DETERMINATION OF ORTHO- AND RETRONASAL DETECTION AND RECOGNITION THRESHOLDS FOR OFF-FLAVOURS IN WINE

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Abstract

The research performed in this thesis had as overall objective to fill the gap in the current literature about flavour thresholds and sensitivity to suprathreshold concentrations. Two groups of tasters were used, one comprised by 15 novices (student panel) and the other by 16 wine experts (professional panel). Detection (DT) and recognition (RT) thresholds were established in wine for six common off-flavour compounds when present in high concentration: acetaldehyde, acetic acid, ethyl acetate, hexanol, hydrogen sulfide and volatile phenols. Thresholds were evaluated orthonasally (ON) and retronasally (RN) by each participant, using the standardized procedures based on the ascending forced-choice (3-AFC) triangular tests. The suprathreshold intensity was obtained ON and RN after testing three measures of individual responsiveness: geometric mean (GM), area-under-curve (AUC) and slope of the response regression line.

DT and RT thresholds yielded overall results within the range reported in literature. Interestingly, in both threshold and suprathreshold tests, contrary to predictions based on reports of an association between expertise and improvement of the olfactory and gustatory sensitivity, the thresholds for the same molecules were similar between tasting panels, indicating that sensitivities were practically not affected by expertise. The relationship between threshold and suprathreshold intensities was also studied. In most cases there was not a relationship, implying that a low threshold does not necessarily lead to greater sensations elicited by suprathreshold concentrations. On the other hand, the results suggested that some subjects perceived the molecules differently on both orthonasal and retronasal pathways, presenting higher variability on the orthonasal evaluation comparing with the retronasal one.

Overall, results highlighted the high variability of individual olfactory responses and the importance of the retronasal pathway on the assessment of off-flavours in wines.

Keywords: threshold, suprathreshold intensity, orthonasal, retronasal, wine off-flavours.

Resumo

O presente trabalho teve como objectivo preencher as lacunas existentes na literatura relativas aos valores de limiares e supra-limiares dos compostos voláteis constituintes do vinho. Neste contexto, foram determinados os limiares de detecção e de reconhecimento para seis dos compostos de defeitos mais comuns em vinho quando presentes em altas concentrações: acetaldeído, ácido acético, acetato de etilo, hexanol, sulfureto de hidrogénio (H_2S) e fenóis voláteis, numa amostra de 15 provadores não treinados e de 16 provadores treinados. Os limiares foram avaliados por cada provador através de duas vias: ortonasal e retronasal, com base na metodologia padrão de comparação direcional de três amostras (3-AFC). As intensidades supra-limiares foram obtidas também pelas vias ortonasal e retronasal, sendo a sensibilidade individual avaliada através de três medidas: média geométrica, área-sob-curva e declive.

Os limiares de detecção e reconhecimento obtidos no presente estudo estão inseridos dentro da gama de resultados publicados na literatura. Curiosamente, nos testes de limiares e supra-limiares, contraditoriamente às conclusões publicadas em alguns artigos relativas à relação entre o conhecimento e a melhoria das sensibilidades olfactiva e gustativa, os valores de limiares para as mesmas moléculas de defeitos foram idênticos entre ambos os painéis de prova, indicando que a sensibilidade não é praticamente afectada pelo conhecimento do provador. A relação entre os limiares e supra-limiares também foi estudada. Em maior parte dos casos não houve relação, implicando que baixos limiares não conduzem necessariamente a grandes sensações relativamente a concentrações de supra-limiares. Por outro lado, os resultados sugeriram que alguns dos provadores apresentam diferenças de intensidade na percepção das moléculas quando comparada a via ortonasal com a retronasal, sendo que a avaliação ortonasal apresentou maior variabilidade. Esta observação indica que a resposta retronasal não deve ser descartada na avaliação de defeitos nos vinhos.

Palavras-chave: limiar, supra-limiar, ortonasal, retronasal, defeitos do vinho.

Resumo alargado

A investigação desenvolvida ao longo da presente tese está inserida num projeto global que tem principal foco estudar e compreender o comportamento do consumidor relativamente a vinhos com características sensoriais incomuns, tendo como referência os defeitos. Nestes vinhos estão incluídos vinhos direccionados para nicho de consumidores que os aceitam mesmo apresentando características sensoriais diferentes dos vinhos ditos comerciais.

Como objectivo inicial, o trabalho desenvolvido focou-se em preencher as lacunas existentes na literatura relativas aos valores de limiares dos compostos voláteis constituintes do vinho. Neste contexto, foram determinados os limiares de detecção (DT) e de reconhecimento (RT) para seis dos defeitos mais comuns em vinho: acetaldeído, ácido acético, acetato de etilo, hexanol, sulfureto de hidrogénio (H_2S) e fenóis voláteis, num painel de 15 provadores não treinados (6 provadores do sexo feminino e 9 do sexo masculino, com idades compreendidas entre os 23 e 36 anos; média 28 ± 4) e de 16 provadores treinados (8 provadores do sexo feminino e 8 do sexo masculino, com idades compreendidas entre os 30 e 65 anos; média 47±13). Os limiares foram avaliados por cada provador através de duas vias de percepção: ortonasal (ON) e retronasal (RN), com base na metodologia padrão de comparação direcional de três amostras (3-AFC), onde as amostras são apresentadas em ordem crescente de concentração e o provador é forçado a escolher uma das três amostras, em cada concentração (uma amostra contém o defeito e as outras duas apenas vinho).

Os resultados dos limiares de detecção e reconhecimento para o painel não treinado foram os seguintes: (i) acetaldeído em vinho branco (mg/L): DT no ON e RN: 12 e 14; RT no ON e RN: 46 e 70; (ii) ácido acético em vinho tinto (mg/L): DT no ON e RN: 302 e 481; RT no ON e RN: 658 e 1035; (iii) acetato de etilo em vinho branco (mg/L): DT no ON e RN: 14 e 17; RT no ON e RN: 27 e 29; (iv) hexanol em vinho branco (µg/L): DT no ON e RN: 72 e 107; RT no ON e RN: 402 e 370; (v) sulfeto de hidrogénio em vinho tinto (µg/L): DT no ON e RN: 83 e 73; RT no ON e RN: 286; e (vi) fenóis voláteis em vinho tinto (4-EF; 4-EG) (µg/L): DT no ON e RN: 29 e 31; RT no ON e RN: 35 e 31.

Os resultados dos limiares de detecção e reconhecimento para o painel treinado foram os seguintes: (i) acetaldeído em vinho branco – 1ª prova (mg/L): DT no ON e RN: 50 e 47; RT no ON e RN: 77 e 61; (ii) acetaldeído em vinho branco – 2ª prova (mg/L): DT no ON e RN: 15 e 14; RT no ON e RN: 27 e 21; (iii) acetaldeído em vinho branco – 3ª prova

(mg/L): DT no ON e RN: 9 e 7; RT no ON e RN: 21 e 16; (iii) acetaldeído em vinho tinto (mg/L): DT no ON e RN: 24 e 18; RT no ON e RN: 26 e 39; (iv) acetato de etilo em vinho branco – 1ª prova (mg/L): DT no ON e RN: 54 e 50; RT no ON e RN: 87 e 65; (v) acetato de etilo em vinho branco – 2ª prova (mg/L): DT no ON e RN: 26 e 32; RT no ON e RN: 26 e 49; (vi) fenóis voláteis em vinho tinto – 1ª prova (4-EF; 4-EG) (µg/L): DT no ON e RN: 41 e 33; RT no ON e RN: 68 e 42; e (vii) fenóis voláteis em vinho tinto – 2ª prova (µg/L): DT no ON e RN: 31 e 33; RT no ON e RN: 49 e 40.

Quanto ao objectivo de caracterizar e entender a sensibilidade individual e a reposta sensorial dos provadores à presença dos defeitos no vinho, três medidas foram avaliadas – média geométrica, área-sob-curva e declive – com base em intensidades supra-limiares. No que diz respeito à analise sensorial com base nas concentrações supra-limiares, a metodologia consistiu na disposição alternada de cinco amostras com concentrações diferentes, em que a avaliação ortonasal foi com o auxílio de uma régua para medir a distância entre o copo da amostra e o nariz do provador, aquando o mesmo sentisse o odor do defeito e a avaliação retronasal foi com base na escala de Likert, que quantifica a intensidade da resposta sensorial numa escala.

Considerando os resultados das correlações entre cada par de medidas, os melhores resultados foram obtidos entre a média geométrica e a área-sob-curva, sendo que as correlações obtidas no que dizem respeito ao declive foram fracas, devido em grande parte à insensibilidade demonstrada pelo painel de provadores no decorrer da análise sensorial. Tendo também em conta todas as limitações relativas às suas determinações, a opção foi a de seleccionar a média geométrica como medida da sensibilidade individual. Individualizando as respectivas respostas sensoriais do painel não treinado com base na média geométrica, foi possível categorizar a sensibilidade dos provadores, contudo foi verificado uma grande variabilidade nas respostas e na percepção ortonasal e retronasal dos defeitos do vinho por parte dos provadores.

Para este estudo, também foi analisada a relação entre os limiares e supra-limiares. Os casos onde se verificaram a respectiva relação foram limitados. Curiosamente, nos testes de limiares e supra-limiares, contraditoriamente às conclusões publicadas em alguns artigos relativas à relação entre o treino e a melhoria das sensibilidades olfactiva e gustativa, os valores de limiares para as mesmas moléculas de defeitos foram idênticos entre ambos os painéis de prova, indicando que a sensibilidade não é praticamente afectada pelo treino do provador. Por outro lado, os resultados também sugeriram que alguns dos provadores apresentam diferenças de intensidade na

percepção das moléculas quando comparada a via ortonasal com a retronasal, sendo que a avaliação ortonasal apresentou maior variabilidade (maiores valores de desvio padrão). Esta observação indica que a resposta retronasal não deve ser descartada na avaliação de defeitos nos vinhos.

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1. INTRODUCTION

In accordance to the movement of society, wines are also following the tendency to meet the essence and purity of the practices, from the vineyard to the winery (Lalas, 2018). As regards viticulture and oenology, biodynamic, organic and natural wines have received increasing attention over the last few years. These styles of wine have become increasingly popular as the customers demand for wines that are made in a minimum-intervention style (Wilson, 2016). However, according to Guzzon *et al.* (2016), many doubts remain among winemakers in relation to its applicability on a large scale for the production of wines and its quality acceptance by consumers.

Generally, the perception of wine quality is easier to recognize than it is to define and it is more related to the intrinsic aspects of wine (Villamor, 2012). Indeed, for example, while the colour, brand or reputation of wine is important to consumers, it is the aroma and taste of wine that have the greatest impact for wine enjoyment. This sensory experience is usually pleasurable. In agreement with Puckette (2014), the first flavours that consumers learn to identify in wine are fruit flavours, perhaps because these are the most gratifying. However, it can at times be a less-than-pleasant surprise with off-flavours, many of which are weird and require an acquired taste (Bartowsky & Pretorius, 2009). Maybe that is one of the reasons why, according to Asimov (2018), wines affected by off-flavours are quickly judged by consumers. Actually, according to Lalas (2018), some "imperfections" or "off-flavours" may rather be associated with some of these wines. However, it is important to take in consideration that the perception of off-flavours depends on the consumer and its capacity to understand them, because the aroma and flavour perceptions are highly individual.

1.1 Chemical basis of wine aroma

Wine aroma is very complex. In agreement with Francis and Newton (2005) and Ferreira *et al.* (2007), at the core of the quality (or the lack of it) of a wine there is a large amount of odour active compounds. One of the long-standing goals of wine aroma research has been to identify those volatile and non-volatiles compounds that are central to particular olfactory attributes of wines, whether perceived ortho- or retronasally, and understand their role in the final perception of the wine.

By the late eighties of last century, chemists had identified more than 800 compounds in the volatile fractions of wines (Schreier, 1979; Maarse & Vischer, 1989). This was a sort of bitter success since so much of that information was apparently mostly useless.

In line with Ferreira *et al.* (2007), the first reason for that apparent failure was because researchers at that time tried to identify all the molecules present in the volatile fraction of wine instead of concentrating on those that really have the power to impact the pituitary tissues. The second one is linked to the complexity of wine flavour: only in a limited number of cases can a single odour molecule be explicitly recognised in the aroma or flavour of the wine. It is not surprising, therefore, that the major successes had come in the identification of off-flavours. The third reason is that at that time it was very difficult to get accurate quantitative data on some of the molecules present at low concentrations. All those limitations have been slowly and progressively solved in the last 10 or 15 years due to the progress made in the quantitative determination of some important odorants and due to the systematic development of gas chromatography-olfactometric (GC-O) methods that have made it possible to screen from all the volatile compounds of wine, those that really have the ability to be flavour active (Ferreira *et al.*, 1998; Lopez *et al.*, 2003; Cullere *et al.*, 2004; Escudero *et al.,* 2004).

The chemical basis of wine aroma according to Ferreira *et al.* (2007), may be explained by the role played by the following compounds:

(i) Impact or highly active compounds.
These are the compounds which can effectively transmit their specific (impact) or primary (highly active) aroma nuance to a given wine without the need of the support of more aroma chemicals. The best example is given by the molecule of linalool.

(ii) Impact groups of compounds.
These are constituted by families of compounds usually having similar chemical structures, with close odour properties and that can impart to the aroma of a wine the specific notes of the family. An example is the group of γ-lactones.

(iii) Subtle compounds.
These are the compounds which fail to transmit their specific aroma nuances to the wine but contribute decisively to the development in wine of some secondary-generic aroma nuance (for instance fruity, sweet).

(iv) Compounds forming the base of wine aroma.
These are the compounds present in all wines at concentrations above their corresponding odour thresholds which, however, are no longer perceived as single entities because their aromas are fully integrated to form the complex concept of wine aroma.

(v) Off-flavour compounds.

These are the compounds whose presence brings a decrease in the overall aroma quality of wine.

The mixture of all the major fermentation compounds at the concentrations at which they are usually found in wine has the typical odour of alcoholic beverages that often is define as vinous (Ferreira *et al.*, 2007). The key point is that this mixture forms what they call an aroma buffer. Both effects of aroma buffer were described by Ferreira *et al.* (2002) and Escudero *et al.* (2004), concluding that the omission or addition of some compounds does not bring about important changes in wine aroma. Therefore, it could be said that wine forms some kind of aromatic buffer towards a wide range of aromas. Table 1.1. shows the effects of omission from the mixture of wine major compounds of one of the odorants, that, in most cases, had no effect, or a just apparent effect that the judges were not able to define. Only in the cases of isoamyl acetate and β-damascenone there were slight effects on the fruitiness of the mixture. Table 1.2. shows the effect of the addition of different aroma compounds to the mixture of wine major compounds. The addition of high amounts of some odorants has nearly no effect, or in some cases the effect is not the perception in the mixture of the added odorant but a decrease on some of the basic attributes of the mixture (except for isoamyl acetate).

According to Escudero *et al.* (2004) and Ferreira *et al.* (2007), the aroma buffer would be caused by the presence in wine of relatively high concentrations of ethanol and other volatiles formed by fermentation such as ethyl esters, fusel alcohols, volatile phenols, β-damascenone, and fatty acids. Fortunately, the aroma of many wines is very rich in aroma nuances that are quite different to the basic 'vinous' aroma of the aroma buffer. This clearly means that some aroma molecules succeed in some wines in breaking the buffer and produce a different sensory perception, transmitting a different aroma nuance. In line with Ferreira *et al.* (2007), the first way to break the buffer is by a single odorant molecule at the concentration at which it can be naturally found in some wines. There are only few aroma chemicals that can act as impact compounds: linalool, c-rose oxide, 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol, sotolon, furfurylthiol, 3-mercaptohexyl acetate, benzylmercaptan, dimethyl sulphide, methional, diacetyl, isoamyl acetate and rotundone. A second way to break the buffer is by the concerted action of a group of molecules sharing chemical and odour properties. The third way to break the buffer is by the concerted action of many chemicals sharing some similarity in any of their generic aroma descriptors. Of course, the buffer can be broken, but in a negative way, by many chemicals playing the role of off-flavours.

Table 1.1. Effects caused by the omission in the wine mixture (the aroma buffer) of one of the wine constituents (adapted from Ferreira *et al.*, 2002).

Compound omitted	Qualitative effect
β-damascenone	Slight decrease in intensity
β-phenylethanol	Inappreciable
Acetaldehyde	None
Butyric acid	Inappreciable
Diacetyl	None
Ethyl acetate	None
Ethyl butyrate	None
Ethyl hexanoate	Inappreciable
Ethyl isovalerate	None
Ethyl octanoate	Inappreciable
Hexanoic acid	Inappreciable
Isoamyl acetate	Slightly less fruity
Isoamyl alcohol	Inappreciable
Methionol	Inappreciable
Octanoic acid	Inappreciable

Table 1.2. Effects caused by the addition of some selected aroma compounds to the wine mixture (adapted from Escudero *et al.*, 2004).

Compound added (level and relative increment)	Effect	Observations
2,6-dimethoxyphenol (2 ppm; 4000x)	Slight	- flowery; - candy
β-damascenone (4.5 ppb; 1x)	None	
Ethyl octanoate (6.0 ppm; 8.6x)	None	
Furaneol (800 ppb; 27x)	None	
Guaiacol (15 ppb; 71x)	Slight	- candy; - flowery
Isoamyl acetate (5.5 ppm; 2.2x)	Slight	+ banana
Sotolon (140 ppb; 28x)	Slight	- fruity; - candy

1.2 Off-flavours in wine

One important prerequisite related with marketing and commercial implications for wine and similar products of high quality is to be free of aroma imperfection. As explained by Rapp (1990), these "imperfections" in wine are of a complex nature because the undesirable aromas can be varietal specific flavours (e.g. black pepper) or can be formed during the production and bottling of wine (e.g. corkiness, mousiness). The

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effect depends on the style of the wine, the concentration of the aroma compound, the sensitivity of the consumers and, as it affects the wine enjoyment, it is extremely difficult for the consumers to make an accurate judgement of the wine.

The first thing that should be said is that the concept of off-flavour is relative, because it is, at least in part, related to the previous experiences and expectations of the consumer. As mentioned by Ferreira *et al.* (2007) there are many examples of this. Many local producers and 'traditional' consumers of Spanish wines from Rioja became so familiar with the presence of small amounts of ethyl phenols in their wines, that for them the phenolic-note produced by those compounds is something essential in the wines. The same is also observed among producers of Beaujolais. Similarly, some producers of wines from Sauvignon Blanc are quite happy with the earthy and black-pepper notes introduced by methoxypyrazines. In some other cases, however, there is a large world-wide consensus about the negative role of some molecules, such as TCA (trichloroanisole; cork taint). It also necessary to point out that some apparently 'bad' molecules can sometimes play an interesting role on wine aroma, such as dimethyl sulfide that enhance the fruitiness in some red wines (Escudero *et al.*, 2007).

The second point that should be remarked is that, in general, the negative role of many aroma molecules is noted at concentrations well below the level at which those molecules are clearly perceived in wine. Before reaching such recognition threshold, the effect of these molecules is to decrease some positive sensory characteristics of the wine, and sometimes even to unbalance the wine aroma.

The final comment is that sometimes the off-flavours can be produced by relatively large amounts of fermentation by-products (isoacids, acetoin, vinylphenols, some alcohols) acting in a concerted way, so the individual compounds do not need to be present at the concentrations at which they usually are considered a risk to quality. This phenomenon was described by Escudero *et al.* (2007) and apparently could be one of the most important causes of low quality observed in many wines.

Therefore, according to Capone *et al.* (2010), off-flavours are unacceptable odours or tastes that result from product components formed by chemical or biological processes (e.g. microbiological spoilage) within the product, attributed to poor winemaking practices and/or inadequate storage conditions. Compounds causing off-flavours in wine are often present at higher concentrations that can lead to undesirable sensory characteristics. Table 1.3. summarises the different wine compounds that have been

documented as potential wine off-flavours that generally strongly influence the wine quality. The compounds off-flavours described in the next sections are the focus of the present overall study where this research is included.

Table 1.3. Most common off-flavours in wine (Ferreira *et al.*, 2007).

Off-flavour/Compound	Descriptors	Possible causes	Sensory thresholds
Acetaldehyde/Oxidation	Green apple, stuck ferment aroma	-Bacterial spoilage (genus *Acetobacter*, *Gluconobacter*); -Oxidation.	500 µg/L (in water/ethanol) (Guth, 1997)
Acetic Acid	Vinegar odour	-Bacterial spoilage (genus *Acetobacter*, *Gluconobacter*); - Yeast spoilage; -Oxidation.	0.2 g/L (in water/ethanol) (Guth, 1997)
Cork taint (*2,4,6-trichloranisole*: TCA)	Musty, earthy, moldy odours	-Presence of fungi on cork stopper.	2.1 ng/L (detection threshold in red wine) (Prescott *et al.*, 2005)
Ethylacetate	Nail polish remover odour	-Yeast spoilage (apiculate yeasts); - Bacterial spoilage.	6.4 mg/L; 13.3 mg/L (detection and recognition thresholds in air, respectively) (Hellman & Small, 1973)
Geosmin (earthy-type taint)	Earthy, musty odours	-Metabolite of *Botrytis cinerea*, soil bacteria and algae.	25 ng/L (in red wine) (AWRI, 2018)
Geranium	Crushed geranium leaves odour	-Metabolism of sorbic acid by lactic acid bacteria.	100 ng/L (AWRI, 2018)
Hexanol	Herbaceous odour	-Grape maturation.	8 mg/L (in water/ethanol) (Guth, 1997)
Mousy taint (2-acetyltetrahydro-pyridine ACTPY), 2-ethyltetrahydropyridine (ETPY), 2-acetylpyrroline (ACPY)	Caged mice, cracker biscuit odours	-Bacterial spoilage (genus *Lactobacillus* and *Oenococcus oeni*); -Yeast spoilage (*Brettanomyces*); -Oxidation (unknown mechanism).	ACTPY: 4.8-106 µg/L; ETPY: 2.7–18.7 µg/L; ACPY: 7.8 µg/L (in wine) (AWRI, 2018)
Sulfur compounds (H_2S, ethyl mercaptan, dimethysulfide)	Rotten egg, garlic, onion, cooked vegetable	-Produced in excess by yeasts during fermentation; -Reduction.	H_2S: 5.7 – 7.9 µg/L (detection threshold in air) (Young & Adams, 1966)
Volatile phenols (4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG))	Barnyard, horse, wet leather odours	-Yeast spoilage during wine storage tank, barrique or bottle (genus *Brettanomyces, Dekkera*).	4-EP + 4-EG: 114.3 µg/L (in wine) (Csikor, Pusztai & Barátossy, 2018)

1.2.1 Acetaldehyde

Acetaldehyde, also known as ethanal, is the most important aldehyde occurring in wine. It is primarily a product of yeast metabolism of sugars during the first stages of alcoholic fermentation (Jackson, 2008). It is the last precursor in yeast fermentation before ethanol is formed, and is produced when pyruvate is converted by the enzyme alcohol dehydrogenase (ADH) to acetaldehyde. Conversely, a secondary source of acetaldehyde production, which usually occurs after wine ageing, is oxidation of ethanol, once again facilitated by the enzyme ADH (Jackowetz *et al.*, 2010). It is sporadically associated with spoilage by acetic acid bacteria. As an intermediate in the bacterial formation of acetic acid and under low-O_2 conditions and/or alcohol levels greater than 10% v/v, tends to accumulate instead of being oxidised to acetic acid (Theron, 2017).

1.2.2 Volatile acidity and ethyl acetate

Volatile acidity (VA) is one of the most important characteristics of wines, contributing directly and indirectly to their quality. It is not possible to produce wine without VA, since acetic acid, the major acid compound (90%), can be primarily produced in low concentrations during the alcoholic fermentation step by yeasts and also formed by bacterial metabolism, such as acetic acid bacteria (AAB) and lactic acid bacteria (Miranda *et al.*, 2017). The AAB oxidize the ethanol to acetic acid and based on the findings of Bartowsky and Henschke (2008), there are two membrane-bound enzymes catalysing the reactions: alcohol dehydrogenase (ethanol to acetaldehyde) and acetaldehyde dehydrogenase (acetaldehyde to acetic acid). The recognition of VA in wines, namely the "acetic nose", is not exclusively the result of the acetic acid levels, but severally depends on the acetic acid and ethyl acetate ratio (Zoecklein, 2012).

Ethyl acetate is the most common ester present in wine and results from the combination between the acetic acid formed with the alcohol remaining in the wine. According to Nogueira and Nascimento (1999), the concentration of both compounds in wine depends severally on the yeast strain, since that, as acetic acid, ethyl acetate can also be formed by the action of apiculate yeasts during fermentation and depends on the action of AAB during ageing. As explained by Cliff and Pickering (2006), the presence of both compounds below perceptible levels can enhance the fruitiness flavour and add complexity to wine, while above it can be considered undesirable and may evidence microbiological problems. Although the incidence of excess ethyl acetate is statistically lower than that of the acetic acid, the first one is considered a more serious problem because of its greater organoleptic impact, given its ability to completely degrade the typicalty of a wine.

1.2.3 Hexanol

Hexanols are the major C_6-alcohols compounds (higher alcohols) and donate a herbaceous odour to certain wines due to problems in grape ripening. However, they may be present in healthy grapes but rarely occur in significant amounts (Jackson, 2008). Part of the compounds present in wines has six carbon atoms deriving from grape polyunsaturated fatty acids (primarily originated from membrane lipids), namely linoleic and α-linolenic acids (Oliveira *et al.*, 2006).

1.2.4 Sulfur compounds

As a consequence of yeast metabolism, heat treatment, light exposure, or other nonenzymatic reactions, a wide diversity of volatile sulfur-containing compounds may be produced during fermentation, ageing, and post-bottling. Although generally occurring in trace amounts, their high volatility and low sensory thresholds, can impart a great significance (Jackson, 2008). The principal offending compounds are reductive aromas developed by disulphides, mercaptans and hydrogen sulfide (H_2S). The last one is the most common volatile sulfur compound in wine and it may be formed during fermentation, as yeasts reduce residual elemental sulfur (Schütz & Kunkee, 1977), or metabolize sulfur-containing amino acids, notably cysteine and methionine (Henschke & Jiranek, 1991).

1.2.5 Volatile phenols

Of the spoilage yeasts, *Brettanomyces* spp. (imperfect state of *Dekkera*) are probably the most notorious (Larue *et al.*, 1991). As explained by Kheir *et al.* (2013), in some cases, depending the conditions and the available precursors (hydroxycinnamic acids, also called phenolic acids such as ferulic and *p*-coumaric acids), these yeasts can produce undesirable metabolites (volatile phenols) when growing and/or ageing in wine. In agremeent with Loureiro and Malfeito-Ferreira (2006), the recognition of the role played by the species in red wine spoilage due to the production of "horse sweat" taint has presented a new challenge to winemakers in the last decade. Surprising to those who detect the malodorous by-products of *Brettanomyces* metabolism, some winemakers appear to appreciate its effects on their wines (Steele, 2004). As identified by Suarez *et al.* (2007), their origin involve the sequential action of two enzymes on a hydroxycinnamic acid substrate. Hydroxycinnamate decarboxylase first turns these acids into vinylphenols (4-vinylphenol and 4-vinylguaiacol), possibly followed by reduction to ethylphenols (4-ethylphenol and 4-ethylguaiacol) by vinylphenol reductase (Chatonnet *et al.*, 1992). Although several bacteria and yeasts are capable of

metabolizing hydroxycinnamates to vinylphenols, only a few yeasts, notably those belonging to the genus *Brettanomyces* spp., can convert substantial amounts to ethylphenols (Chatonnet *et al.*, 1992).

1.3 Orthonasal and retronasal aroma perception

Although closely related, flavour, aroma, smell and taste are not exactly interchangeable (Puckette, 2014). Flavour is the result of the simultaneous stimulation of three principal sensory systems: taste, olfaction (aroma) and the trigeminal system. The latter comprises chemical, thermal and tactile sensations (Cerf-Ducastel & Murphy, 2001). Taste refers to the senses inside our mouth including the tongue (Puckette, 2014). Every human tongue has two kinds of receptors: one type is for taste, known as 'taste buds', which can be found all over the tongue. While the other receptor is for tactile sensations that refers to the free nerve endings sensed all over the inside of the mouth and tongue. In simple language, taste buds focus primarily on sweet, salt, bitter and sour, while the mouthfeel, as a sensory propriety, focuses on viscosity (i.e. body), tannins, and the overall texture of the wine (Puckette, 2014). Aroma and *bouquet* are just pleasant words used to describe the odours (Puckette, 2014). Odours are volatilized compounds sensed in our brain by the limbic system which deals with emotion, behaviour, motivation and long-term memory. An odour molecule may reach the olfactory epithelium via the nose (orthonasal olfaction), referred to the external world, or via the mouth (retronasal olfaction), referred to the oral cavity (Rozin, 1982). The illusion that retronasally perceived odours are localized to the mouth is so powerful that people routinely mistake retronasal olfaction for "taste" (Rozin, 1982). As an example, we may say that we like the "taste" of a wine because of its fruity or spicy notes. However, taste refers only to the sensations of sweet, sour, salty and bitter, and thus the pleasant "taste" to which we refer is actually a pleasant odour sensed retronasally.

The perception of volatiles and non-volatiles aroma compounds by orthonasal and retronasal pathways has been of particular interest in flavour research and strong evidence has shown that odours are perceived differently when presented in ortho- versus retronasal modes (Rozin, 1982; Halpern, 2004; Heilmann & Hummel, 2004; Small *et al.* 2004; Small *et al.* 2005; Sun & Halpern, 2005; Hummel & Heilmann, 2008; Welge-Lussen *et al.*, 2009). In 1982, Rozin was the first one that observed that "olfaction is the only dual sensory modality, in that it senses both objects in the external world and objects in the mouth" and thus proposed that "the same olfactory stimulation may be perceived and evaluated in two qualitatively different ways depending on

whether it is referred", which suggested that orthonasal and retronasal olfactory perceptions are two different systems. However, several studies testing Rozin's hypothesis have yielded mixed results, with several authors concluding that orthonasal and retronasal olfaction differ only in the efficiency with which odours are delivered to the olfactory epithelium (Burdach *et al.*, 1984; Voirol & Daget, 1986; Pierce & Halpern, 1996; Bojanowski & Hummel, 2012). So for a while no support was found for the 'olfactory duality' hypothesized by Rozin (1982). Most studies comparing orthonasal versus retronasal have focused upon qualities of the experience that provide information about the quantity or identity of the sensory stimulus, whereas the key distinction Rozin had made was that route of delivery influenced not what the stimulus was but rather where the stimulus was perceived. Generally, in these studies were found greater intensity for orthonasal than for retronasal stimulation. Specially, Voirol and Daget (1986) have shown that perception intensity depends on the absolute quantity of odorant reaching the olfactory epithelium, which corresponds to the difference in the air flow between sniffing and exhalating, inducing higher intensity judgement for the orthonasal mode. Given the proposed importance of retronasal in the oral perception of wines, usually the retronasal identification accuracy is inferior to orthonasal under normal breathing conditions which may seem counterintuitive. Nonetheless, this was observed repeatedly in Pierce and Halpern (1996): the vapour phase has been reported to be less efficiently sensed by the retronasal than by the orthonasal.

Although several studies have examined brain responses to retronasal olfactory stimulation (de Araujo *et al.*, 2003; Small *et al.*, 2004), none have directly compared orthonasal and retronasal in the same subjects or considered the possibility that the effects of route of stimulation depend on the way that odours are typically sensed. The findings of Small *et al.* (2005) clearly support all these notions by demonstrating that the neural response evoked by an odour may be influenced by its route of administration. First, imaging techniques like functional magnetic resonance imaging (fMRI) allowed to identify that activation due to retronasal stimulation was found at the base of the central sulcus, corresponding to the primary representation of the oral cavity, possibly reflecting that retronasal odours are referred to the mouth. In second, the stimulus studied "chocolate" was perceived as similarly intense and pleasant across both orthonasal and retronasal administration, thus the only perceptual difference was related to where the stimulus was referred (nose vs mouth). In Small *et al.* (2004) and Small *et al.* (2005) studies, they identified the preferential activation in different brain areas in response to ortho- and retronasally presented stimulus. It was observed

deactivation in the orbitofrontal cortex, insula and anterior cingulate cortex for taste-orthonasal stimulus, and supra-additive responses at the same regions for the combination of taste-retronasal stimulus. The results clearly indicate that the neural processing of an odour is influenced by its route of administration.

1.4 Suprathreshold and threshold sensory measures

Individual perception of aroma and taste are challenging to measure and there is no single method to assess it. Indeed, five distinct methods have been used by researchers to understand individual's sensitivity to tastes: detection threshold (DT) and recognition threshold (RT), suprathreshold intensity measure, 6-n-propylthiouracil (PROP) taster status and fungiform papillae (FP) number (Webb *et al.*, 2015). In the present study, the methods used were the detection and recognition thresholds and suprathreshold intensity regarding flavour active molecules. An individual sensing an odour is directly aware of only two sensory properties of the odour: intensity (how strong it smells) and character (what it smells like). These sensations are interpreted based on prior experience and expectations. Most studies about flavour perception (Prescott *et al.*, 2005; Pickering *et al.*, 2007; Ross *et al.*, 2014; Chrysanthou *et al.*, 2016) do not reflect the prime importance of odour intensity. Instead, they emphasize odour threshold: a measure of dilution needed to reduce the perceived odour intensity to some defined probability of detection level. It resulted from an earlier assumption that threshold multiples are measures of odour intensity. In line with Dravnieks and Jarke (1980), it is now known not to be true. The perceived odour intensities, as on a typical scale of none/very faint/faint/easily noticeable/strong/very strong, relate to the threshold multiples differently for different odorants. Because of the lack of direct relation between odour thresholds and intensities, there is a growing interest in measuring the perceived odour intensities directly (Dravnieks & Jarke, 1980).

Detection threshold (DT) and recognition threshold (RT) provide estimates of the lowest chemical concentration that can be perceived by an individual. For example, a solution may contain a substance at a concentration undetectable to the general population, but as the concentration is increased, a DT is attained such that the solution can be discriminated from pure solvent in a forced choice task. As the concentration is further increased, a RT is attained, and this is the point where the substance is both perceived and identifiable (Keast & Roper, 2007). It is widely accepted that individuals with lower detection and recognition thresholds are more sensitive to a chemical than those with a higher detection and recognition thresholds. Thresholds are determined using a classic psychophysical protocol - ASTM E 679 - described by the American Society for

Testing and Materials (ASTM, 2004), defined as the method of limits with an ascending concentration series using 3-alternative forced-choice (3-AFC) at each concentration. In a forced-choice method, the judgment is made after smelling several stimulus, including blanks. This offers an advantage over methods in which one stimulus is judged at a time and progressively stronger odorous sample concentrations can be systematically evaluated. The forced-choice method also reduces the problems stemming from odour adaptation.

Suprathreshold intensity refers to the perceived intensity (magnitude) of a substance at concentration above threshold. As the stimulus concentration increases, it is expected that the perceived intensity will also increase, eventually reaching to a terminal threshold for the stimulus: it occurs at concentration between the recognition threshold and the terminal threshold (suprathreshold) (Webb *et al.*, 2015). Odour intensities are expressed by scales based on descriptive categories or on estimates of magnitude. In all cases, the measurement is a sensory evaluation (Turk *et al.*, 1980). However, intensity is an individual difference characteristic defined in terms of the typical strength of an individual's responsiveness (Larsen & Diener, 1987) and relatively to the individual sensitivity measurement there are few studies. In Webb *et al.* (2015), suprathreshold taste intensity concentrations were presented based on geometric mean, however they did not relate with the individual sensitivity. Geometric mean (GM) is often used to evaluate data covering several orders of magnitude, and sometimes for evaluating ratios and percentage changes. The geometric mean, unlike the arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) was calculated (Costa, 2018). In Peng *et al.* (2016) the area-under-curve (AUC) measure was used to serve as a quantifier of people's sensitivity for detecting stimulus. With AUC the sensitivity can be computed across all the possible suprathreshold and threshold intensities and the correspondent plot curve and area under the curve, as an effective measure of sensitivity, have been considered with meaningful interpretations. This curve plays a central role in diagnostic test evaluation, finding the optimal cut off values, and comparing two alternative diagnostic tasks when each task is performed on the same subject (adapting, for example, for comparisons between orthonasal and retronasal flavour perceptions) (Hajian-Tilaki, 2013). Besides these two sensitivity measures, in this study the slope of the intensity plot will be evaluated also as an individual sensitivity measure. Although no study refers the slope as a measure of individual sensitivity, Turk *et al.* (1980) used it to relate the magnitude of sensations with the intensity of the stimuli.

1.5 Objectives

The research performed in this thesis was the initial step of a global project aimed at understanding the consumer behaviour regarding wines with unusual sensory features like off-flavours or off-tastes. These wines include those directed to niche consumers that are willing to accept wines with sensory characteristics distinct from the flawless international commercial ones. The first overall objective was to fill the gap in the current literature about thresholds and its real representativity in wines matrixes by both smell pathways (orthonasal and retronasal), since most of the published values are measured in water/ethanol solutions and only by the orthonasal route. Another overall research objective was to characterize and understand the sensitivity and sensory response to the presence of off-flavours related with the above mentioned niche wines. In particular, the specific objectives of this study were:

(i) To evaluate sensory thresholds (detection and recognition) of different off-flavours, for both orthonasal and retronasal pathways in wine;

(ii) To evaluate different measures of individual sensitivity to flavours in wine;

(iii) To understand the relation between suprathreshold flavour sensitivity and sensory thresholds.

2.1 Panel selection

The tasting sessions involved a total of 15 students selected from the first year of the Master of Viticulture and Enology held at the Instituto Superior de Agronomia in 2017/2018 (ISA, University of Lisbon). The tasting panel comprised 6 females and 9 males ranging in age from 23 to 36 years old (28 ± 4). None of the students had previously been trained in experiments on odour and taste sensitivity, however they reported that were aware of the fundamental concepts related to wine tasting. In parallel, another panel was used comprising 16 wine professionals between 30 and 65 years old (8 females and 8 males, 47±13), with more than five years of experience in wine tasting. The untrained panel will be mentioned as the student panel and the trained panel as the professional one. In both cases the number of tasters was higher than 9, which is regarded as the minimum necessary to obtain a sufficient number of data points for sample comparisons if each panelist evaluates only once, according to Dravnieks and Jarke (1980).

2.2 Sensory sessions

All the sessions were conducted in the Laboratory of Microbiology (ISA) and lasted 1 h each weekly session from March to May 2018. Each session took place in the morning and was divided in three phases, corresponding each phase to a different test that involved both orthonasal and retronasal evaluations. The first one corresponded to the intensity test, where the subjects evaluated different suprathreshold concentrations of off-flavours. The second and third phases corresponded to the detection and recognition threshold tests, respectively. Tasters were provided each weekly session with a different off-flavour to evaluate.

For all tastings, the samples were presented to the panel coded with three-digit random numbers and without further information. Sample volume was 25 mL of wine served at room temperature (23 ± 2 ºC) in tasting glasses according the requirements of ISO 3591:1997 standard and covered with a petri dish for 1 h before tasting. Tasters were given unsalted crackers, a spitter and demineralized water to cleanse the palate before the tests and between the samples. A break was enforced between each odorant concentration and between each test to minimise sensory saturation. Adapting some of the minimum requirements from ISO 8589:2010 standard, the samples were prepared in a near room separated from the tasting area and were evaluated on the benches of a well-ventilated classroom.

2.3 Sample preparation

The wines were produced in the ISA experimental winery following conventional winemaking techniques. White wine produced in the year 2016 was made from Macabeu variety while a blend of Cabernet Sauvignon and Syrah from the year 2017 was used in the red wine. The physical-chemical analysis of both wines is shown in Table 2.1. and was performed by Laboratory Ferreira Lapa, in Instituto Superior de Agronomia. Previous sensorial tasting by the laboratory trained staff did not reveal the presence of off-flavours in the base wines. However, analysing the results in Table 2.2. from ETSIAAB in Universidad Politécnica de Madrid, in the wines there were effectively the presence of the molecules acetaldehyde, ethyl acetate and volatile phenols.

Table 2.1. Composition of the base wines used in the threshold and suprathreshold tests.

Wine	pH	Total acidity (g tartaric acid/L)	Reducing substances (g/L)	Alcoholic strength (%, v/v)	Volatile acidity (g acetic acid/L)	Free SO$_2$ (mg/L)	Total SO$_2$ (mg/L)
White	3.52	5.3	0.7	11.3	0.23	39	105
Red	3.52	8.0	1.9	14.6	0.43	26	80

Table 2.2. Quantification of the volatile compounds in the base wines - acetaldehyde, ethyl acetate, hexanol and volatile phenols, and respective correspondence to the suprathreshold added concentration.

Molecule	Added concentration (mg/L)				
	0	125	250	500	1000
Acetaldehyde (in white wine)	37	28	127	109	202
Ethyl acetate (in white wine)	19	165	216	523	855
	Added concentration (µg/L)				
	125	250	500	1000	2000
Hexanol (in white wine)	142	266	451	976	1679
Volatile phenols (4-EF) (in red wine)	624	842	1036	1760	3326

A total of six off-flavour compounds were tested (acetaldehyde, acetic acid, ethyl acetate, hexanol, hydrogen sulfide, volatile phenols (4-ethylphenol and 4-ethylguaiacol in 10:1) by spiking the basic white and red wines as described in Table 2.3. Five concentration steps were made with a constant 2-fold dilution factor throughout the range. The concentration series for each odorant was initially established with reference to the detection threshold range reported in the literature. All solutions were prepared approximately 24 hours before the sensory session and tested by three members of the laboratory staff to check the presence of the odour.

Table 2.3. Added concentrations of the off-flavour molecules used in the threshold and suprathreshold sensory tests.

Molecule	Solution´s information	Test	Concentration level				
			1st	2nd	3rd	4th	5th
Acetaldehyde in white wine (mg/L)	Fluka product CAS Number: 75-07-0	Suprathreshold	0	125	250	500	1000
		Threshold	15.625	31.25	62.5	125	250
Acetic acid in red wine (mg/L)	Acetic acid glacial; PanReac product code: 131008	Suprathreshold	250	500	1000	2000	4000
		Threshold	125	250	500	1000	2000
Ethyl acetate in white wine (mg/L)	BDH chemicals product code: 28311	Suprathreshold	0	125	250	500	1000
		Threshold	15.625	31.25	62.5	125	250
Hexanol in white wine (µg/L)	1-hexanol; Sigma-aldrich product CAS Number: 111-27-3	Suprathreshold	0	500	1000	2000	4000
		Threshold	62.5	125	250	500	1000
Hydrogen sulfide in red wine (µg/L)	Sigma-aldrich product code: 742546	Suprathreshold	125	250	500	1000	2000
		Threshold	62.5	125	250	500	1000
4-ethylphenol (4-EF); 4-ethylguaiacol (4-EG) (10:1) in red wine (µg/L)	4-EF and 4-EG; Sigma-aldrich products CAS Number: 123-07-9 and 2785-89-9, respectively	Suprathreshold	125	250	500	1000	2000
		Threshold	31.25	62.5	125	250	500

2.4 Sensory methodology for ortho- and retronasal perceptions

2.4.1 Suprathreshold flavour intensity test

The determination of flavour suprathreshold intensities was performed using 5 glasses with different concentrations in random order across tasters, as illustrated in Figure 2.1. The orthonasal intensity was measured by placing the glass with a 45º angle without swirling and gently approaching the glass to the nose. When the odour started to be felt, the distance (cm) was measured with a ruler from the top of the glass to the nose. The further away from their nose the glass was, the greater the aromatic intensity of the wine. For the retronasal test, the tasters used a nose clip (Decathlon; made in Malaysia), put the wine in the mouth and chewed for 3 times and then spitted. The intensity value was drawn by a vertical mark in the Likert scale (Likert, 1932), used to scaling intensity responses, between "barely detectable" and "strongest imaginable" on the retronasal intensity sheet (Annex 1.).

In order to measure the individual sensitivity, the geometric mean (GM), the area-under-curve (AUC) and the regression slope were used. The first measure of individual sensitivity was obtained by calculating the geometric mean of the intensity scores

across all suprathreshold concentrations of the compounds (Webb *et al.*, 2015). The individual sensitivity was also expressed by calculating the area under the curve, derived from plotting the measured intensity values as a function of suprathreshold concentrations of the compounds. This plot also allowed to determine the slope of the regression line obtained by fitting with method of minimum squares. The AUC and slope results were obtained with Software R and the GM mean was calculated with Microsoft Excel.
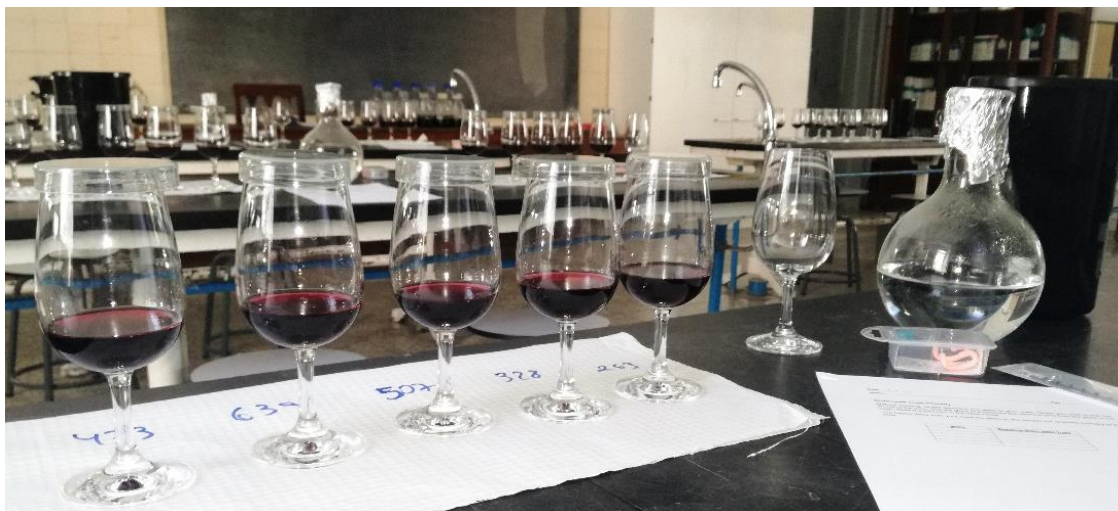
Figure 2.1. Suprathreshold intensity sample presentation scheme.

2.4.2 Detection and recognition thresholds test

The protocol ASTM E-679 (ASTM, 2004) used for determination of thresholds consists of an ascending forced-choice (3-AFC) triangular tests. Each triad contains one target sample (with the off-flavour) and two blank samples (with clean wine), as illustrated in Figure 2.2. For each concentration, tasters were asked first to identify the spiked sample by smelling the triad (orthonasal evaluation) and in second, by retronasal evaluation, to recognise the sample with the odd smell and give it a name or equivalent descriptor, completing the threshold sheet (Annex 2.). The test was run from the lowest concentration to the higher concentration, avoiding come back.

Individual best estimated thresholds (BETs) were calculated according the method ASTM E-679 (2004) and were determined as the geometric mean of the last incorrect response and the following detected and/or recognised concentration, when this was followed by at least 2 further correct responses (Chrysanthou *et al.*, 2016). Each series of five responses were tabulated and a (-) symbol was used for an incorrect response, (+) for a correct one and (+) painted of grey for a correct recognition of the off-flavour compound. Group BETs were calculated as the geometric mean of the average of

individual BETs. Calculation of individual and group thresholds were carried out using the Microsoft Excel program and statistical analyses were evaluated by ANOVA at $p<0.05$ significance level.
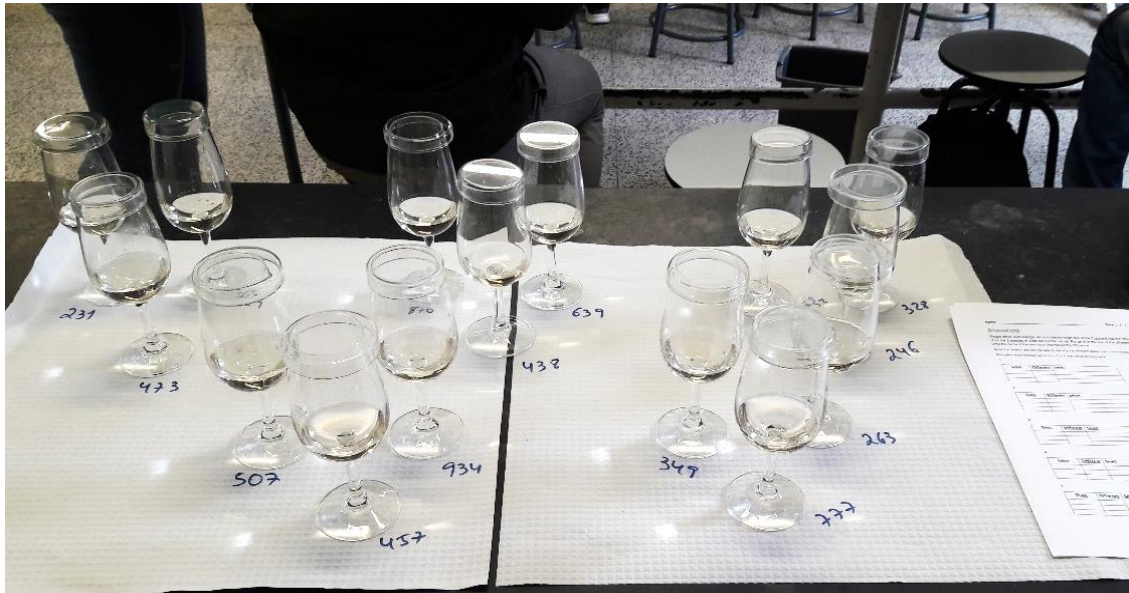
Figure 2.2. Threshold sample preparation scheme.

3. RESULTS AND DISCUSSION

3.1 Ortho- and retronasal detection and recognition thresholds

Professional panel

The results of the several trials to obtain detection and recognition thresholds for the professional panel are shown in Table 3.1. The first tests were done with acetaldehyde in white wine and were repeated 3 times in order to assess the increase in odour sensitivity with training. The lower thresholds were observed in the third experiment which was due not only to the increased taster sensitivity but also to the reduction in the lower range of tested concentrations. In fact, several tasters were able to detect the molecule in the lower concentrations of the first trials and by lowering further the concentration range it was possible to better ascertain their sensitivity (see Annex 3.). The same behaviour was also observed with ethyl acetate in white wines and volatile phenols in red wines (see Annex 4. and Annex 5., respectively). Interestingly, the retronasal route yielded lower nominal results but with no significant difference from the orthonasal pathways. Taking in consideration individual BET's, some tasters showed increased sensitivity and ability to identify the flavour by the retronasal pathway (see Annex 3., Annex 4. and Annex 5.). The acetaldehyde thresholds were higher in red than in white wine after training but, as illustrated in Table 3.2, significant differences regarding the training ($p<0.05$) were only observed on detection threshold values. Regarding the matrix effect, no evident differences were observed between the thresholds evaluated in white and in red wine ($p<0.05$).

Table 3.1. Detection and recognition BET thresholds values for orthonasal and retronasal (professional panel).

Base Wine	Molecule	Detection threshold				p-value	Recognition threshold				p-value
		Orthonasal		Retronasal			Orthonasal		Retronasal		
		mean	sd[a]	Mean	sd[a]		mean	sd[a]	mean	sd[a]	
White Wine	Acetaldehyde (mg/L)	50	1	47	1	0.56	77	2	61	3	0.65
		15	2	14	1	0.74	27	3	21	4	0.72
		9	3	7	3	0.61	21	3	16	6	0.83
	Ethyl acetate (mg/L)	54	1	50	1	0.63	87	3	65	3	0.57
		26	2	32	2	0.43	26	2	49	4	0.12
Red Wine	Acetaldehyde (mg/L)	24	3	18	2	0.44	26	3	39	3	0.47
	Volatile phenols (4-EF, 4-EG) (µg/L)	41	2	33	2	0.53	68	3	42	3	0.40
		31	2	33	2	0.78	49	2	40	2	0.44

[a] Standard deviation of $\log_{10}$ BET.

The standard deviation of log BET may be interpreted as a measure of the variability between tasters (Table 3.1). Values were higher for the recognition than for the detection thresholds indicating that some individuals would require more training to correctly identify certain off-flavours.

Table 3.2. Significance of the difference (p-values) between (a) the first and third sensory sessions for acetaldehyde (in white wine) and between (b) threshold determination of acetaldehyde in white and in red wine (professional panel).

Molecule		Detection		Recognition	
		Orthonasal	Retronasal	Orthonasal	Retronasal
Acetaldehyde	(a)	0.0002	0.0001	0.054	0.12
	(b)	0.09	0.1	0.9	0.5

Student panel

The threshold results of the student panel were obtained in only one session per molecule to assess the sensitivity without previous training (Table 3.3, and more detailed in Annex 6. to Annex 11.). The threshold values were equal by both routes (p>0.05) and nominal values were mostly higher for the retronasal route, contrarily to the professional panel. Interestingly, thresholds for the same molecules were similar between tasting panels, indicating that sensitivities were practically not affected by training.

Table 3.3. Detection and recognition BET thresholds for orthonasal and retronasal (student panel).

Base Wine	Molecule	Detection threshold				p-value	Recognition threshold				p-value
		Orthonasal		Retronasal			Orthonasal		Retronasal		
		mean	sd[a]	mean	sd[a]		mean	sd[a]	mean	sd[a]	
White Wine	Acetaldehyde (mg/L)	12	1	14	1	0.19	46	5	70	5	0.53
	Ethyl acetate (mg/L)	14	2	17	2	0.39	27	3	29	3	0.90
	Hexanol (µg/L)	72	3	107	3	0.37	402	4	370	4	0.83
Red Wine	Acetic acid (mg/L)	302	3	481	3	0.32	658	4	1035	3	0.41
	Hydrogen sulfide (µg/L)	83	2	73	3	0.87	286	5	461	4	0.58
	Volatile phenols (4-EF, 4-EG) (µg/L)	29	2	31	2	0.77	35	2	31	2	0.78

[a] Standard deviation of $\log_{10}$ BET.

Beyond the medium used in the threshold determination, sensory threshold values are a function of sample preparation, individual sensitivities and, of course, they are estimate for particular wines that highly influence the odour threshold values. The threshold values for acetaldehyde and acetic acid estimated in the present study were higher than the values reported by Guth (1997) (500 μg/L and 200 mg/L, respectively). On the contrary, for the molecule hexanol, the detection and recognition threshold values obtained were much lower than the reported by Guth (1997) (8000 μg/L). The difference between both results can be explained by the methodology used by Guth (1997): (i) the sessions involved only six trained judges; (ii) the compounds were dissolved in ethanol and added to a mixture of water/ethanol, (iii) the sensory evaluation (triangular tests) was performed only retronasally and (iv) the samples were presented in order of decreasing concentrations.

The detection and recognition threshold values for ethyl acetate obtained in white wine were close to the values determined in air by Hellman and Small (1973) (6.4 mg/L and 13.3 m/L, respectively). These values were obtained using a force-choice (3-AFC) method with ascending series and a panel of five trained tasters. The threshold values for hydrogen sulfide measured in this study were more than hundred times higher than the detection threshold values in air suggested by Young & Adams (1966) (5.7 – 7.9 μg/L). The differences between both values can be explained by the procedure used: (i) large size of the panel (N=81 tasters); (ii) presentation mode of the molecule was by a mask and (iii) the methodology was performed in trials of ascending series (without forced-choice). Analysing the results for volatile phenols in wine obtained in this study, the threshold values were lower than the reported by Csikor *et al*. (2018) also in wine (4-EP + 4-EG: 114.3 μg/L). The values were determined in a sample of 260 wines using a high-performance liquid chromatography with a fluorimetric detector and sensory sessions was performed by five trained judges.

3.2 Comparison of individual flavour thresholds

In order to understand the distribution of taste sensitivity among the student tasters, for each off-flavour molecule the average of the group log (BET) values of the panel was used as the cut-off value between classes of high and low sensitive tasters. Computing all responses for all molecules, the tasters were aggregated in 2 sensitivity classes - high (H) and low (L), as shown in Annex 12. The comparison among the behaviour of the tasters was done qualitatively by the number of times each one was considered as of high or of low sensitivity for all off-flavours (Table 3.4). The responses varied according to taster, molecule and mode of wine administration and evidenced the high

individual variability on the orthonasal and retronasal perceptions of the flavours. The cases where tasters were always within one class were rare, comprising taster 4 with low recognition thresholds, taster 8 with low sensitivity in the detection, and tasters 11 and 14 with low sensitivity on detection and high sensitivity on the recognition.

Table 3.4. Number of threshold determination where the tasters were of high (H) or low (L) sensitivity for the orthonasal and retronasal pathways (student panel).

Taster	Detection				Recognition			
	Orthonasal		Retronasal		Orthonasal		Retronasal	
	L	H	L	H	L	H	L	H
1	1	3	1	3	2	2	4	-
2	3	2	1	4	2	3	2	3
3	3	3	4	2	4	2	4	2
4	4	1	3	2	5	-	5	-
5	-	4	1	3	-	4	2	2
6	1	-	1	-	1	-	1	-
7	1	1	2	-	-	2	-	2
8	5	-	5	-	4	1	2	3
9	4	-	2	2	3	1	2	2
10	2	2	-	4	1	3	-	4
11	4	1	4	1	-	5	-	5
12	3	1	3	1	3	1	4	-
13	2	3	3	2	2	3	4	1
14	4	1	4	2	-	5	-	5
15	4	1	3	2	2	3	2	3
Total	41	23	37	28	29	35	32	32

3.3 Evaluation of the sensitivity to suprathreshold concentrations

The individual sensitivity to off-flavour suprathreshold concentrations of the student tasting panel was measured for all 6 molecules regarding the orthonasal and retronasal pathways. Results were highly variable with patterns that can be summarised by 3 representative individuals as shown in Figure 3.1, concerning orthonasal and retronasal intensities of ethyl acetate. The absolute intensity values of orthonasal and retronasal pathways can only be qualitatively compared because of the different scale measures of intensity (ruler and Likert's scale, respectively). However, the slope of the regression curve can be compared between both pathways. There were tasters showing increasing response to the stimulus in both routes, while others were orthonasally insensitive and retronasally sensitive or fully insensitive. The sensitivity could be evaluated by the magnitude of the slope of the regression line. Sensitive individuals showed a positive slope while insensitive ones showed a null or negative slope.
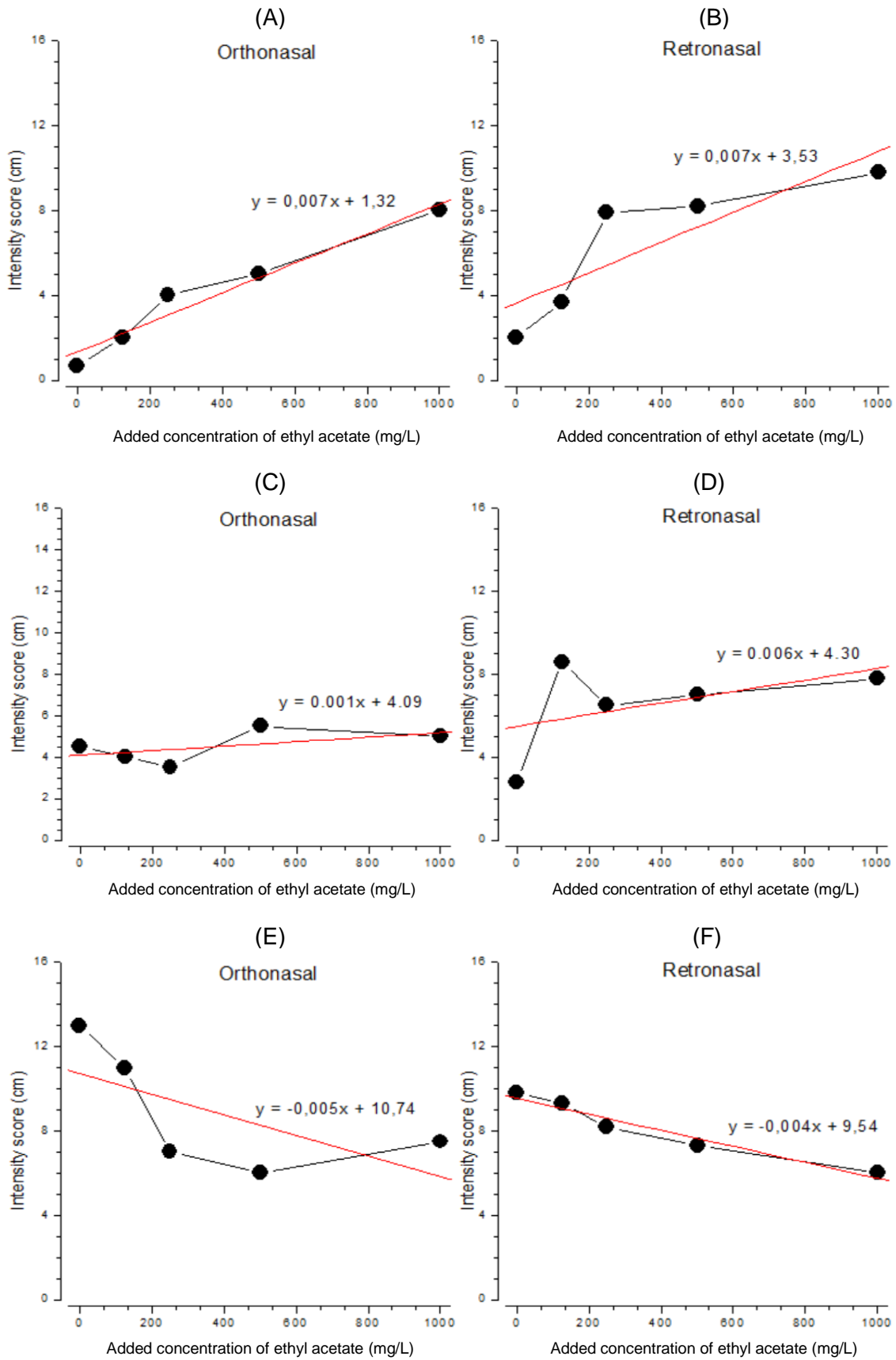
Figure 3.1. Orthonasal and retronasal flavour intensity responses of three classes of subjects (A and B; C and D; E and F) for ethyl acetate (student panel).

Taking in consideration the number of individuals that showed negative slopes of the regression curves which makes little physiological sense and could be ascribed to the individual ability to use the methodology, other measures of sensitivity were used. The GM and AUC have already been used in other works related with taste responsiveness (Webb *et al.*, 2015, Peng *et al.*, 2016, respectively) and it would be interesting to check if they could be used in flavour tests. The correlations between each pair of measures taking as example the molecule ethyl acetate are shown in Figure 3.2. and the correlations factors for all molecules are shown in Table 3.5. The best correlations were obtained between GM and AUC which was expected given the mathematical similarity of the determinations, although it was low regarding the retronasal intensity of acetic acid. The correlations with the slope were poor showing that possibly this is not a reliable measure of individual flavour sensitivity. Probably, this is due to the high number of individuals that did not show an increasing response to concentration leading to null or negative slopes (see Table 3.6). For GM and AUC, both measures yield positive values being an illustrative measure of the responsiveness to the stimulus.

Table 3.5. Correlation values (r^2 values) for orthonasal and retronasal intensity measure for each molecule (student panel).

Molecule	Orthonasal			Retronasal		
	GM[a] vs AUC[b]	GM[a] vs Slope	AUC[b] vs Slope	GM[a] vs AUC[b]	GM[a] vs Slope	AUC[b] vs Slope
Acetaldehyde	0.98	0.04	0.21	0.75	-0.11	0.34
Acetic acid	0.95	0.21	0.50	0.40	-0.64	0.30
Ethyl acetate	0.95	-0.64	-0.46	0.82	-0.04	0.37
Hexanol	0.99	-0.02	-0.002	0.93	0.24	0.57
Hydrogen sulfide	0.99	-0.16	-0.12	0.98	-0.19	-0.26
Volatile phenols (4-EF, 4-EG)	0.99	0.14	0.24	0.95	-0.07	0.15

[a] Geometric mean.
[b] Area-under-curve.
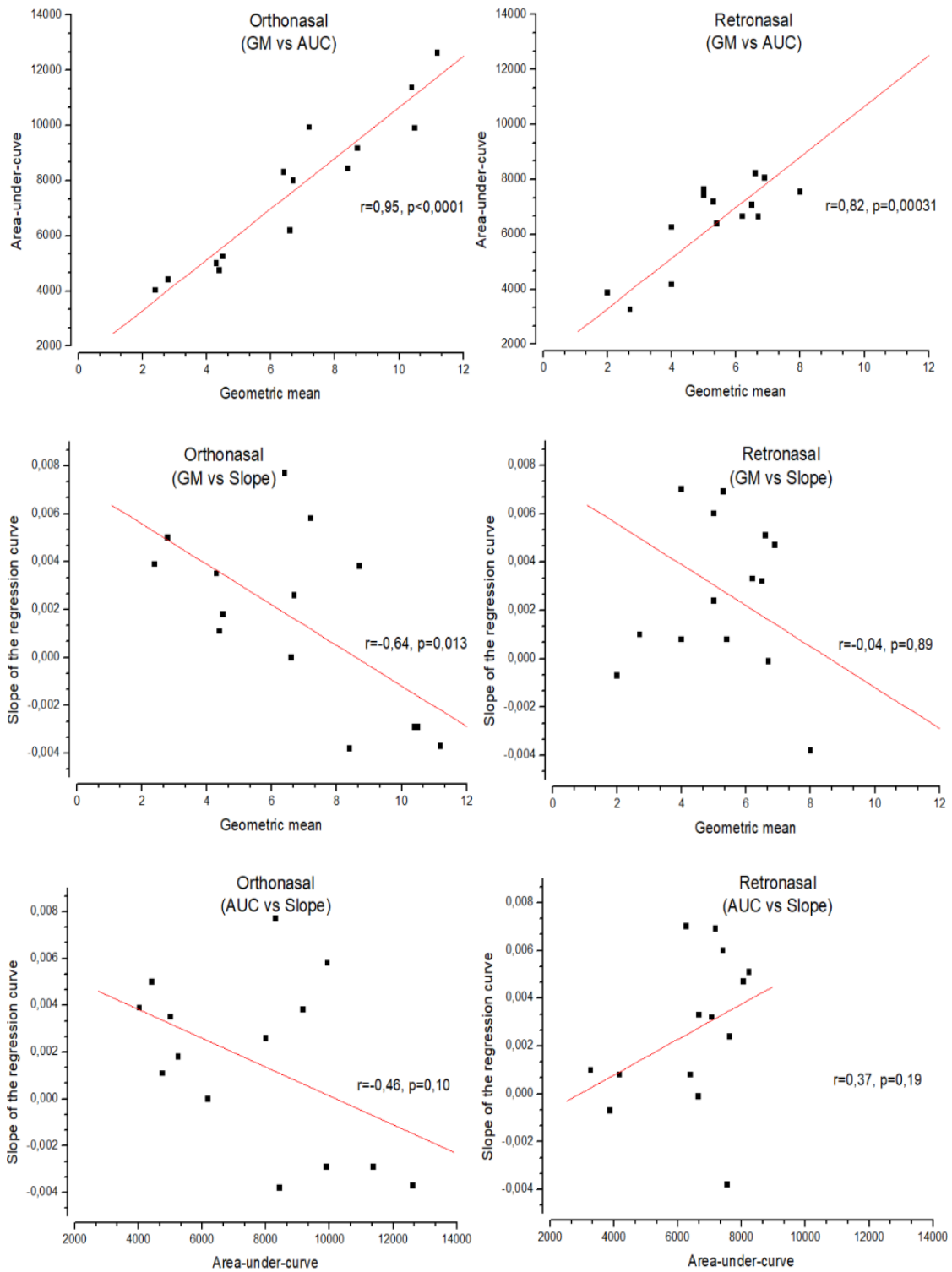
Figure 3.2. Correlation values for ethyl acetate, between each pair of measure, for orthonasal and retronasal intensity (GM, geometric mean; AUC, area under the curve; Slope, slope of the regression line of the intensity scores) (student panel).

The determination of these measures also raises the question of the methodology to detect sensitivity to off-flavours that probably may not be directly adapted from the responses to taste molecules. For instance, the flavour concentrations were in a random order, as advised for tastants (de Graaf *et al.*, 1994), but probably the nose "cleaning" is not so effective as the palate cleaning, even assuring that the effects are minimized by adequate olfactory cleansing between samples. Another fact that may have contributed to these results is related with the sensitivity of the tasters. As shown in Figure 3.1, for the same off-flavour compound, there were subjects with very different intensity responses, more variable when compared to taste responsiveness, mainly regarding the negative slope responses. Considering this individual variability, it becomes more complicated to find one single measure to quantify all the individual sensitivity. Probably, this is the reason why, when studying flavour sensitivity, research is almost restricted to sensory thresholds and do not mention suprathreshold determinations.

Another reason taken in consideration for the selection of the sensitivity measures was related with the absolute concentration of the off-flavour compounds, because if the area-under-curve was used as sensitivity measure, it would not be appropriate to compare different areas that corresponded to different absolute concentrations. Considering all results and having in mind the limitations of each determination to be a good measure of the suprathreshold sensitivity to the flavour stimulus, the option was to use the GM as an individual sensitivity measure.

3.4 Individual and group suprathreshold intensity evaluation

When all individual responses were merged an overall panel sensitivity may be obtained enabling to compare the stimulus elicited by each molecule in both pathways. Figure 3.3. shows the panel response to each one of the molecules and Table 3.6 lists the intensity measure scores of the student tasters. The acetaldehyde response shows a typical saturation curve where after an initial responsiveness increase there is a constancy in the perceived intensities. The other molecules elicited practically constant responses across all concentrations, with practically null slopes, showing that tasters were not highly sensitive to the increase in flavour concentration. The differences between the first and last tested concentrations are listed in Table 3.7 and reflect the low slope values across all flavour levels.
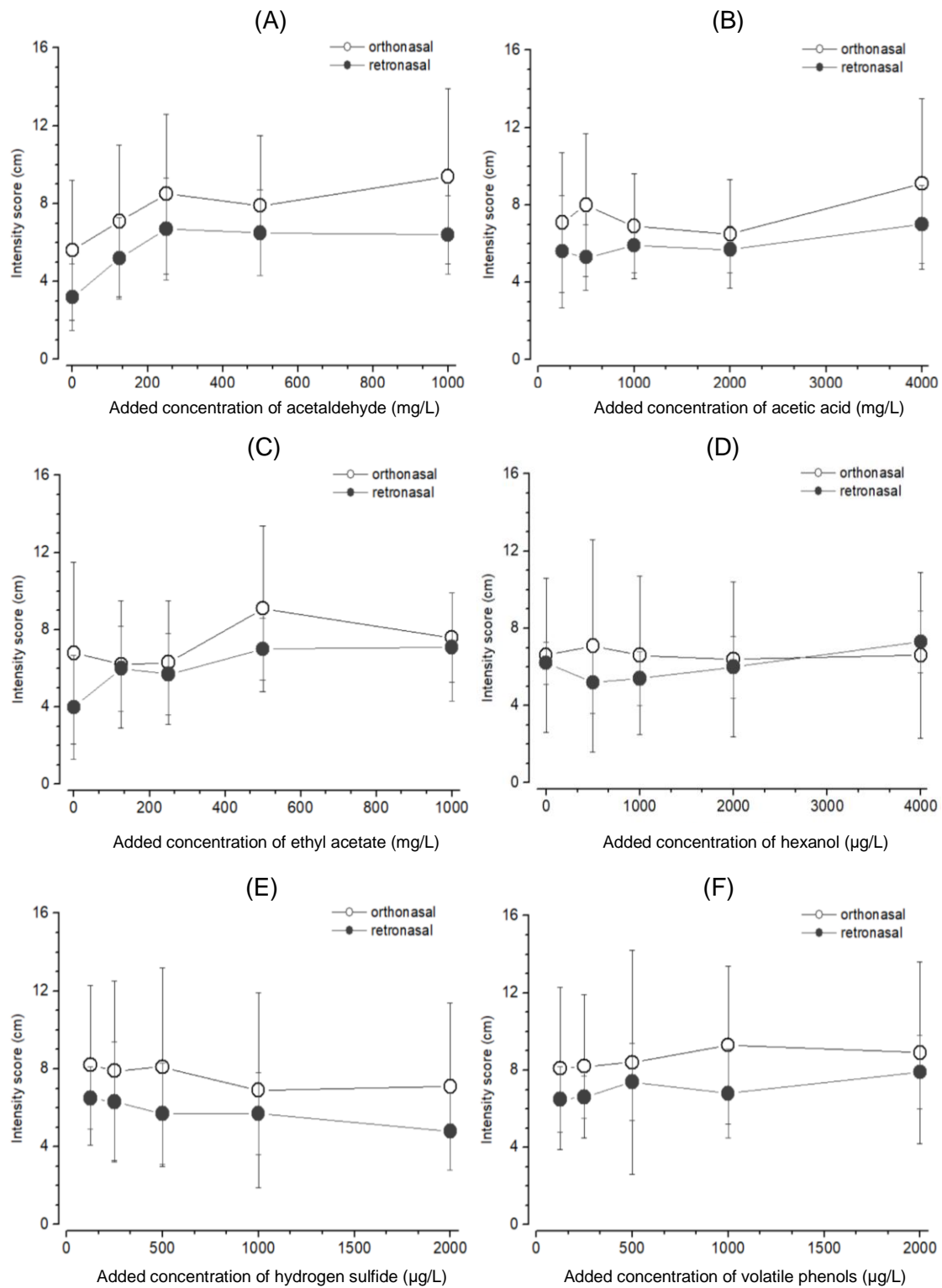
Figure 3.3. Intensity responses of the panel across all suprathreshold concentrations of acetaldehyde (A), acetic acid (B), ethyl acetate (C), hexanol (D), hydrogen sulfide (E) and volatile phenols (F) in wine solution, for orthonasal and retronasal pathways (student panel).

Table 3.6. Slope, area-under-curve and geometric mean average values for orthonasal and retronasal intensity measure of the student tasting panel.

Molecule	Orthonasal			Retronasal		
	Slope	Area-under-curve	Geometric mean	Slope	Area-under-curve	Geometric mean
Acetaldehyde	0.003	8144	7.2	0.002	6144	5.0
Acetic acid	0.0004	27910	7.2	0.0004	22663	5.5
Ethyl acetate	0.002	7694	6.7	0.003	6469	5.5
Hexanol	-0.0001	26350	6.5	0.0004	24500	5.9
Hydrogen sulfide	-0.001	13756	7.4	-0.001	10400	5.5
Volatile phenols (4-EF, 4-EG)	0.001	16619	8.4	0.001	13469	6.8

Table 3.7. Significance of the difference between the first and last concentration for orthonasal and retronasal intensity measure (student panel).

Molecule	p-value	
	Orthonasal	Retronasal
Acetaldehyde	0.02	0.0001
Acetic acid	0.25	0.19
Ethyl acetate	0.59	0.01
Hexanol	0.99	0.06
Hydrogen sulfide	0.60	0.09
Volatile phenols (4-EF, 4-EG)	0.75	0.16

The relatively high standard deviation of the mean values (see Figure 3.3.) indicated a large variability in smell and flavour perceptions by analysing all the molecules in the 5 suprathreshold concentrations. Comparisons between the mean values confirmed differences between orthonasal and retronasal, sustaining the theory of Rozin (1982) for the qualitative difference and the duality in the ortho- and retronasal olfactory senses. In order to understand the distribution of these responses, the geometric mean (GM) of each taster response across the five suprathreshold concentrations of each molecule provided a measure of individual sensitivity. For each molecule, in orthonasal and retronasal pathways, the GM average of the tasting panel was used as the cut-off value between classes of high and low sensitive tasters. Computing all responses for all molecules, the tasters were aggregated in 2 sensitivity classes - high (H) and low (L), as shown in Annex 13. Table 3.8. shows the results merging the classes of all

flavours. The outputs were: (i) low sensitivity on orthonasal and high sensitivity on retronasal for tasters number 2, 4, 12; (ii) high sensitivity on orthonasal and low sensitivity on retronasal for tasters number 7, 8; (iii) high sensitivity on ortho- and retronasal for tasters number 3, 11, 15; (iv) low sensitivity on ortho- and retronasal for tasters number 5, 6, 10, 13, 14. For tasters number 1 and 9, the attribution of a specific sensitivity category for retronasal was inconclusive because of the similarity between numbers in each H and L classes. By individuating the overall responses and considering the consistence of each taster in all molecules, only tasters number 2, 3, 5, 10, 11, 13, 15 were coherent in all their orthonasal response and/or in all their retronasal response, for all molecules (with some exceptions).

Concerning the differences between the chemical composition of the molecules and their aroma perception by each subject, there was a different individual behaviour for each molecule that affects the coherence of the tasting. When the results were analysed individually, not all the subjects were consistent in all their ortho- and retronasal responses, presenting different odour perceptions for each off-flavour.

Table 3.8. Number of suprathreshold determination where the tasters were of high (H) or low (L) sensitivity for the orthonasal and retronasal pathways (student panel).

Taster	Orthonasal		Retronasal	
	L	H	L	H
1	4	-	2	2
2	5	-	-	5
3	-	6	2	4
4	3	-	1	2
5	3	1	3	1
6	2	1	2	1
7	1	3	3	1
8	2	3	3	2
9	-	4	2	2
10	4	-	3	1
11	-	5	-	5
12	3	1	1	3
13	4	1	4	1
14	5	-	3	2
15	-	5	-	5
Total	36	30	29	37

3.5 Comparison between ortho- and retronasal intensity evaluation

The Figures 3.4 to 3.9 correspond each one to a different molecule and each one includes four plots (A, B, C, D). The panel was divided in two classes according their sensitivity. Plot A shows the intensity scores for each suprathreshold concentration on orthonasal for both low and high sensitivity tasters. Plot B shows the respective intensity scores for each suprathreshold concentration on retronasal route for the subjects within the sensitivity groups of Figure A. On orthonasal via, the different sensitivity between both groups was not evident on retronasal olfaction. Plot C illustrates the intensity scores for each suprathreshold concentration on retronasal smell for low and high sensitivity tasters. Plot D shows the intensity scores for each suprathreshold concentration on orthonasal route for the same subjects of Plot C. Analysing all the results for all molecules, low and high sensitivity tasters on retronasal smell had similar sensitivity and when compared on orthonasal route.
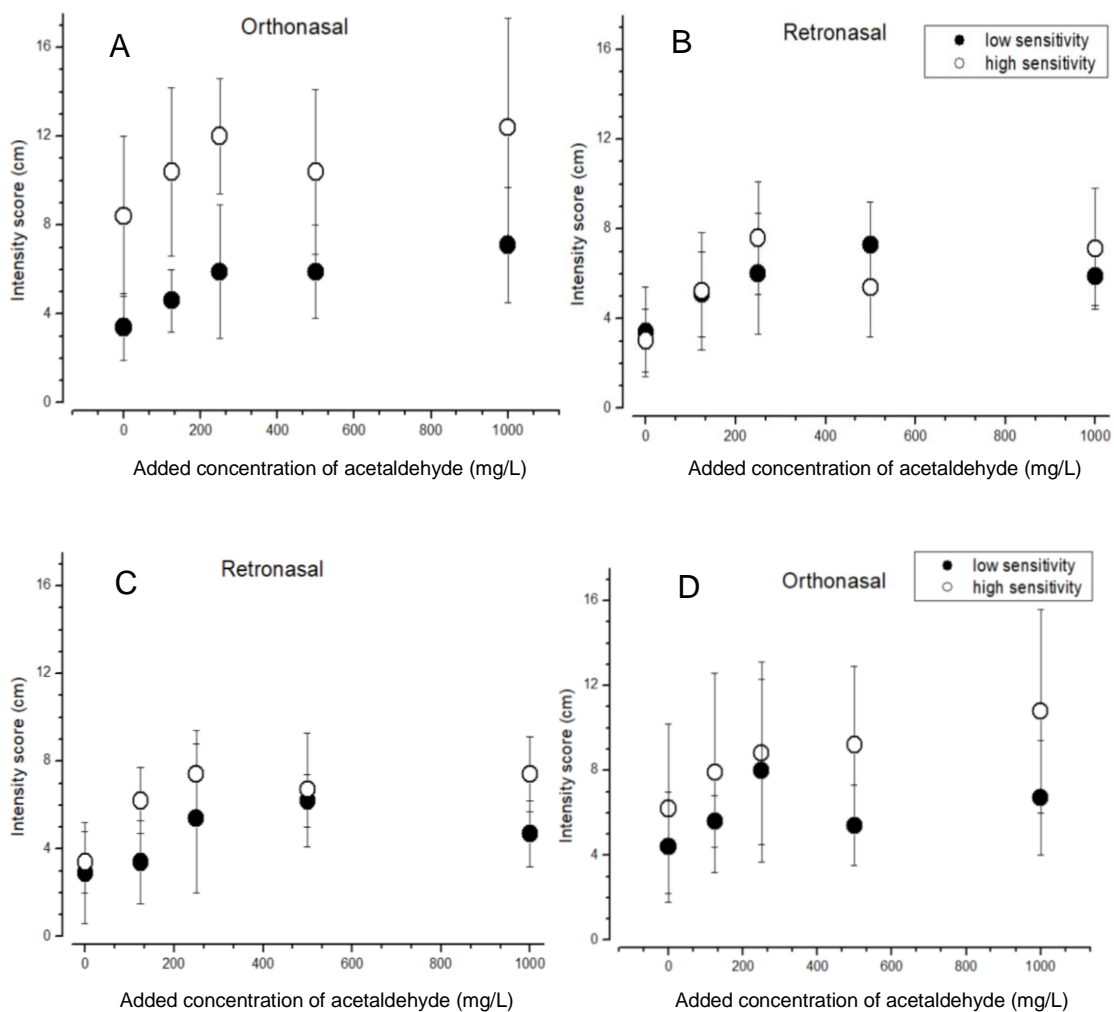
Figure 3.4. Intensity scores for each suprathreshold concentration of acetaldehyde on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.
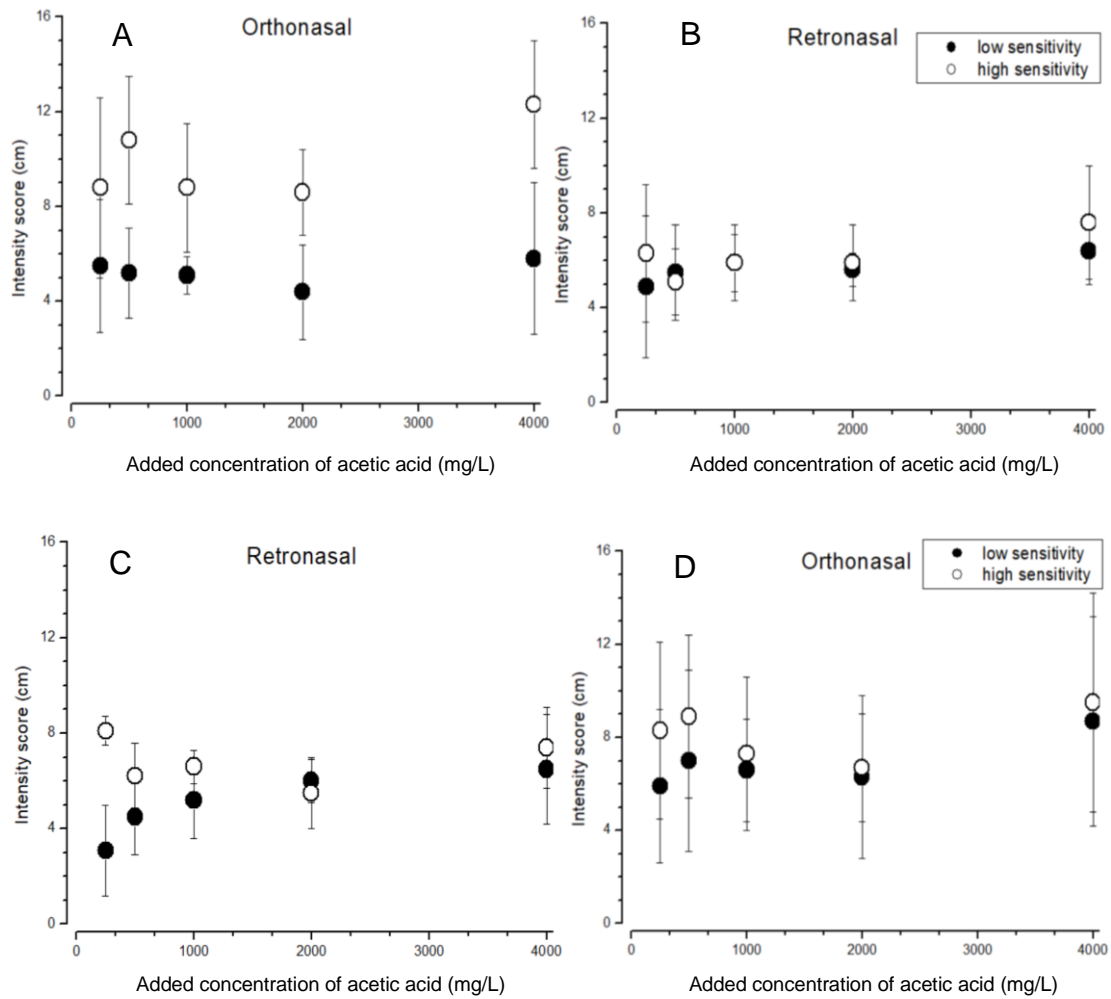
Figure 3.5. Intensity scores for each suprathreshold concentration of acetic acid on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.
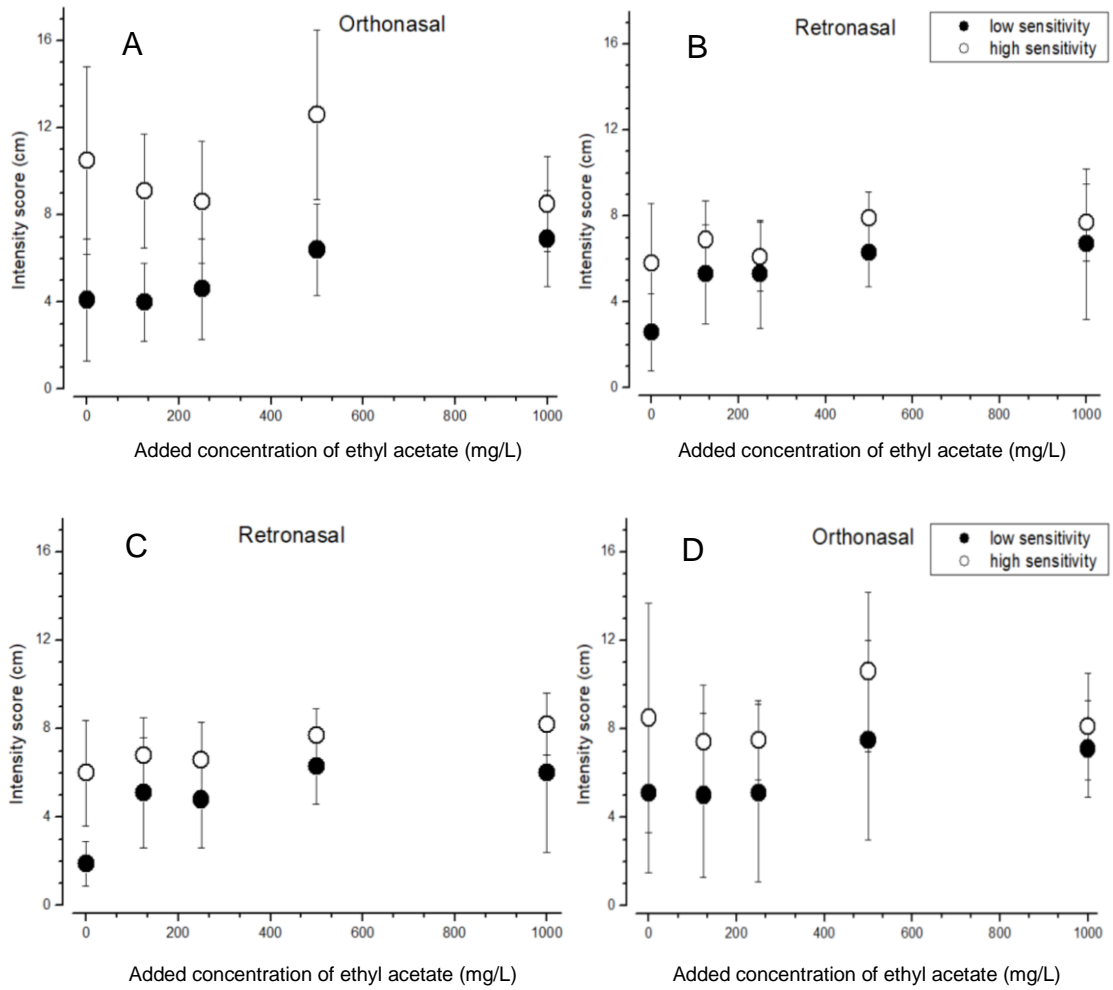
Figure 3.6. Intensity scores for each suprathreshold concentration of ethyl acetate on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.
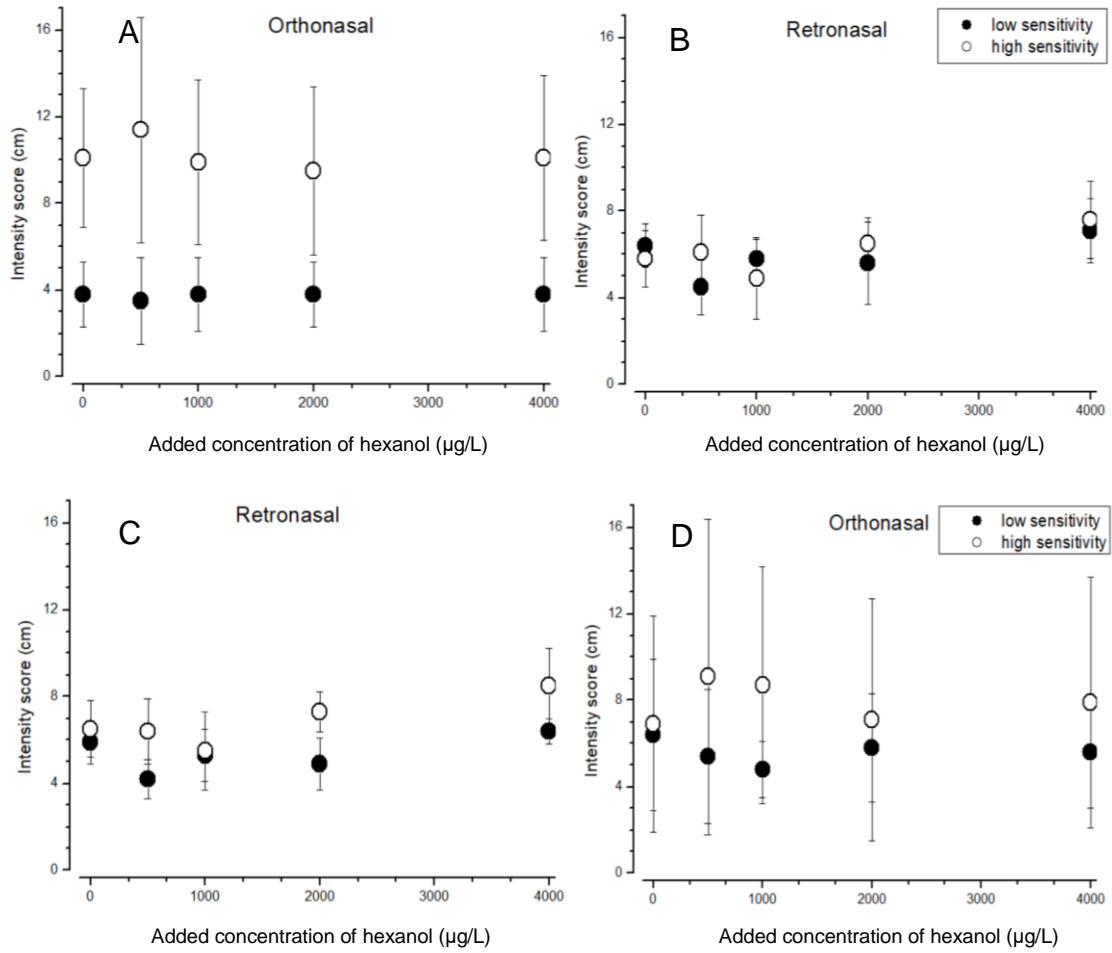
Figure 3.7. Intensity scores for each suprathreshold concentration hexanol on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.
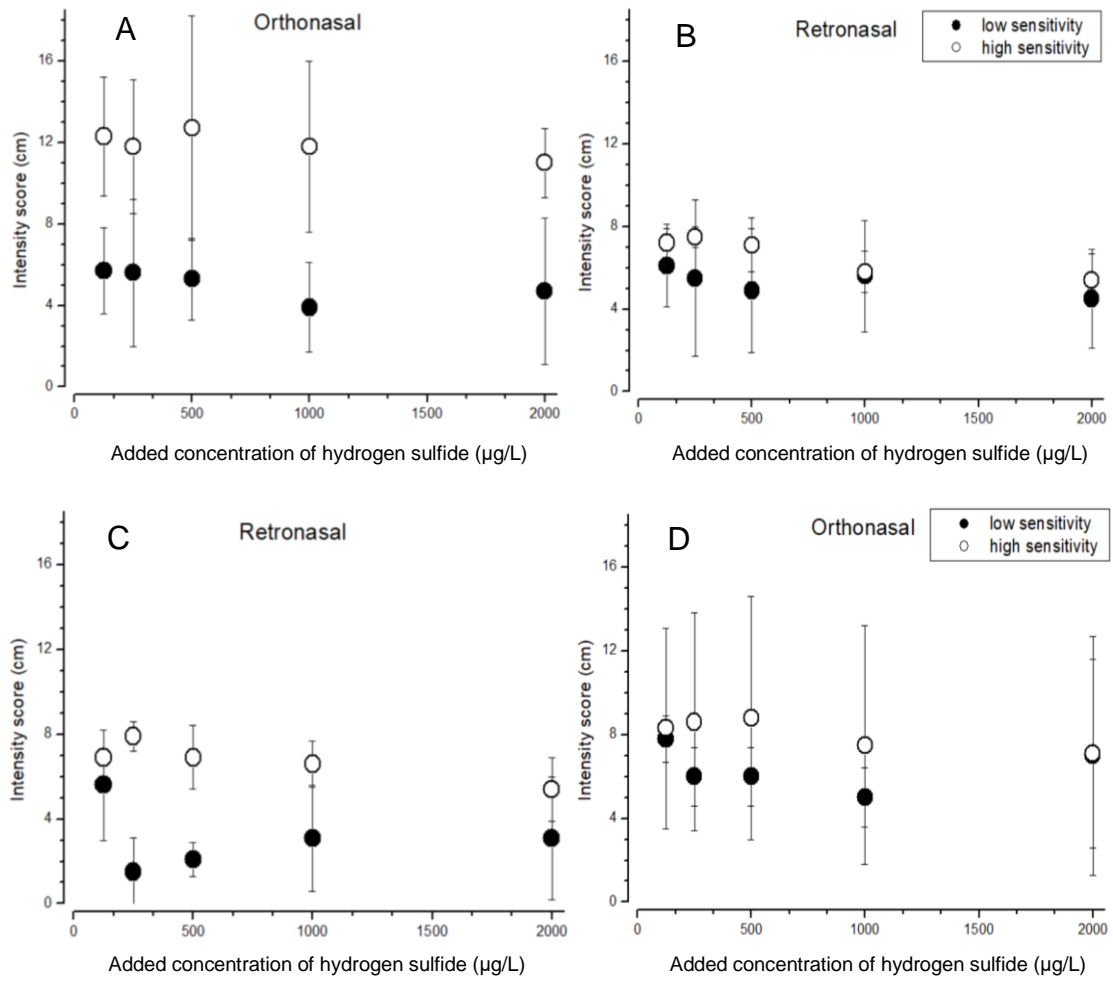
Figure 3.8. Intensity scores for each suprathreshold concentration of hydrogen sulfide on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.
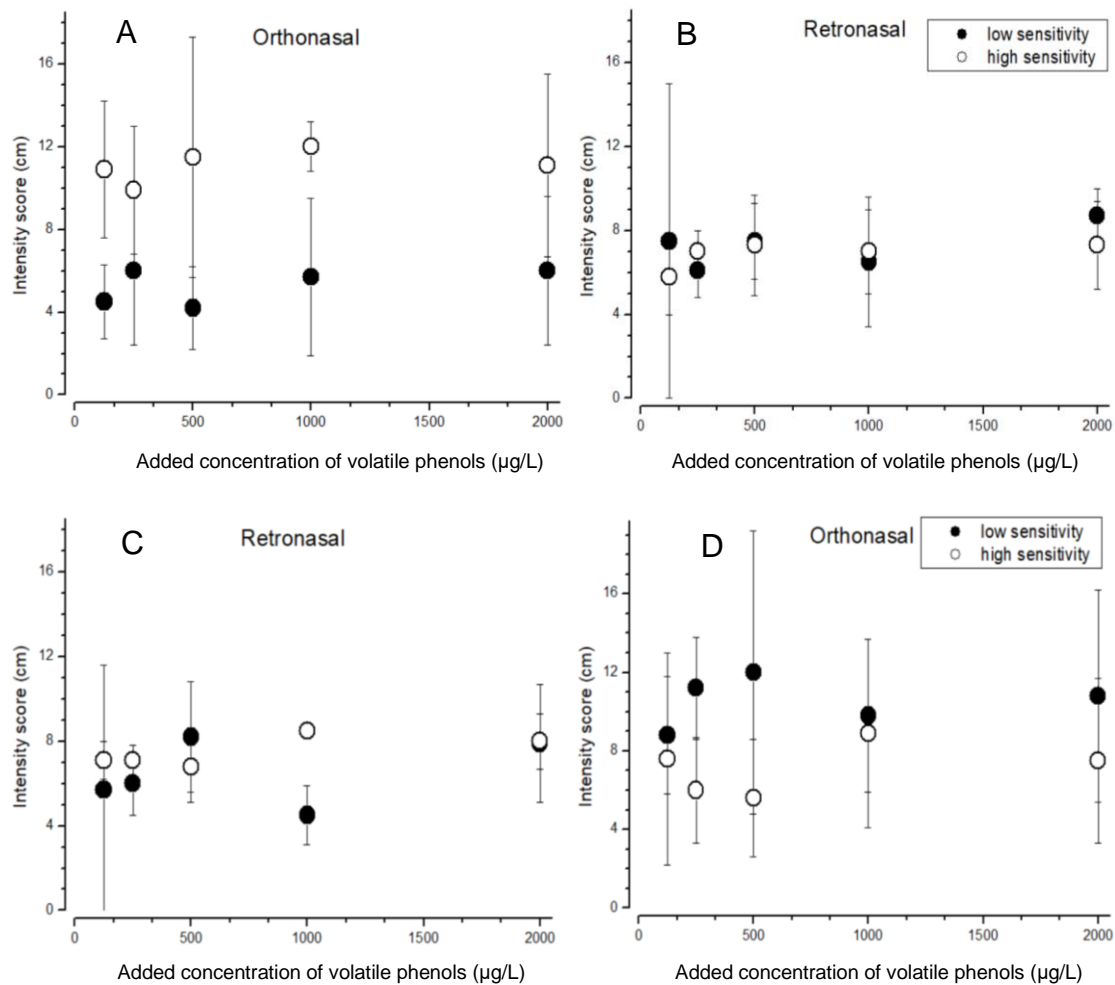
Figure 3.9. Intensity scores for each suprathreshold concentration of volatile phenols on orthonasal (A) and correspondent distribution on retronasal (B) and on retronasal (C) and correspondent distribution on orthonasal (D), for the same low and high sensitivity subjects.

Analysing the results for all molecules in Table 3.9, the mean and standard deviation values were always higher on orthonasal than on retronasal mode. The orthonasal mode presented also a higher variability (with higher standard deviation values) in the intensity response of the subjects, for suprathreshold concentrations. The values of the coefficient of variation (CV) illustrated in Table 3.10. corroborated this: on orthonasal the respective values were higher than on retronasal, meaning that the panel was more heterogeneous on orthonasal pathway. As most of the coefficient of variation values were higher than 25%, the panel comprised medium to high dispersion between the subjects (Romano *et al.*, 2005), evidencing their higher individual variability. The difference between the high and low sensitive untrained tasters for the orthonasal perception was significant for all molecules (evaluated by ANOVA at p=0.05 level of significance). On the contrary, for the retronasal pathway, no distinction of sensitivity was observed between the tasters for acetaldehyde, acetic acid and volatile phenols.

Table 3.9. Mean and standard deviation values (cm) for each molecule, on orthonasal and retronasal, for all type of sensitivity tasters (student panel).

Molecule	Orthonasal				Retronasal			
	All tasters	Highly sensitive tasters	Low sensitive tasters	p-value (for high and low)	All tasters	Highly sensitive tasters	Low sensitive tasters	p-value (for high and low)
	Mean ± sd[a]	Mean ± sd[a]	Mean ± sd[a]		Mean ± sd[a]	Mean ± sd[a]	Mean ± sd[a]	
Acetaldehyde	7.7 ± 4.0	10.7 ± 3.7	5.4 ± 2.1	0.001	5.6 ± 2.1	6.2 ± 1.8	4.5 ± 2.1	0.11
Acetic acid	7.5 ± 3.4	9.8 ± 2.7	5.2 ± 2.1	0.0003	5.9 ± 1.8	6.8 ± 1.2	5.1 ± 1.7	0.05
Ethyl acetate	7.2 ± 3.5	9.8 ± 3.2	5.2 ± 2.2	0.001	5.9 ± 2.3	7.1 ± 1.7	4.8 ± 2.2	0.03
Hexanol	6.7 ± 4.4	10.2 ± 4.0	3.7 ± 1.7	0.0004	6.0 ± 1.5	6.9 ± 1.4	5.3 ± 1.0	0.045
Hydrogen sulfide	7.6 ± 4.6	11.9 ± 3.5	5.0 ± 2.7	0.0003	5.8 ± 2.3	6.7 ± 1.2	3.0 ± 2.1	0.002
Volatile phenols (4-EP, 4-EG)	8.6 ± 4.5	11.1 ± 3.6	5.3 ± 3.0	0.00001	7.0 ± 1.8	7.5 ± 1.0	6.5 ± 2.8	0.21

[a] Standard deviation.

Table 3.10. Coefficient of variation values for each molecule and all type of sensitivity tasters, on orthonasal and retronasal pathways (student panel).

Molecule	Orthonasal			Retronasal		
	All tasters	Highly sensitive tasters	Low sensitive tasters	All tasters	Highly sensitive tasters	Low sensitive tasters
	CV[a]	CV[a]	CV[a]	CV[a]	CV[a]	CV[a]
Acetaldehyde	52%	35%	39%	38%	29%	47%
Acetic acid	45%	28%	40%	31%	18%	33%
Ethyl acetate	50%	33%	42%	39%	24%	46%
Hexanol	66%	39%	46%	25%	20%	19%
Hydrogen sulfide	61%	30%	54%	40%	18%	70%
Volatile phenols (4-EP, 4-EG)	52%	32%	57%	26%	13%	43%

[a] Coefficient of variation.

The same methodological approach was performed with the professional panel to compare with the behaviour of the untrained tasters. The analysis made for the trained panel is illustrated in Table 3.11. and Table 3.12. Supporting the results for the student panel, the orthonasal route also presented a higher variability (with higher standard deviation values) in the intensity response of the trained subjects. The coefficient of variation values illustrated in Table 3.13. corroborated this: on orthonasal the respective values were higher than on retronasal, meaning that the panel was more heterogeneous on orthonasal intensity measure. The difference between the high and low sensitive trained tasters was significant for all molecules, on orthonasal perception (Table 3.11, evaluated by ANOVA at p=0.05 level of significance). On the contrary, the retronasal pathway showed no distinction of sensitivity between the high and low sensitive individuals for acetaldehyde, ethyl acetate and volatile phenols (like the student panel).

The comparison of the intensity responses between the student and professional panel appears to yield similar results (p>0.05, evaluated by ANOVA), with exception for the orthonasal perception of hydrogen sulfide and volatile phenols (Table 3.13). Students showed a higher sensitivity to hydrogen sulphide and lower sensitivity to volatile phenols. Interestingly, Tempere *et al.* (2012) reported that the sensitivities of graduated experts to some wine odorant compounds were improved over several tastings. These differences between taster experience were not observed for the retronasal route (except for all tasters with hydrogen sulphide).

Table 3.11. Mean and standard deviation values (cm) for each molecule, on orthonasal and retronasal, for all type of sensitivity tasters (professional panel).

Molecule	Orthonasal				Retronasal			
	All tasters	Highly sensitive tasters	Low sensitive tasters	p-value (for high and low)	All tasters	Highly sensitive tasters	Low sensitive tasters	p-value (for high and low)
	Mean ± sd[a]	Mean ± sd[a]	Mean ± sd[a]		Mean ± sd[a]	Mean ± sd[a]	Mean ± sd[a]	
Acetaldehyde	8.5 ± 4.0	11.5 ± 3.4	6.0 ± 2.1	0.001	6.6 ± 2.0	6.6 ± 2.1	6.7 ± 2.1	0.93
Acetic acid	7.8 ± 4.0	10.9 ± 3.4	5.5 ± 2.1	0.00001	5.9 ± 1.9	6.8 ± 1.5	4.3 ± 1.5	0.02
Ethyl acetate	7.3 ± 3.5	9.6 ± 2.3	4.7 ± 2.3	0.002	5.4 ± 2.1	5.8 ± 1.9	4.9 ± 2.4	0.50
Hydrogen sulfide	6.0 ± 3.0	8.6 ± 2.0	4.0 ± 1.5	0.000001	4.4 ± 2.1	6.0 ± 1.5	2.9 ± 1.4	0.0001
Volatile phenols (4-EP, 4-EG)	9.4 ± 5.2	14.7 ± 2.3	6.5 ± 2.6	0.0000002	5.7 ± 2.3	5.6 ± 3.0	5.5 ± 1.6	0.90

[a] Standard deviation.

Table 3.12. Coefficient of variation values for each molecule and all type of sensitivity tasters, on orthonasal and retronasal (professional panel).

Molecule	Orthonasal			Retronasal		
	All tasters	Highly sensitive tasters	Low sensitive tasters	All tasters	Highly sensitive tasters	Low sensitive tasters
	CV[a]	CV[a]	CV[a]	CV[a]	CV[a]	CV[a]
Acetaldehyde	47%	30%	35%	30%	32%	32%
Acetic acid	51%	31%	38%	32%	22%	35%
Ethyl acetate	48%	24%	49%	39%	33%	49%
Hydrogen sulfide	50%	23%	38%	48%	25%	48%
Volatile phenols (4-EP, 4-EG)	55%	16%	40%	40%	54%	29%

[a] Coefficient of variation.

Table 3.13. p-values of the difference between the intensity scores of the student and professional tasters.

Molecule	Orthonasal			Retronasal		
	All tasters	Highly sensitive tasters	Low sensitive tasters	All tasters	Highly sensitive tasters	Low sensitive tasters
Acetaldehyde	0.44	0.42	0.58	0.33	0.76	0.05
Acetic acid	0.61	0.27	0.45	0.17	0.98	0.39
Ethyl acetate	0.75	0.77	0.64	0.64	0.28	0.95
Hydrogen sulfide	0.001	0.00002	0.025	0.004	0.17	0.82
Volatile phenols (4-EP, 4-EG)	0.04	0.0002	0.03	0.19	0.16	0.35

3.6 Comparison between suprathreshold and threshold sensitivities

It was hypothesized by Webb *et al.* (2015) that those who were able to detect and recognize a taste compound at a lower concentration (more sensitive) would consequently perceive a greater intensity when presented suprathreshold concentrations of the same stimulus. However, these authors did not found evidence to support their initial speculation. Notably, earlier research had also failed to find relationships between the taste thresholds and suprathresholds intensity ratings (Bartoshuk, 1978; Mattes, 1985; Mojet *et al.,* 2005). Interestingly, based on the findings of Mojet *et al.* (2005), for the young people, threshold sensitivity was unrelated to suprathreshold intensity for all tastants and in all experimental conditions. However, for the elderly, in a few cases a relationship was found between threshold sensitivity and suprathreshold intensity, but only when subjects wore a nose clip. In the present study, the positive relation between odour thresholds and suprathreshold intensities by analysing the tables in Annex 12. and Annex 13., respectively, was only observed for taster number 8 concerning acetaldehyde; for taster number 15 concerning acetic acid and hexanol; for taster number 3 concerning ethyl acetate; for taster number 9 concerning hydrogen sulfide; and for taster number 6 concerning volatile phenols. For these tasters, low threshold values lead to greater sensations of suprathreshold concentrations. Therefore, our results considering flavour molecules were in accordance to the speculations reported by Bartoshuk (1978), Mattes (1985) and Webb *et al.* (2015), including the exceptions described by Mojet *et al.* (2005).

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Detection and recognition threshold values in wines of six off-flavour compounds were obtained, filling the existing gap in the current literature. The values from this study were similar for ethyl acetate, lower for hexanol and volatile phenols and higher for acetaldehyde, acetic acid and hydrogen sulfide than the previously reported in literature, taking in consideration that were mediated by different wine styles and evaluation modes (orthonasal versus retronasal). In both threshold and suprathreshold intensity tests, some differences were found between orthonasal and retronasal evaluations and between trained and untrained tasters, suggesting that subjects perceive the molecules differently. However, there was a higher variability on the orthonasal evaluation comparing with the retronasal, suggesting that the retronasal route is less variable among tasters. This observation indicates that retronasal responses should not be dismissed on the evaluation of off-flavours in wines. In fact, the tendency to have higher threshold sensitivities by the retronasal via shows that orthonasal evaluations must be followed by wine ingestion.

This study presented a new insight into the individual flavour perception by revealing the differences in intensity measures between sensitivity groups and between different routes of flavour perception (orthonasal and retronasal). Furthermore, the study showed that sensitivity to one off-flavour compound does not indicate sensitivity to another off-flavour compound. There was a large individual sensitivity that affected the orthonasal and retronasal perception of the off-flavours in wine by the consumers. In order to access that individual sensitivity, the geometric mean was the measure that captured better the totality of the sense of aroma and taste.

This study had some limitations that may have influenced the lack of relationships between threshold and suprathreshold intensity tests and between orthonasal and retronasal evaluations. Many of the off-flavour molecules tested have strong aroma intensity what become more complicated for the tasters to clean the olfactory and palate between each test. This factor may have affected their sensitivity and the results reported in this study. In second, the population of the study was composed by few numbers of tasters. More research is needed to improve the methods of flavour intensity assessment in order to minimise what appeared to be the difficulty in performing the requested tasks.

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1) Tasting sheet for suprathreshold intensity test.

Date___/__/_____

Name_____**Age** _____

Orthonasal smell Intensity

Without swirling, slowly approach the glass to your nose. When you start to feel the aroma of the wine, stop the glass and measure the distance (cm) from the glass to the nose. Write the measure in the table below.

Wine	**Distance from nose (cm)**

<u>Retronasal Aroma Intensity</u>

Close the nose with your fingers. Put the wine in your mouth. Masticate for 3 times and spit. Remove the fingers and breath. Put a vertical mark in the scales.

Wine n. _____

Barely Detectable   Strongest Imaginable

|—————|—————|—————|—————|

Wine n. _____

Barely Detectable   Strongest Imaginable

|—————|—————|—————|—————|

Wine n. _____

Barely Detectable   Strongest Imaginable

|—————|—————|—————|—————|

Wine n._____

Barely Detectable   Strongest Imaginable

|—————|—————|—————|—————|
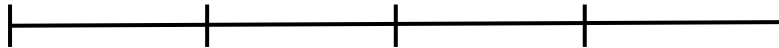
Wine n. _____

Barely Detectable   Strongest Imaginable

|—————|—————|—————|—————|

2) Tasting sheet for threshold test.

Name _____   **Date**___/__/_____

Orthonasal smell

Please smell each triangle set and identify which one of the 3 glasses has the different smell (2 of the 3 glasses in each set are the same). Put an X in the box of the different glass and write the name of the descriptor that makes the difference. Note that even if you are not able to identify the different glass, you must choose one. Complete each triangle set in from 1 to 5 and avoid coming back.

1

Glass	**Different**	**Smell**

2

Glass	**Different**	**Smell**

3

Glass	**Different**	**Smell**

4

Glass	**Different**	**Smell**

5

Glass	**Different**	**Smell**

Retronasal smell

Repeat the process but put the wine in the mouth, masticate and spit without swallowing.

1

Glass	Different	Smell

2

Glass	Different	Smell

3

Glass	Different	Smell

4

Glass	Different	Smell

5

Glass	Different	Smell

3) Individual and group best estimated thresholds for acetaldehyde, on orthonasal and retronasal pathways, over several sensory sessions performed by professional panel.

a. Individual and group best estimated orthonasal thresholds for acetaldehyde on first experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	44.2	1.65	88.4	1.95
2	+	+	+	+	+	44.2	1.65	44.2	1.65
3	-	+	+	+	+	88.4	1.95	88.4	1.95
4	+	+	+	+	+	44.2	1.65	176.8	2.25
5	+	+	+	+	+	44.2	1.65	44.2	1.65
6	-	+	+	+	+	88.4	1.95	707.1	2.85
7	+	+	+	+	+	44.2	1.65	44.2	1.65
8	+	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	44.2	1.65	44.2	1.65
10	+	+	+	+	+	44.2	1.65	88.4	1.95
proportion correct for detection	0.80	1.00	1.00	1.00	1.00	Mean log (BET)	1.70	Mean log (BET)	1.89
proportion correct for recognition	0.50	0.80	0.90	0.90	1.00	Antilog (BET)	50	Antilog (BET)	77

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for acetaldehyde on first experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	44.2	1.65	44.2	1.65
2	+	+	+	+	+	44.2	1.65	44.2	1.65
3	-	+	+	+	+	88.4	1.95	88.4	1.95
4	+	+	+	+	+	44.2	1.65	44.2	1.65
5	+	+	+	+	+	44.2	1.65	44.2	1.65
6	+	+	+	+	+	44.2	1.65	353.6	3.15
7	+	+	+	+	+	44.2	1.65	44.2	1.65
8	+	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	44.2	1.65	44.2	1.65
10	+	+	+	+	+	44.2	1.65	44.2	1.65
proportion correct for detection	0.90	1.00	1.00	1.00	1.00	Mean log (BET)	1.67	Mean log (BET)	1.79
proportion correct for recognition	0.80	0.90	0.90	1.00	1.00	Antilog (BET)	47	Antilog (BET)	61

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

c. Individual and group best estimated orthonasal thresholds for acetaldehyde on second experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	11.0	1.04	44.2	1.65
2	+	+	+	+	+	11.0	1.04	11.0	1.04
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	+	-	+	+	+	44.2	1.65	44.2	1.65
5	+	+	+	+	+	11.0	1.04	11	1.04
6	+	+	+	+	+	11.0	1.04	353.6	2.55
7	+	+	-	+	+	11.0	1.04	88.4	1.95
8	-	+	+	+	+	22.1	1.34	22.1	1.34
9	+	+	+	+	+	11.0	1.04	11.0	1.04
10	+	-	+	+	+	44.2	1.65	44.2	1.65
proportion correct for detection	0.90	0.80	0.90	1.00	1.00	Mean log (BET)	1.17	Mean log (BET)	1.43
proportion correct for recognition	0.60	0.50	0.80	0.90	0.90	Antilog (BET)	15	Antilog (BET)	67

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

d. Individual and group best estimated retronasal thresholds for acetaldehyde on second experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	11.0	1.04	22.1	1.34
2	+	+	+	+	+	11.0	1.04	11.0	1.04
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	-	+	+	+	+	22.1	1.34	22.1	1.34
5	+	+	+	+	+	11.0	1.04	11.0	1.04
6	-	+	+	+	+	22.1	1.34	353.6	3.15
7	+	+	+	+	+	11.0	1.04	11.0	1.04
8	-	+	+	+	+	22.1	1.34	22.1	1.34
9	+	+	+	+	+	11.0	1.04	22.1	1.34
10	-	+	+	+	+	22.1	1.34	22.1	1.34
proportion correct for detection	0.60	1.00	1.00	1.00	1.00	Mean log (BET)	1.15	Mean log (BET)	1.32
proportion correct for recognition	0.40	0.90	0.90	0.90	0.90	Antilog (BET)	14	Antilog (BET)	21

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

e. Individual and group best estimated orthonasal thresholds for acetaldehyde on third experiment (professional panel, N=9).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	3.91	7.81	15.625	31.25	62.5	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	-	+	-	22.1	1.34	88.4	1.95
2	+	-	-	+	+	22.1	1.34	88.4	1.95
3	-	-	-	+	+	22.1	1.34	22.1	1.34
4	+	+	+	+	+	2.8	0.44	88.4	1.95
5	+	+	+	+	+	2.8	0.44	2.8	0.45
6	+	+	-	+	+	22.1	1.34	88.4	1.95
7	-	-	+	+	+	11.0	1.04	11.0	1.04
8	-	+	-	+	+	22.1	1.34	22.1	1.34
9	-	+	+	+	+	5.5	0.74	11.0	1.04
proportion correct for detection	0.56	0.67	0.44	1.00	0.89	Mean log (BET)	0.96	Mean log (BET)	1.32
proportion correct for recognition	0.22	0.33	0.33	0.67	0.56	Antilog (BET)	9	Antilog (BET)	21

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

f. Individual and group best estimated retronasal thresholds for acetaldehyde on third experiment (professional panel, N=9).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	3.91	7.81	15.625	31.25	62.5	BET[a]	log (BET)	BET[a]	log (BET)
1	-	-	-	+	+	22.1	1.34	22.1	1.34
2	-	-	-	+	+	22.1	1.34	22.1	1.34
3	-	+	+	-	+	5.5	0.74	5.5	0.74
4	+	+	+	+	+	2.8	0.44	88.4	1.95
5	+	+	+	+	+	2.8	0.44	11.0	1.04
6	-	+	-	+	-	22.1	1.34	88.4	3.15
7	-	-	+	+	+	11.0	1.04	11.0	1.04
8	-	+	-	+	+	22.1	1.34	22.1	1.34
9	+	+	+	+	+	2.8	0.44	2.8	0.45
proportion correct for detection	0.33	0.67	0.56	0.89	0.89	Mean log (BET)	0.84	Mean log (BET)	1.20
proportion correct for recognition	0.11	0.33	0.44	0.78	0.78	Antilog (BET)	7	Antilog (BET)	16

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

4) Individual and group best estimated thresholds for ethyl acetate, on orthonasal and retronasal pathways, over several sensory sessions performed by professional panel.

a. Individual and group best estimated orthonasal thresholds for ethyl acetate on first experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	44.2	1.65	88.4	1.95
2	+	+	+	+	+	44.2	1.65	44.2	1.65
3	-	+	+	+	+	88.4	1.95	88.4	1.95
4	-	+	+	+	+	88.4	1.95	176.8	2.25
5	+	+	+	+	+	44.2	1.65	44.2	1.65
6	-	+	+	+	+	88.4	1.95	1414.2	3.15
7	+	+	+	+	+	44.2	1.65	44.2	1.65
8	+	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	44.2	1.65	88.4	1.95
10	+	+	+	+	+	44.2	1.65	88.4	1.95
proportion correct for detection	0.70	1.00	1.00	1.00	1.00	Mean log (BET)	1.73	Mean log (BET)	1.94
proportion correct for recognition	0.40	0.80	0.90	0.90	0.90	Antilog (BET)	54	Antilog (BET)	87

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for ethyl acetate on first experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	44.2	1.65	88.4	1.95
2	+	+	+	+	+	44.2	1.65	44.2	1.65
3	+	+	+	+	+	44.2	1.65	44.2	1.65
4	-	+	+	+	+	88.4	1.95	88.4	1.95
5	+	+	+	+	+	44.2	1.65	44.2	1.65
6	-	+	+	+	+	88.4	1.95	176.8	3.15
7	+	+	+	+	+	44.2	1.65	44.2	1.65
8	+	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	44.2	1.65	44.2	1.65
10	+	+	+	+	+	44.2	1.65	44.2	1.65
proportion correct for detection	0.80	1.00	1.00	1.00	1.00	Mean log (BET)	1.70	Mean log (BET)	1.82
proportion correct for recognition	0.70	0.90	1.00	1.00	1.00	Antilog (BET)	50	Antilog (BET)	65

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

c. Individual and group best estimated orthonasal thresholds for ethyl acetate on second experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	+	+	+	22.1	1.34	22.1	1.34
2	+	+	+	+	+	11.0	1.04	11.0	1.04
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	-	-	-	+	+	88.4	1.95	88.4	1.95
5	-	-	+	+	+	44.2	1.65	44.2	1.65
6	-	-	+	+	+	44.2	1.65	44.2	1.65
7	+	-	+	+	+	44.2	1.65	44.2	1.65
8	-	+	+	+	+	22.1	1.34	22.1	1.34
9	-	+	+	+	+	22.1	1.34	22.1	1.34
10	-	+	+	+	+	22.1	1.34	22.1	1.34
proportion correct for detection	0.30	0.60	0.9.	1.00	1.00	Mean log (BET)	1.41	Mean log (BET)	1.41
proportion correct for recognition	0.30	0.60	0.90	1.00	1.00	Antilog (BET)	26	Antilog (BET)	26

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

d. Individual and group best estimated retronasal thresholds for ethyl acetate on second experiment (professional panel, N=10).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	+	+	+	22.1	1.34	44.2	1.65
2	+	+	+	+	+	11.0	1.04	44.2	1.65
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	+	-	-	+	+	88.4	1.95	88.4	1.95
5	+	-	+	+	+	44.2	1.65	44.2	1.65
6	-	-	-	+	+	88.4	1.95	88.4	3.15
7	-	+	+	+	+	22.1	1.34	22.1	1.34
8	-	-	-	+	+	88.4	1.95	88.4	1.95
9	-	+	+	+	+	22.1	1.34	22.1	1.34
10	-	+	-	+	+	88.4	1.95	88.4	1.95
proportion correct for detection	0.40	0.60	0.60	1.00	1.00	Mean log (BET)	1.51	Mean log (BET)	1.69
proportion correct for recognition	0.30	0.40	0.60	1.00	1.00	Antilog (BET)	32	Antilog (BET)	49

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

5) Individual and group best estimated thresholds for volatile phenols (4-ethylphenol; 4-ethylguaiacol), on orthonasal and retronasal pathways, over several sensory sessions performed by professional panel.

a. Individual and group best estimated orthonasal thresholds for volatile phenols on first experiment (professional panel, N=9).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
1	-	-	-	+	+	176.8	2.25	176.8	2.25
2	-	+	+	+	+	44.2	1.65	88.4	1.95
3	-	-	+	+	+	88.4	1.95	88.4	1.95
4	+	-	+	+	+	88.4	1.95	707.1	2.85
5	+	+	+	+	+	22.1	1.34	22.1	1.34
6	+	+	+	+	+	22.1	1.34	44.2	1.65
7	-	+	+	+	+	44.2	1.65	88.4	1.95
8	+	+	+	+	+	22.1	1.34	44.2	1.65
9	+	+	+	+	+	22.1	1.34	22.1	1.34
proportion correct for detection	0.56	0.67	0.89	1.00	1.00	Mean log (BET)	1.62	Mean log (BET)	1.83
proportion correct for recognition	0.22	0.44	0.78	0.89	0.89	Antilog (BET)	41	Antilog (BET)	68

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for volatile phenols on first experiment (professional panel, N=9).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
1	-	-	-	+	+	176.8	2.25	176.8	2.25
2	+	+	+	+	+	22.1	1.34	44.2	1.65
3	+	+	+	+	+	22.1	1.34	22.1	1.34
4	+	-	+	+	+	88.4	1.95	707.1	2.85
5	+	+	+	+	+	22.1	1.34	22.1	1.34
6	+	+	+	+	+	22.1	1.34	22.1	1.34
7	+	+	+	+	+	22.1	1.34	22.1	1.34
8	-	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	22.1	1.34	22.1	1.34
proportion correct for detection	0.78	0.78	0.89	1.00	1.00	Mean log (BET)	1.52	Mean log (BET)	1.62
proportion correct for recognition	0.56	0.78	0.78	0.89	0.89	Antilog (BET)	33	Antilog (BET)	42

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

c. Individual and group best estimated orthonasal thresholds for volatile phenols on second experiment (professional panel, N=11).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	22.1	1.34	22.1	1.34
2	+	+	+	+	+	22.1	1.34	22.1	1.34
3	+	-	+	+	+	88.4	1.95	88.4	1.95
4	-	+	+	+	+	44.2	1.65	44.2	1.65
5	+	+	+	+	+	22.1	1.34	44.2	1.65
6	+	-	+	+	+	22.1	1.34	353.6	2.55
7	+	+	+	+	+	22.1	1.34	22.1	1.34
8	-	+	+	+	+	44.2	1.65	176.8	2.25
9	+	+	+	+	+	22.1	1.34	44.2	1.65
10	-	+	+	+	+	44.2	1.65	44.2	1.65
11	-	+	+	+	+	44.2	1.65	44.2	1.65
proportion correct for detection	0.64	0.82	1.00	1.00	1.00	Mean log (BET)	1.50	Mean log (BET)	1.69
proportion correct for recognition	0.36	0.73	0.82	0.91	1.00	Antilog (BET)	31	Antilog (BET)	49

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

d. Individual and group best estimated retronasal thresholds for volatile phenols on second experiment (professional panel, N=11).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	22.1	1.34	22.1	1.34
2	-	-	+	+	+	88.4	1.95	88.4	1.95
3	+	-	+	+	+	88.4	1.95	88.4	1.95
4	-	+	+	+	+	44.2	1.65	44.2	1.65
5	+	+	+	+	+	22.1	1.34	22.1	1.34
6	+	+	+	+	+	22.1	1.34	22.1	1.34
7	+	+	+	+	+	22.1	1.34	22.1	1.34
8	-	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	22.1	1.34	44.2	1.65
10	+	+	+	+	+	22.1	1.34	88.4	1.95
11	-	+	+	+	+	44.2	1.65	44.2	1.65
proportion correct for detection	0.64	0.82	1.00	1.00	1.00	Mean log (BET)	1.52	Mean log (BET)	1.60
proportion correct for recognition	0.36	0.73	1.00	1.00	1.00	Antilog (BET)	33	Antilog (BET)	40

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

6) Individual and group best estimated thresholds for acetaldehyde, on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for acetaldehyde on first experiment (student panel, N=13).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	+	+	+	22.1	1.34	22.1	1.34
2	+	+	+	+	+	11.0	1.04	11.0	1.04
3	+	+	+	+	+	11.0	1.04	353.6	2.55
4	+	+	+	+	+	11.0	1.04	11.0	1.04
5	-	+	+	+	+	22.1	1.34	353.6	2.55
8	+	+	+	+	+	11.0	1.04	11.0	1.04
9	+	+	+	+	+	11.0	1.04	353.6	2.55
10	+	+	+	+	+	11.0	1.04	44.2	1.65
11	+	+	+	+	+	11.0	1.04	353.6	2.55
12	+	+	+	+	+	11.0	1.04	11.0	1.04
13	+	+	+	+	+	11.0	1.04	11.0	1.04
14	+	+	+	+	+	11.0	1.04	353.6	2.55
15	+	+	+	+	+	11.0	1.04	353.6	2.55
proportion correct for detection	0.85	1.00	1.00	1.00	1.00	Mean log (BET)	1.08	Mean log (BET)	1.66
proportion correct for recognition	0.38	0.69	0.46	0.46	0.31	Antilog (BET)	12	Antilog (BET)	46

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for acetaldehyde on first experiment (student panel, N=13).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	+	+	+	22.1	1.34	22.1	1.34
2	+	+	+	+	+	11.0	1.04	353.6	2.55
3	+	+	+	+	+	11.0	1.04	353.6	2.55
4	+	+	+	+	+	11.0	1.04	11.0	1.04
5	-	+	+	+	+	22.1	1.34	353.6	2.55
8	+	+	+	+	+	11.0	1.04	11.0	1.04
9	-	+	+	+	+	22.1	1.34	353.6	2.55
10	-	+	+	+	+	22.1	1.34	353.6	2.55
11	-	+	+	+	+	22.1	1.34	353.6	2.55
12	+	+	+	+	+	11.0	1.04	11.0	1.04
13	+	+	+	+	+	11.0	1.04	11.0	1.04
14	+	+	+	+	+	11.0	1.04	353.6	2.55
15	+	+	+	+	+	11.0	1.04	353.6	2.55
proportion correct for detection	0.62	1.00	1.00	1.00	1.00	Mean log (BET)	1.15	Mean log (BET)	1.84
proportion correct for recognition	0.31	0.39	0.54	0.39	0.23	Antilog (BET)	14	Antilog (BET)	70

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

7) Individual and group best estimated thresholds for ethyl acetate, on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for ethyl acetate on first experiment (student panel, N=13).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	11.0	1.04	22.1	1.34
2	-	+	+	+	+	22.1	1.34	22.1	1.34
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	-	+	+	+	+	22.1	1.34	22.1	1.34
5	+	-	-	+	+	88.4	1.95	88.4	1.95
8	+	+	+	+	+	11.0	1.04	11.0	1.04
9	+	+	+	+	+	11.0	1.04	11.0	1.04
10	+	+	+	+	+	11.0	1.04	88.4	1.95
11	+	+	+	+	+	11.0	1.04	353.6	2.55
12	+	+	+	+	+	11.0	1.04	11.0	1.04
13	+	+	+	+	+	11.0	1.04	11.0	1.04
14	+	+	+	+	+	11.0	1.04	88.4	1.95
15	+	+	+	+	+	11.0	1.04	88.4	1.95
proportion correct for detection	0.85	0.92	0.92	1.00	1.00	Mean log (BET)	1.14	Mean log (BET)	1.43
proportion correct for recognition	0.39	0.62	0.62	0.92	0.92	Antilog (BET)	14	Antilog (BET)	27

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for ethyl acetate on first experiment (student panel, N=13).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	15.625	31.25	62.5	125	250	BET[a]	log (BET)	BET[a]	log (BET)
1	+	+	+	+	+	11.0	1.04	11.0	1.04
2	-	+	+	+	+	22.1	1.34	22.1	1.34
3	+	+	+	+	+	11.0	1.04	11.0	1.04
4	-	+	+	+	+	22.1	1.34	22.1	1.34
5	-	+	+	+	+	22.1	1.34	88.4	1.95
8	+	+	+	+	+	11.0	1.04	88.4	1.95
9	+	+	+	+	+	11.0	1.04	11.0	1.04
10	+	-	-	+	+	88.4	1.95	88.4	1.95
11	+	+	+	+	+	11.0	1.04	44.2	1.65
12	+	+	+	+	+	11.0	1.04	11.0	1.04
13	+	+	+	+	+	11.0	1.04	11.0	1.04
14	+	-	+	+	+	44.2	1.65	88.4	1.95
15	-	+	+	+	+	22.1	1.34	353.6	2.55
proportion correct for detection	0.70	0.85	0.92	1.00	1.00	Mean log (BET)	1.22	Mean log (BET)	1.46
proportion correct for recognition	0.38	0.54	0.62	0.92	0.85	Antilog (BET)	17	Antilog (BET)	29

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

8) Individual and group best estimated thresholds for hexanol, on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for hexanol on first experiment (student panel, N=13).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	-	-	-	+	+	88.4	1.95	1414.2	3.15
2	+	+	+	+	+	44.2	1.65	1414.2	3.15
3	-	+	+	+	+	88.4	1.95	88.4	1.95
4	+	+	+	+	+	44.2	1.65	44.2	1.65
5	-	+	+	+	+	88.4	1.95	1414.2	3.15
10	-	-	-	-	-	1414.2	3.15	1414.2	3.15
11	+	+	+	+	+	44.2	1.65	1414.2	3.15
12	+	+	+	+	+	44.2	1.65	44.2	1.65
13	-	+	+	+	+	88.4	1.95	1414.2	3.15
14	+	+	+	+	+	44.2	1.65	707.1	2.85
15	+	+	+	+	+	44.2	1.65	353.6	2.55
proportion correct for detection	0.55	0.82	0.82	0.91	0.91	Mean log (BET)	1.86	Mean log (BET)	2.60
proportion correct for recognition	0.18	0.27	0.27	0.36	0.45	Antilog (BET)	72	Antilog (BET)	402

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for hexanol on first experiment (student panel, N=13).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	-	+	+	353.6	2.55	353.6	2.55
2	-	+	-	+	+	353.6	2.55	353.6	2.55
3	-	+	+	+	+	88.4	1.95	1414.2	3.15
4	+	+	+	+	+	44.2	1.65	44.2	1.65
5	-	-	-	+	+	353.6	2.55	353.6	2.55
10	-	-	-	-	-	1414.2	3.15	1414.2	3.15
11	+	+	+	+	+	44.2	1.65	1414.2	3.15
12	+	+	+	+	+	44.2	1.65	44.2	1.65
13	-	+	+	+	+	88.4	1.95	1414.2	3.15
14	+	+	+	+	+	44.2	1.65	707.1	2.85
15	+	+	+	+	+	44.2	1.65	353.6	2.55
proportion correct for detection	0.45	0.82	0.64	0.91	0.91	Mean log (BET)	2.03	Mean log (BET)	2.57
proportion correct for recognition	0.18	0.27	0.18	0.55	0.64	Antilog (BET)	107	Antilog (BET)	370

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

9) Individual and group best estimated thresholds for acetic acid, on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for acetic acid on first experiment (student panel, N=12).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	125	250	500	1000	2000	BET[a]	log (BET)	BET[a]	log (BET)
1	+	-	-	+	+	707.1	2.85	707.1	2.85
3	-	+	-	+	+	707.1	2.85	707.1	2.85
4	+	+	-	+	+	88.4	1.95	88.4	1.95
7	+	+	+	-	+	88.4	1.95	2828.4	3.45
8	-	+	+	-	+	176.8	2.25	1414.2	3.15
9	-	+	+	+	+	176.8	2.25	176.8	2.25
10	-	-	+	-	-	2828.4	3.45	2828.4	3.45
11	-	+	+	-	-	176.8	2.25	2828.4	3.45
12	-	-	-	+	+	707.1	2.85	707.1	2.85
13	-	-	-	+	+	707.1	2.85	707.1	2.85
14	+	-	-	+	+	707.1	2.85	2828.4	3.45
15	+	+	+	+	+	88.4	1.95	88.4	1.95
proportion correct for detection	0.42	0.58	0.50	0.67	0.83	Mean log (BET)	2.48	Mean log (BET)	2.82
proportion correct for recognition	0.25	0.33	0.17	0.50	0.58	Antilog (BET)	302	Antilog (BET)	658

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for acetic acid on first experiment (student panel, N=12).

Subject	Concentration (mg/L)					Detection threshold		Recognition threshold	
	125	250	500	1000	2000	BET[a]	log (BET)	BET[a]	log (BET)
1	-	+	-	+	+	707.1	2.85	707.1	2.85
3	-	+	-	+	+	707.1	2.85	707.1	2.85
4	-	-	-	+	+	707.1	2.85	707.1	2.85
7	+	+	+	-	+	88.4	1.95	2828.4	3.45
8	-	+	+	+	+	176.8	2.25	2828.4	3.45
9	-	-	+	-	-	2828.4	3.45	2828.4	3.45
10	-	-	+	-	+	2828.4	3.45	2828.4	3.45
11	-	-	+	+	+	353.5	2.55	1414.2	3.15
12	-	-	-	+	+	707.1	2.85	707.1	2.85
13	-	-	-	+	+	707.1	2.85	707.1	2.85
14	+	-	-	+	+	707.1	2.85	2828.4	3.45
15	+	+	+	+	+	88.4	1.95	88.4	1.95
proportion correct for detection	0.25	0.42	0.50	0.75	0.92	Mean log (BET)	2.68	Mean log (BET)	3.02
proportion correct for recognition	0.08	0.17	0.08	0.50	0.58	Antilog (BET)	481	Antilog (BET)	1035

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

10) Individual and group best estimated thresholds for hydrogen sulfide, on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for hydrogen sulfide on first experiment (student panel, N=8).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
2	-	+	+	+	+	88.4	1.95	1414.2	3.15
3	-	+	+	+	+	88.4	1.95	88.4	1.95
7	-	+	+	+	+	88.4	1.95	1414.2	3.15
8	+	+	+	+	+	44.2	1.65	44.2	1.65
9	+	+	+	+	+	44.2	1.65	44.2	1.65
11	-	+	-	-	+	707.1	2.85	1414.2	3.15
13	-	+	+	+	+	88.4	1.95	353.6	2.55
14	+	+	-	+	+	44.2	1.65	1414.2	3.15
proportion correct for detection	0.38	1.00	0.75	0.88	1.00	Mean log (BET)	1.92	Mean log (BET)	2.46
proportion correct for recognition	0.26	0.38	0.38	0.50	0.50	Antilog (BET)	83	Antilog (BET)	286

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated retronasal thresholds for hydrogen sulfide on first experiment (student panel, N=8).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	62.5	125	250	500	1000	BET[a]	log (BET)	BET[a]	log (BET)
2	-	+	-	-	-	1414.2	3.15	1414.2	3.15
3	-	+	+	+	+	88.4	1.95	88.4	1.95
7	+	+	+	+	+	44.2	1.65	1414.2	3.15
8	+	+	+	+	+	44.2	1.65	1414.2	3.15
9	+	+	+	-	+	44.2	1.65	44.2	1.65
11	+	+	-	-	+	44.2	1.65	1414.2	3.15
13	-	+	+	+	+	88.4	1.95	353.6	2.55
14	+	+	-	-	-	44.2	1.65	1414.2	3.15
proportion correct for detection	0.63	1.00	0.63	0.50	0.75	Mean log (BET)	1.86	Mean log (BET)	2.66
proportion correct for recognition	0.13	0.25	0.25	0.25	0.38	Antilog (BET)	73	Antilog (BET)	461

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

11) Individual and group best estimated thresholds for volatile phenols (4-ethylphenol; 4-ethylguaiacol), on orthonasal and retronasal pathways, performed by student panel.

a. Individual and group best estimated orthonasal thresholds for hydrogen sulfide on first experiment (student panel, N=7).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
2	+	+	+	+	+	22.1	1.34	88.4	1.95
3	+	+	+	+	+	22.1	1.34	22.1	1.34
4	+	+	+	+	+	22.1	1.34	22.1	1.34
5	-	+	+	+	+	44.2	1.65	44.2	1.65
6	+	+	+	+	+	22.1	1.34	22.1	1.34
8	+	+	+	+	+	22.1	1.34	22.1	1.34
17	+	-	+	+	-	88.4	1.95	88.4	1.95
proportion correct for detection	0.86	0.86	1.00	1.00	0.86	Mean log (BET)	1.46	Mean log (BET)	1.54
proportion correct for recognition	0.86	0.71	1.00	1.00	0.86	Antilog (BET)	29	Antilog (BET)	35

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

b. Individual and group best estimated orthonasal thresholds for hydrogen sulfide on first experiment (student panel, N=7).

Subject	Concentration (µg/L)					Detection threshold		Recognition threshold	
	31.25	62.5	125	250	500	BET[a]	log (BET)	BET[a]	log (BET)
2	+	-	+	+	+	88.4	1.95	88.4	1.95
3	+	+	+	+	+	22.1	1.34	22.1	1.34
4	+	+	+	+	+	22.1	1.34	22.1	1.34
5	+	+	+	+	+	22.1	1.34	22.1	1.34
6	+	+	+	+	+	22.1	1.34	22.1	1.34
8	+	+	+	+	+	22.1	1.34	22.1	1.34
17	+	-	+	+	-	88.4	1.95	88.4	1.95
proportion correct for detection	1.00	0.71	1.00	1.00	0.86	Mean log (BET)	1.49	Mean log (BET)	1.49
proportion correct for recognition	1.00	0.71	1.00	1.00	0.86	Antilog (BET)	31	Antilog (BET)	31

[a]BET, best estimated threshold; correct choice indicated by + and incorrect by -; highlighted grey cells indicate recognition of off-flavour.

12) **Taster characterization according sensitivity classes (H-high; L-low) for each molecule on orthonasal and retronasal pathways, regarding detection and recognition threshold results (student panel).**

Subject	Acetaldehyde Detection Ortho	Acetaldehyde Detection Retro	Acetaldehyde Recognition Ortho	Acetaldehyde Recognition Retro	Acetic acid Detection Ortho	Acetic acid Detection Retro	Acetic acid Recognition Ortho	Acetic acid Recognition Retro	Ethyl acetate Detection Ortho	Ethyl acetate Detection Retro	Ethyl acetate Recognition Ortho	Ethyl acetate Recognition Retro	Hexanol Detection Ortho	Hexanol Detection Retro	Hexanol Recognition Ortho	Hexanol Recognition Retro	Hydrogen sulfide Detection Ortho	Hydrogen sulfide Detection Retro	Hydrogen sulfide Recognition Ortho	Hydrogen sulfide Recognition Retro	Volatile phenols Detection Ortho	Volatile phenols Detection Retro	Volatile phenols Recognition Ortho	Volatile phenols Recognition Retro
1	L	L	H	H	L	L	L	H	H	H	H	H	L	L	L	H	-	-	-	-	-	-	-	-
2	H	H	H	L	-	-	-	-	L	L	H	H	H	L	L	H	L	L	L	L	H	L	L	L
3	H	H	L	L	L	L	L	H	H	H	H	H	L	H	H	L	L	L	H	H	H	H	H	H
4	H	H	H	H	H	L	H	H	L	L	H	H	H	H	H	H	-	-	-	-	H	H	H	H
5	L	L	L	L	-	-	-	-	L	L	L	L	L	L	L	H	-	-	-	-	L	H	L	H
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H
7	-	-	-	-	H	H	L	L	-	-	-	-	-	-	-	-	L	H	L	L	-	-	-	-
8	H	H	H	H	H	H	L	L	H	H	H	L	-	-	-	-	H	H	H	L	H	H	H	H
9	H	L	L	L	H	L	H	L	H	H	H	H	-	-	-	-	H	H	H	H	-	-	-	-
10	H	L	H	L	L	L	L	L	H	L	L	L	L	L	L	L	-	-	-	-	-	-	-	-
11	H	L	L	L	H	H	L	L	H	H	L	L	H	H	L	L	L	H	L	L	-	-	-	-
12	H	H	H	H	L	L	L	H	H	H	H	H	H	H	H	H	-	-	-	-	-	-	-	-
13	H	H	H	H	L	L	L	H	H	H	H	H	L	H	L	L	L	L	L	H	-	-	-	-
14	H	H	L	L	L	L	L	L	H	L	L	L	H	H	L	L	H	H	L	L	-	-	-	-
15	H	H	L	L	H	H	H	H	H	L	L	L	H	H	H	H	-	-	-	-	L	L	L	L

13) Taster characterization according sensitivity classes (H-high; L-low) for each molecule on orthonasal and retronasal pathways, regarding suprathreshold intensity concentration (student panel).

Taster	Acetaldehyde		Acetic acid		Ethyl acetate		Hexanol		Hydrogen sulfide		Volatile phenols	
	Ortho	Retro	Ortho	Retro	Ortho	Retro	Ortho	Retro	Ortho	Retro	Ortho	Retro
1	L	H	L	L	L	H	L	L	-	-	-	-
2	L	H	-	-	L	H	L	H	L	H	L	H
3	H	H	H	H	H	H	H	L	H	H	H	L
4	-	-	L	H	-	-	L	L	-	-	L	H
5	L	L	-	-	L	L	H	H	-	-	L	L
6	L	L	-	-	L	L	-	-	-	-	H	H
7	H	L	H	L	H	H	-	-	L	L	-	-
8	H	H	L	L	H	H	-	-	L	L	H	L
9	H	H	H	L	H	L	-	-	H	H	-	-
10	L	H	L	L	L	L	L	L	-	-	-	-
11	H	H	H	H	H	H	H	H	H	H	-	-
12	L	H	H	H	L	L	L	H	-	-	-	-
13	L	L	L	L	L	L	H	L	L	H	-	-
14	L	L	L	H	L	L	L	L	L	H	-	-
15	H	H	H	H	H	H	H	H	-	-	H	H