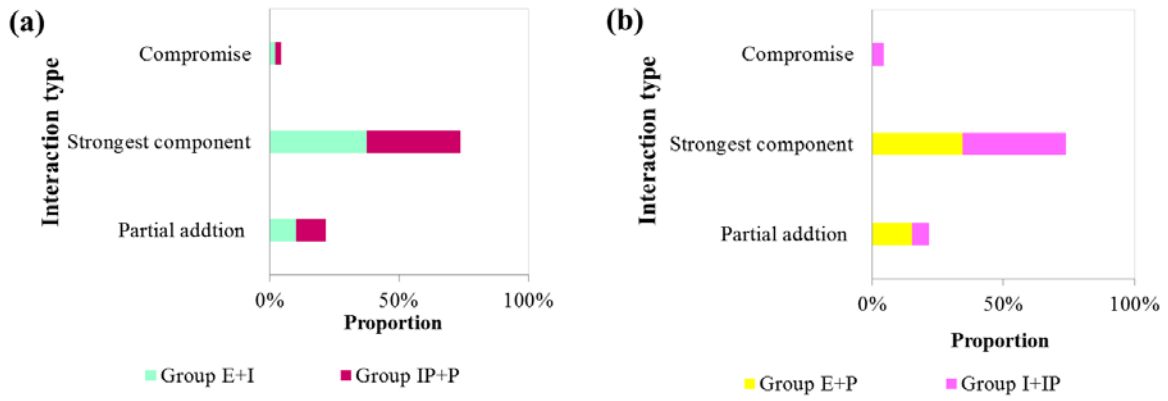



**Supplementary Figure 15** Percent of mixture-induced odor intensity change (IC, refer to Equation 2) for the odor of a given individual odorant (in row, light blue background) perceived within a binary mixture with another odorant (in column). **(a)** Odorants in row and column were ordered by average intensity. **(b)** Odorants in row and column were ordered by average pleasantness.

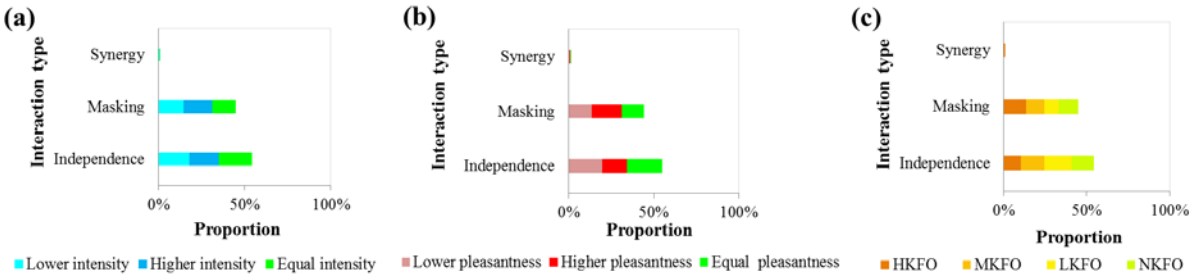



**Supplementary Figure 16** Mixture-induced intensity effects in the 198 different binary mixtures. The color in the figure reflects the degree of hypo-addition ($D_{HA}$, equation 11), which was calculated to illustrate the deviation in the overall intensity of the mixture compared to the strongest component. $D_{HA}$ is equal to 0 (light green color) when the intensity of the mixture followed the strongest component. When $D_{HA}$ is greater than 0 (yellow to red color), partial addition occurred, and when $D_{HA}$ is less than 0 (light blue to dark blue color), compromise occurred.

$$D_{HA} = \frac{I_{AB} - \max(I_A, I_B)}{\max(I_A, I_B)} \times 100 \qquad \text{(Equation 11)}$$




**Supplementary Figure 17 (a)** Frequency of mixture-induced effects on the overall intensity of binary mixtures as a function of components' intensity and pleasantness: Group E+I (green color ) gathered mixtures made with odorants not significantly different in their unmixed odor pleasantness and group IP+P (red color) gathered mixtures made with odorants significantly different in their unmixed odor pleasantness. **(b)** Frequency of mixture-induced effects on the overall intensity of binary mixtures as a function of components' intensity and pleasantness: Group E+P (yellow color ) gathered mixtures made with odorants not significantly different in their unmixed odor intensity and group I+IP (pink color) gathered mixtures made with odorants significantly different in their unmixed odor intensity.




**Supplementary Figure 18** The proportion of mixture-induced odor intensity effect (masking, independence, and synergy) as a function of relative intensity, pleasantness and frequent value for one component. **(a)** Proportion of 3 levels of relative intensity of components on 3 main types of interaction in mixture. For a given binary mixture, if there is no significant difference ($p$> 0.05) between $I_A$ and $I_B$, odorant A and B were labelled as equal ; if there is a significant difference between $I_A$ and $I_B$ , the odorant with higher intensity was labelled as higher and the other one as lower. **(b)** Proportion of 3 levels of relative pleasantness of components on 3 main types of interaction in mixture. For a given binary mixture, if there is no significant difference ($p$> 0.05) between $P_A$ and $P_B$, odorant A and B were labelled as equal ; if there is a significant difference between $P_A$ and $P_B$ , the odorant with higher pleasantness was labelled as higher and the other one as lower. **(c)** Proportion of 4 levels of frequent value of components on 3 main types of interaction in mixture. The odorant's frequent value was also considered as a classification criteria. To balance the number of samples in each category, a frequent value above 10% was labelled as high (HKFO, 15 odorants), a value between 3% and 10% was labelled as medium (MKFO, 12 odorants), and a value below 3% was labelled as low (LKFO, 15 odorants). The odorants for which frequent value has not been reported were labelled as Non-KFO (NKFO, 30 odorants).