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Improved omission testing for understanding
the relative contribution of volatiles and
tastants to sweet and savoury flavours

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Abstract

This research project fully defines and evaluates a new approach in sensory omission testing, based on the same-different test (ASTM E2139-05 2011) and the Thurstonian measure d' . The applications of this new approach were investigated to fully characterise sweet and savoury flavour models and to investigate interactions between flavour compounds. Panels of naïve assessors conducted a series of omission tests using both a strawberry (9 volatiles) and a savoury (10 volatiles) flavour model.

Using the Thurstonian d' as a measure of the sensitivity of the discrimination test, results showed that the new approach using the same-different test was more sensitive compared to the more traditional approach using the triangle test: the d' values obtained using the same-different test were 1.2 to 3.5 times higher than the d' values obtained using the triangle test. It was hypothesised that the evaluation of three samples in the triangle test generated additional noise related to carry-over, sensory fatigue and memory effects. In particular, the triangle test requires that the three successive stimulus sensations are stored into memory until the discrimination test has been completed.

The same-different approach was then successfully applied to (i) determine the relative importance of individual volatiles in ortho- and retronasal flavours (ii) assess interactions between volatiles in mixtures, and (iii) investigate interactions between congruent tastes and aromas in flavours.

Results showed that cis-3-hexen-1-ol, 4-hydroxy-2,5-dimethyl-3-furanone and ethyl butanoate play a key role in the strawberry flavour, while sulfur compounds play a major role in the savoury flavour. For both the sweet and the savoury flavours, orthonasal perception was more sensitive to the removal of individual volatiles and this was attributed to different efficiency in delivery to the olfactory receptors. The same-different approach highlighted synergistic, suppressive and blending interactions between volatiles within flavour mixtures. In particular, the presence of 4-hydroxy-2,5-dimethyl-3-furanone increased the assessor sensitivity to the removal of other individual volatiles in the savoury flavour. Cross-modal interactions were highlighted within the strawberry flavour, particularly where congruency between taste and aroma could be identified.

The omission approach brings a novel contribution to sensory science as it allows further analyses and a deeper understanding of flavour. This study pioneers the use of the Thurstonian d' for omission experiments, enabling the relative importance of the individual components of flavour perception to be determined.

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List of symbols and abbreviations

AEDA: Aroma Extract Dilution Analysis

AMP: Adenosine 5-MonoPhosphate

COD: Comparison Of Distances

d': Thurstonian distance

DOD: Degree Of Difference

ENaC: Amiloride-sensitive epithelial sodium channel

FA: False Alarm

FID: Flame Ionisation Detector

GC: Gas Chromatography

GC - FID: Gas Chromatography - Flame Ionisation Detection

GC - MS: Gas Chromatography - Mass Spectrometry

GC - O: Gas Chromatography - Olfactometry

GC - OH: Gas Chromatography - Olfactometry of static Headspace

GMP: Guanosine 5-MonoPhosphate

GPCRs: G-protein-coupled receptors

H: Hit

IMP: Inosine 5-MonoPhosphate

K_{aw} : Air/water partition coefficient

LR model: Likelihood-Ratio model

LMS: Labelled Magnitude Scale

MFT: 2-Methyl-3-FuranThiol

MFT-MFT: bis(2-methyl-3-furyl)disulphide

MS: Mass Spectrometer

MSD: Mass Spectrometer Detector

MSG: MonoSodium L-Glutamate

MPS: Multi Purpose Sampler

n: Original flavour model

n - 1: Omission sample

n - 0.5: Fractional omission sample

NST: Nucleus of the Solitary Tract

OAV: Odour Activity Value

oOAV: Orthonasal Odour Activity Value

rOAV: Retronasal Odour Activity Value

OITE: Odour Induced Taste Enhancement

ORN: Olfactory Receptor Neurons

P_1 : Probability of responding 'different' to a 'same' pair in the same-different test

P_2 : Probability of responding 'different' to 'different' pair in the same-different test

P_c : Proportion of correct answers

P_d : Proportion of 'true' discriminators in a triangle test

$P_{s/s}$: Proportion of assessors who answered 'same' for the 'same' pair of samples in a same-different test

PEA: Phenyl Ethyl Alcohol

PG: Propylene Glycol

RF: Response Factor

RI: Retention Index

ROC: Receiver Operating Characteristic

RT: Retention Time

SDT: Signal Detection Theory

TRC: Taste Receptor Cell

α : Type I error, or the probability of concluding that a perceptible difference exists when one does not.

β : Type 2 error, or the probability of concluding that a perceptible difference does not exist when one exists.

δ : Size of the difference of interest to calculate the number of assessors required using the Thurstonian approach

Δ : Minimum difference in proportion that the researcher wants to detect in a same-different test

Chapter 1. General introduction

1.1 Introduction

A new approach in sensory omission testing was developed at the University of Nottingham (O'Mahony, 2012), based upon the same-different test (ASTM E2139-05 2011) associated with a sureness ratings. The research project presented here aims to fully define and evaluate this new approach to help gain a better understanding of flavours. The new approach was characterised and compared with a more traditional approach based on triangle tests. In this thesis, the approach using the same-different test in omission testing will be referred to as the 'same-different approach', while the approach using the triangle test will be referred to as the 'triangle approach'.

The same-different approach was applied to (1) assess the relative contribution of individual volatiles in flavours delivered ortho- or retronasally, (2) assess interactions between volatiles in mixtures and (3) investigate interactions between volatiles and tastants.

This introductory chapter is organised into four main sections. Section 1.2 reviews flavour perception and cross-modal interactions between sensory modalities. Section 1.3 details methods used to develop food flavour models, with a focus on sensory omission testing. Section 1.4 introduces sensory discrimination testing and Thurstonian modelling, in the particular context of omission testing and section 1.5 details the specific objectives of the experimental work carried out during this PhD project.

1.2 Flavour perception

Flavour has been defined as the 'psychological interpretation of a physiological response to a physical stimulus' (Noble, 1996). This chapter describes the modalities involved in the perception of flavour and how they interact at different levels. In the context of the project, a particular interest is given to the cross-modal interactions between taste and aroma.

1.2.1 Definitions

Flavour is the overall impression experienced from the perception of aroma and taste when a food product is sniffed or consumed. A third factor known as the trigeminal sensation is now considered as a component of flavour (Green, 2004). The terms 'taste' and 'flavour' are often used by non-specialists interchangeably. However, taste is a completely separate sense and has a specialised gustatory system to perceive the five main tastes: sweet, sour, salt, bitter (Bachmanov and Beauchamp, 2007), and the more recently discovered umami taste (Nelson et al., 2002).

Aromas are perceived when volatiles released from a product stimulate neurons in the olfactory bulb through receptors in the nasal cavity. Aromas can be perceived through tasting in the oral cavity (retronasal delivery) and through inhalation (orthonasal delivery). Most of the flavour is perceived through the olfactory sense and aromas alone are sufficient to elicit flavour perception, contrarily to taste alone.

In this thesis, 'strawberry flavour' and 'savory flavour' are used to refer to the strawberry and savory flavour model systems. The term 'volatile' is used to refer to individual volatile compounds in a flavour mixture. The term 'aroma' is used to refer to the odour of a single volatile, perceived either ortho- or retronasally.

1.2.2 Sensory modalities

Three chemosensory modalities contribute to flavour perception: olfactory, gustatory and trigeminal senses. These chemical senses are unique because the stimulus is a chemical compound that interacts directly with the receptors. Thanks to those senses, humans can differentiate between consumable food necessary for the body and harmful substances that should be rejected. The olfactory sense is also extremely important as a defence mechanism. For example, the smell of smoke during a fire or rotten food can act as an alarm for a danger (Li, 2014).

1.2.2.1 Gustatory sense

The five principal tastes are sweet, salt, sour, bitter and umami. Umami is the pleasant taste of sodium glutamate originally derived from the Japanese. The pleasant tastes of sweet and umami signal for nutrients required and easily digested (sugars and amino acids, respectively). Sweet taste indicates the presence of fast-acting carbohydrates, and it has been suggested that the umami taste could signal the presence of proteins in food (Beauchamp, 2009). Saltiness and sourness are related to ionic and pH environment in the mouth,

and bitterness can be interpreted as a warning signal of the food ingested (Chaudhari and Roper, 2010).

Gustatory perception is caused by soluble substances in the mouth perceived by Taste Receptor Cells (TRCs) in taste buds. TRCs are grouped into taste buds located on papillae. Taste buds contain 50 to 150 cells surrounded by the epithelial cells of the papilla (Figure 1) (Chandrashekar et al., 2006). There are about 10,000 taste buds in the human mouth. TRCs have a very short life and are continuously replaced in taste buds (Lindemann, 2001). Three types of papillae - fungiform, foliate and circumvallate - carry taste buds. A fourth type of papillae, the filiform papillae, only has a mechanical function and does not carry taste buds (Smith and Margolskee, 2001). Although most taste buds are clustered in fungiform, foliate, and circumvallate papillae, they are also found on the soft palate, epiglottis and pharynx. Contrarily to the olfaction sense, gustatory receptors are immersed in the ingested solution for a few seconds. Furthermore, as gustatory receptors are bathed in saliva, conditions in the mouth affect the taste perception (Meilgaard et al., 2007).

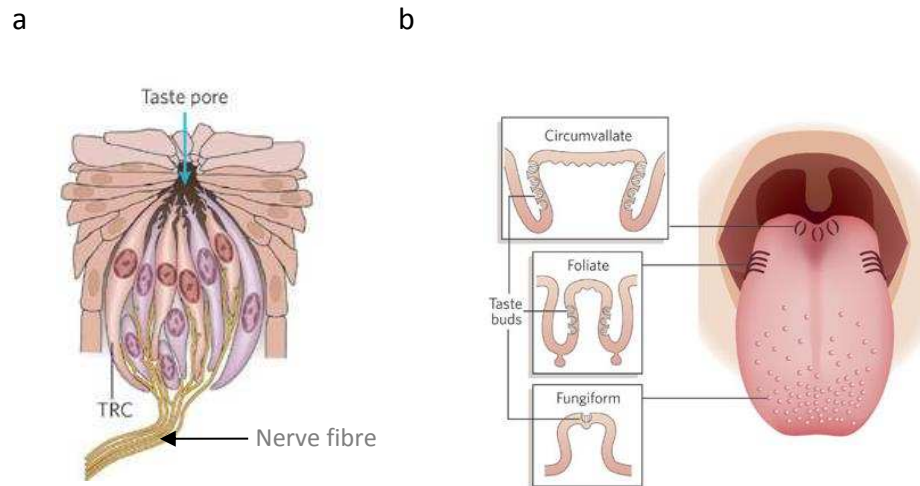


Figure 1: a. Taste bud b. Location of papillae on the tongue. Source: Chandrashekar *et al.* (2006)

TRC are bipolar cells: the microvilli are in contact with the oral cavity, while the synapses are in contact with sensory nerve fibres (Lindeman 2001). The contact between the tastant and its respective receptor triggers a signal transduction across nerve fibres which carries the sensory signal to the brain (Sugita and Shiba, 2005). Different types of proteins serve as receptors for tastants: ion channels, ligand-gated channels, enzymes and G-protein-coupled receptors (GPCRs) (Lindemann, 2001).

Detection of sweet taste

Sweet taste responds mainly to the presence of soluble carbohydrates in the oral cavity, but other non-carbohydrate molecules can also elicit sweet taste. Sugar molecules trigger a G-membrane signalling system via the activation of sucrose receptors (Margolskee, 2002, Lindemann, 1996). The spatial arrangement and the electrostatic character of the sweet molecules induce an electrostatic charge in the receptor cell through transduction pathways.

The GPCRs T1R3 and T1R2 found in mice are good candidates for sweet receptors (Lindemann, 2001, Hoon et al., 1999, Montmayeur et al., 2001). Two transduction pathways are suggested for the activation of TRC by sweet stimuli: one mechanism involves an increase in cyclic nucleotides (cGMP or cAMP), and the other mechanism involves an increase in inositol-1,4,5-trisphosphate (Lindemann, 2001).

Detection of salt taste

Salt taste can be elicited by many ionic species. The salt test elicited by the presence of Na⁺ ions is the most studied (Lindemann, 2001). In rodents, the Na⁺ specific salt taste is mediated by the amiloride-sensitive epithelial sodium channel (ENaC) (Canessa et al., 1994, Lindemann, 2001). ENaC acts as pathway for Na⁺ ions into TRC. The Na⁺ current causes the depolarisation of the TRC and triggers synaptic events.

Detection of sour taste

The detection mechanisms for sour taste are very diverse and illustrate the complexity of taste transduction. Receptors for sour taste can be classified into two groups. The first group comprises ion channels that conduct an inward proton current and activate the TRC channels (Gilbertson et al., 1993). The second group of receptors include H⁺ gated channels (Ugawa et al., 1998, Miyamoto et al., 2000).

Detection of bitter taste

A group of GPCRs from the T2R family acts as receptors for bitter taste in mammals (Adler et al., 2000, Matsunami et al., 2000). T2R receptors have a short amino-terminal domain. A single TRC can express a large number of T2Rs, suggesting that a TRC may be capable of recognizing multiple tastants. Some bitter peptides can interact directly with G-proteins without activating GPCRs. Quinine and caffeine for example can permeate the cell membrane and directly activates G-proteins (Naim et al., 1994, Rosenzweig et al., 1999).

Detection of umami taste

It was hypothesised that the taste receptor for L-glutamate was related to the glutamate receptor mGluR4 (Bigiani et al., 1997, Lin and Kinnamon, 1999). MGLuR4 is a GPCR and is abundant in the central nervous system. The transduction signal for the detection of umami taste is complex. One mechanism involves the closure of an unspecific cation channel, causing hyperpolarization of the TRC (Bigiani et al., 1997, Lin and Kinnamon, 1999). Other glutamate and amino-acid receptors were also found in TRC (Brand, 2000, Zviman et al., 1996, Hayashi et al., 1996, Smith, 2000).

Sensory nerve fibres carry the impulse triggered by the contact between the receptor and the tastant to the brain. The chorda tympani nerve conducts signals from the front and sides of the tongue and the glossopharyngeal nerve conducts signals from the back of the tongue. Signals from taste receptors in the mouth and larynx are transmitted by the vagus nerve. The chorda

tympani nerve, the glossopharyngeal nerve, and the vagus nerve make contact in the Nucleus of the Solitary Tract (NST). The signals are then transmitted to the frontal lobe and frontal operculum cortex in the brain where gustatory information is processed (Finger, 1987).

1.2.2.2 Olfactory sense

Odour is perceived when volatiles enter the nose and reach the olfactory epithelium, located in the roof of the nasal cavity (Figure 2). The mucosa of the olfactory epithelium is covered by millions of ciliated extensions of the Olfactory Receptor Neurons (ORNs), which contain the olfactory receptors (Figure 2). The receptor sensitivity to different chemicals varies greatly, giving the nose enormous discrimination power. For example, a trained perfumer can identify up to 200 different odour qualities. 17,000 volatiles are known at this time and their combination can elicit a multitude of odours which can be perceived by humans.

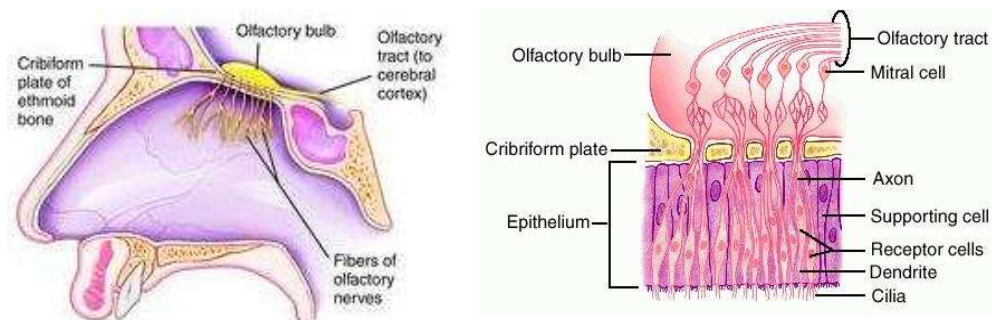


Figure 2: The olfactory system. Source: Mosby et al. (2009)

Once the volatile has reached the olfactory epithelium, the binding between the volatile and the olfactory receptor on the cilia of the ORN generates an

electrical impulse by transduction pathway. The cilia of the olfactory are the site of olfactory signal transduction. The activation of the ORN is mediated by specific G proteins. The interaction between the olfactory receptor and the G proteins initiate a transduction signal, generating an action potential (Buck and Axel, 1991, Boekhoff et al., 1990). The mechanism by which the receptors generate the signal that they send to the brain is still not completely understood (Meilgaard et al., 2007).

The electrical impulse is transferred by the ORNs up to the olfactory bulb through the cribriform plate. Glomeruli are small regions in the olfactory bulb where ORNs converge and integrate, before transferring information to mitral cells. In mammals, millions of receptor cells project between 1,000 and 2,000 glomeruli. Lateral connections between the glomeruli and between the mitral cells permit the input from one odorant to inhibit or reduce the input of another volatile (Laing and Jinks, 2001, Valova et al., 2007). The olfactory bulb is a sophisticated system of neurons where the electrical impulse is processed before being transferred across the olfactory nerve to the brain.

The specific detection of distinct odorant molecules is thought to result from the association of odorant molecules with specific olfactory receptors. One olfactory receptor can interact with multiple volatiles, albeit with different affinities (Buck and Axel, 1991). Different odour molecules are represented by different spatial patterns of receptor activation in the olfactory bulb

(Shepherd, 2006). Such patterns are called 'odour images' or 'odour maps', and are responsible for the uniqueness of the perceived odours.

After being processed by the olfactory system, signals travel to the amygdala, the hippocampus, the hypothalamus, the thalamus and the orbitofrontal cortex which are part of limbic system brain areas. How the brain processes the incoming signals to produce odour perception is not fully understood (de Araujo et al., 2003). The orbitofrontal cortex receives signals from other sensory modalities including gustatory, trigeminal and visual stimuli (Rolls and Baylis, 1994, de Araujo et al., 2003, Small and Prescott, 2005, Abdi, 2002), allowing cross-modal interactions between olfactory and other sensory modalities (this will be discussed in section 1.2.3.2).

Smell has a 'dual nature', as volatiles can reach the olfactory epithelium either via external nares (orthonasal route) or via internal nares (retronasal route) (Figure 3). Orthonasal stimulation involves inhalation of volatiles by sniffing in through the external nares. The orthonasal route is the route to sense odours in the environment. Previous research has indicated that an odour presented orthonasally is easier to identify compared to a retronasal odour (Delwiche, 2004, Rozin, 1982).

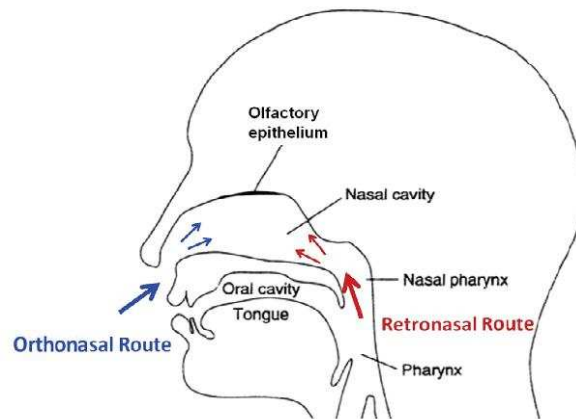


Figure 3: Orthonasal and retronasal routes. Source: Goldstein *et al.* (2010)

Volatiles can also enter the nose via retronasal route, during chewing or swallowing. Volatiles in the mouth enter the nose via two mechanisms. The first mechanism is called velum-tongue movements: food or liquid are subjected to oral processing before swallowing, which causes volatiles to ascend towards the nasal cavity. In the second mechanism, after swallowing, volatiles are ‘pumped’ towards the roof of the nasal cavity through the posterior nares of the nasopharynx. The second mechanism provides the greatest delivery of volatiles from the retronasal route (Smith, 2000).

Most of the flavour is perceived through the olfactory sense. It is the retronasal olfactory system which is responsible for our ability to identify the flavour of food (Shepherd, 2006, Chen and Engelen, 2012). For example, lemon flavour is not perceived from its taste but from the volatile terpene compounds perceived through the retronasal route.

1.2.2.3 Trigeminal system

According to Green *et al.* (2004), trigeminal sensation should be viewed as a component of flavour. It involves chemesthetic effects in the mouth such as tactile, cooling, burning or irritative effects (Petit *et al.*, 2007). Examples are the burn associated with chilli, the cooling of menthol and the pungency of mustard (Green, 2004). Chemesthesis is mediated by nonspecific somatosensory fibres activated by chemical stimulation (Green and Lawless, 1991). The stimulation of these nerves triggers activity in the trigeminal nerve associated with the perception of irritation.

1.2.3 Interactions in flavour perception

In everyday life, humans do not distinguish between the different sensations experienced during eating. This is because the human nervous system is able to integrate information from distinct sensory modalities, giving rise to the perception of flavour. Integrated sensory modalities include gustatory, olfactory, visual, auditory and somatosensory inputs. Interactions between all those sensory modalities allow enhancing the detection and the identification of a stimulus, in particular when the stimulus is ambiguous, incomplete, or with low perceptibility (Small and Prescott, 2005). Psychophysical, neuroimaging and neurophysiological studies have provided an understanding of the mechanisms of integration in flavour (Small and Prescott, 2005).

Interactions can occur at different levels, from physico-chemical interactions within the food product to peripheral interactions at a receptor level and

cognitive interactions at a central level. This chapter gives a particular focus on taste-aroma interactions, as one of the main objectives of this thesis was to assess the effect of taste on the perception of flavour.

1.2.3.1 Different levels of interactions

1.2.3.1.1 Physico-chemical interactions

Physico-chemical interactions between flavour compounds can occur within the food product before the product is consumed. Interactions between volatiles and other components in the food matrix have been widely reported (Friel et al., 2000, Hollowood et al., 2002). These interactions can lead to changes in volatile release (Da Porto et al., 2006). An illustration of interactions in food matrices is the well-known 'salting-out' phenomenon. The presence of salt in solution decreases the availability of water molecules and thereby increases the release of volatiles in the gas phase (Saint-Eve et al., 2009, Ventanas et al., 2010a, Ventanas et al., 2010b). The term 'salting-out effect' has been generalised to the effect of other compounds that can decrease the availability of water molecules, such as MonoSodium L-Glutamate (MSG) (Maga and Lorenz, 1972, Maga, 1983) or sucrose (Nahon et al., 1998, Da Porto et al., 2006).

The presence of tastant molecules in flavour mixtures can also impact the physico-chemical properties of the food matrix and thereby affect the release of volatile compounds. For example, the change in pH caused by citric acid can modify the partition coefficient of certain volatiles (Guyot et al., 1996,

Baldwin et al., 1973, Leksrisompong, 2008). The presence of sucrose could increase the viscosity of the food matrix and thereby decrease the perceived intensity of volatiles (Hollowood et al., 2002). Proteins and carbohydrates can also interact with volatiles through binding and decrease their release into the headspace (Taylor and Linforth, 2010, Jones et al., 2008, Guth and Fritzler, 2004, Heng et al., 2004, Seuvre et al., 2004, Frost et al., 2005).

1.2.3.1.2 Peripheral interactions at receptor level

Tastants can interact at receptor level, as tastant compounds can interfere with the receptors or transduction mechanisms associated with other compounds (Keast and Breslin, 2003, Lindemann, 2001). For example, suppression between sucrose and sodium chloride occurs at both peripheral (receptor) and central (cognitive) levels (Gillan, 1982). Bitter taste can be suppressed by other tastant compounds at receptor level (Keast and Breslin, 2002, Keast et al., 2001, Breslin, 1996).

Interactions between volatiles at a receptor level are thought to play a major role in the processing of volatile mixtures (Oka et al., 2004, Brodin et al., 2009, Chaput et al., 2012). The competition between volatiles for receptor binding can induce agonists or antagonists effects (Cruz and Lowe, 2013, Chaput et al., 2012, Brodin et al., 2009, Laing and Jinks, 2001). If the volatiles are both agonists, they may be perceived as weaker in a mixture, or the volatile with a greater affinity for the receptor would dominate in the mixture (Laing and Jinks, 2001). If competition occurs between one agonist and one

antagonist, the antagonist can bind olfactory receptors without activation and result in suppressive interactions (Oka et al., 2004).

The activation of certain olfactory receptors can also inhibit or reduce the input of another volatile, via lateral connections between the glomeruli and between the mitral cells (Takeuchi et al., 2009, Laing and Jinks, 2001, Valova et al., 2007). This mechanism is called lateral inhibition.

1.2.3.1.3 Interactions at a cognitive level

The integration of stimuli from different senses can give rise to the perception of a single unit. One example is face recognition, where the brain is able to integrate separate line features into a single pattern (McBride and MacFie, 1990). The same phenomenon happens when gustatory and olfactory stimuli give rise to the perception of a flavour.

It is now established that there is no measurable physico-chemical interaction between taste and aroma in systems with relatively low tastant concentrations (100 g/L) (Green et al., 2012, Friel et al., 2000). Therefore, sensory and neuroimaging experiments have focused attention on cognitive explanations for cross-modal interactions in flavour. Brain imaging showed that some brain areas are activated by both taste and aroma stimuli, suggesting cognitive interactions between those senses (de Araujo et al., 2003). Eldeghaidy *et al.* (2011) have shown cortical enhancement of aroma by taste. Differences in activity in the insula, amygdala and orbitofrontal cortex have also been measured when taste and smell were presented alone or in

combination (Delwiche, 2004). Dalton *et al.* (2000) provided further evidence for the cognitive integration of taste and smell. Finally, the importance of congruency between taste and smell and learning through exposure confirm that interactions occur at a cognitive level.

A key question is whether the interactions occur at a neural or cognitive level. At a neural level, the first hypothesis is that cross-modal perception relies on information coded by the network formed by sensory specific unimodal neurons, modulated by other sensory input. Another hypothesis is the existence of multimodal neurons that may receive converging sensory information and respond specifically to the combinations of different sensory inputs (Small and Prescott, 2005).

Integration between the different sensory modalities also occurs at a cognitive level, when flavour is processed in the sensory-specific cortex. The chemosensory regions of the brain - insula, operculum, orbitofrontal cortex and anterior cingulate cortex - are suspected to play a key role in integrating the sensory inputs from different sensory modalities (Small and Prescott, 2005). In particular, the orbitofrontal cortex is the area of the brain where all taste, smell, trigeminal information and vision can interact (Abdi, 2002, Rolls and Baylis, 1994, de Araujo *et al.*, 2003, Small and Prescott, 2005).

1.2.3.2 Cross-modal interactions

1.2.3.2.1 Taste-aroma interactions

There is strong evidence for the integration between aromas and tastes when they are experienced in mixtures compared to when they are experienced individually. Studies have shown that the overall intensity of a mixture of taste and aroma tends to be slightly lower than the added intensities of the single taste and aroma compounds (Delwiche, 2004, Gillan, 1983).

One source of evidence for interaction between taste and smell is the so-called 'Odour Induced Taste Enhancement' (OITE). The presence of congruent aromas can increase the perception of tastes at threshold and subthreshold levels (Djordjevic et al., 2004a, Prescott, 2003). Strawberry, vanilla and caramel aromas enhanced the perceived sweetness (Frank and Byram, 1988, Prescott, 1999, Stevenson et al., 1999). Cocoa increased bitterness (Labbe et al., 2006). Meat, fish or cheese aromas enhanced saltiness (Lawrence et al., 2009, Nasri et al., 2011). Djordjevic *et al.* (2004b) showed that imagined aromas can influence taste perception in the same way as perceived odours.

Another manifestation of taste-odour interaction is 'taste induced odour enhancement'. The presence of a tastant has been shown to enhance the perception of an aroma and lower its detection threshold. Dalton *et al.* (2000) demonstrated that the presence of sodium saccharin at a subthreshold level lowered the orthonasal detection threshold of benzaldehyde. The addition of sucrose and/or acid has been shown to increase the intensity of fruit flavours

(Pfeiffer et al., 2006, Delwiche, 2004, Murphy et al., 1977, Stampanoni, 1995) and manipulating the sucrose concentration delivered retronasally over time in a banana flavoured solution resulted in changes in the perceived banana flavour (Hort and Hollowood, 2004). Sour taste has also been shown to enhance the perceived intensity of apple and lemon flavour (Cayeux and Mercier, 2003).

It must be noted that interactions between retronasal olfaction and taste have sometimes been attributed primarily to halo dumping (Green et al., 2012). The halo dumping effect is the rating of changes in an attribute on an inappropriate scale. The assessor rates the perceived sensation (for example sweetness intensity) on the only available scale (for example flavour intensity). However, halo dumping can be avoided by including appropriate response categories for both tastes and odours (Green et al., 2012). Interactions still occurred when assessors had appropriate response categories (Cayeux and Mercier, 2003, Petit et al., 2007).

1.2.3.2.2 Interactions in savoury flavours

Only a few publications deal with taste-aroma interactions in savoury flavours. Research has mostly focused on the use of savoury aromas to counterbalance the decrease of salt in food products. It has been shown that savoury aromas can enhance the perceived saltiness of salt solution (Pionnier et al., 2004), savoury products like cheese (Lawrence et al., 2009) or savoury bouillons (Batenburg and van der Velden, 2011). On the other hand, sodium

chloride, MSG, and nucleotides such as Adenosine 5-MonoPhosphate (AMP), Guanosine 5-MonoPhosphate (GMP) and Inosine 5-MonoPhosphate (IMP) are considered as flavour enhancers, because of their ability to increase the perceived intensity of savoury flavour (Bellisle, 1999, Reineccius, 2005).

Sodium chloride is a major ingredient for the food industry. Sodium chloride is generally present in significant quantities in products like bread, soup, cheese and sausages. In addition to taste enhancing properties, sodium chloride also has a role in texture, volatile release, and preservation of food. It is recognised that salt contributes more to sensory perception than adding to salty taste. Batenburg *et al.* (2011) showed that reducing salt content in beef and chicken bouillons resulted in the decrease of characteristic flavour attributes (such as roasted grain and fenugreek), and a decrease in the fullness of the flavour. Ventanas *et al.* (2010a) showed that the addition of salt enhanced overall flavour intensity and flavour attributes ('broth-like' and 'saltiness', 'mushroom flavour', 'nutty' and 'cocoa flavours') of a beef broth flavour model. This effect was partly explained by the 'salting out' phenomenon.

Umami is the Japanese word for savoury and delicious and is popularly referred to as savoury. Umami taste is perceived in a diverse range of foods rich in glutamate, like fish, meat (beef, cured ham), cheese (Parmegiano Reggiano, Emmental), tomatoes and some vegetables (Maga, 1983). MSG and 5'-ribonucleotides such as IMP and GMP are the most important compounds

associated to umami taste (Yamaguchi and Ninomiya, 2000). Mixtures of MSG, IMP, and GMP are commonly used in the food industry to enhance the flavour of culinary products, snacks, sauces, soups, and seasonings. Umami tastes have been reported to have complex interactions with aromas (Ventanas et al., 2010a, Niimi et al., 2014). When MSG was given in combination with a savoury vegetable odour, the resulting flavour was more intense and more pleasant (McCabe and Rolls, 2007). MSG was able to enhance salty taste, sweet taste, and potato flavour (Jung et al., 2010). MSG also enhanced nutty, cocoa, and potato flavour intensities in a model broth (Ventanas et al., 2010a). Cheese aroma was shown to significantly enhance umami perception (Niimi et al., 2014). Green *et al.* (2012) and Lim *et al.* (2011) suggested that nutritive tastes such as sweet, salty, and umami were able to enhance retronasal aromas.

1.2.3.2.3 Importance of congruency and associative learning

In contrast to vision and audition, representation of taste and smell occurs in regions of the limbic brain largely associated with emotion and memory functions, such as the hippocampus and amygdala. This explains why the odours are highly associated with memory and emotions and suggests that taste-odour integration is influenced by experience and affective factors (Shepherd, 2006).

Congruency between stimuli (i.e. when the stimuli are commonly encountered together) plays a major role in the perception of flavour (Petit et

al., 2007, Delwiche, 2004). For example, the aroma of strawberry, but not peanut butter, enhanced the perceived sweetness of sucrose (Frank and Byram, 1988) and interactions between taste and odour was more evident for stimuli frequently encountered together (Delwiche, 2004, Petit et al., 2007). Breslin et al. (2001) showed that integration between benzaldehyde and MSG did not occur in a replication of the experiment carried out by Dalton and coworkers (2000), suggesting that congruency is necessary for taste-odour integration. However, sensitivity enhancement has also been associated with incongruent odour-taste pairs, such as pineapple-broth (Delwiche and Heffelfinger, 2005).

Evidence shows that associative learning plays a role in odour-taste interaction and that congruency can be learned through exposure (Delwiche, 2004, Prescott, 2001, Petit et al., 2007). As an example, pairing between the cooling sensation and pineapple aroma was shown to be learned through regular exposure (Petit et al., 2007).

1.2.3.2.4 Interactions between other sensory stimuli

Visual and auditory stimulations can also have an effect on the perceived flavour (Delwiche, 2004). Several studies have shown that colour can affect flavour identification (Philipsen et al., 1995, Zellner et al., 1991, Delwiche, 2004, Petit et al., 2007), as learned associations between colour and flavour can impact on perceived taste. For example, Johnson *et al.* (1982) showed that red colour enhanced the perceived sweetness in cherry juices. Colouring

a white wine in red also caused assessors to use a different set of descriptive terms corresponding to red wine (Morrot et al., 2001).

Chemesthetic sensations experienced by the trigeminal system are also a component of flavour (Green, 2004). Previous research has shown the existence of perceptual interactions between flavour and chemesthetic sensations. Irritants like capsaicin can inhibit the perceived intensity of savoury flavour (Cain and Murphy, 1980) whereas cooling appears to enhance fruit flavour perception (Petit et al., 2007).

1.3 Development of food flavour models

1.3.1 Creation of artificial food flavour

1.3.1.1 Aromas in food

The perceived aroma of a food results from a complex mixture of volatiles. Table 1 presents the number of identified volatile compounds in a range of different foods. More than 7,000 aroma constituents have been identified so far, with a large number of different chemical structures, physical and chemical properties (Taylor and Linfoth, 2010). Simple flavours such as strawberry or grape flavours can contain 100 to 300 volatiles, whereas more complex flavours like the flavours created from Maillard reactions (coffee, meat, chocolate) can totalise more than 900 volatiles (Taylor and Linfoth, 2010).

Over 1,000 aroma compounds have been identified in meat. The characteristic flavour of cooked meat derives from thermally induced

reactions during heating, principally the Maillard reaction and the degradation of lipid. The Maillard reaction occurs between amino compounds and reducing sugars and leads to the formation of a wide range of aroma compounds, which accounts for the large number of volatile compounds found in cooked meat. Heterocyclic compounds and sulfur compounds are formed during the Maillard reaction and are important flavour compounds contributing to cooked foods (cooked meat) (Gasser and Grosch, 1988, Gasser and Grosch, 1990) and beverages (coffee) (Blank et al., 1992), providing savoury, meaty, roast and boiled flavours. Lipid degradation provides compounds which give fatty aromas to cooked meat and determine differences between the odours of meat from different species.

The level of complexity of flavour models varies from 15 volatiles in simple fruit flavour to 100 volatiles in a more complex flavour (such as the flavour of cooked food) (Taylor and Linforth, 2010). The major difference between sweet and savoury flavours is the presence of sulfur compounds in savoury flavours generated from the Maillard reaction, such as disulphides. One consequence of the high reactivity of these compounds is the instability of savoury flavours. In this PhD study, the use of a sweet flavour (strawberry flavour) and a savoury flavour implies assessing the stability of both flavours before conducting sensory experiments, as the stability could vary between the two flavours.

Table 1: Number of volatiles identified in different food products. Source: van Straten *et al.* (1977)

Food product	Total volatiles identified
Tomato	194
Strawberry	252
Potato	134
White bread	161
Cheddar cheese	152
Beer	235
Coffee	540
Heated beef	372

1.3.1.2 Recombination protocol

Only a small proportion (less than 5 %) of the volatiles in food contribute to its flavour (Grosch, 1999, Grosch, 2001). In the creation of food flavour models, the challenge is to determine which compounds are needed to reproduce the flavour. Research has shown that 10 to 30 volatiles are needed to replicate the character of the aroma of any food studied (Grosch, 2001). Recombination protocols involve analysing aroma profiles of a food product in order to create a recombined aroma model.

Previously, it was hypothesised that Odour Activity Values (OAVs) (the ratio between the concentration of the volatile in the flavour and the detection threshold of the volatile) could be used to indicate the contribution of individual aromas to the overall sensory percept (Acree *et al.*, 1984).

Currently used recombination protocols choose volatiles with OAVs above 1 for recombination experiments and the higher the OAV value, the more important the compound is considered (Guth and Grosch, 1999, Lytra et al., 2012).

Grosch (2001) reviewed the different approaches involved in the analytical procedure used to create flavour models.

- The first step of flavour model recombination is the screening for potent volatiles. The volatile fraction is extracted from the food and separated by high-resolution gas chromatography.
- The potent volatiles are selected by charm analysis or Aroma Extract Dilution Analysis (AEDA). Gas Chromatography - Olfactometry of static Headspace (GC-OH) can then be used to detect highly volatile volatiles.
- The potent volatiles in the extract are identified with a Mass Spectrometer (MS) and quantified in the food product, and their OAVs are calculated. The identification step requires the comparison of the odour quality of the analyte in the volatile fraction with the odour quality of an authentic standard.
- A recombination aroma model is prepared based on the data obtained from instrumental analysis. The recombined model can then be compared sensorially against the original product (Buttery et al., 1990).

1.3.2 Sensory omission studies

Research has mostly focused on instrumental analysis to create flavour models, as more resource, time and space in published studies are allocated to analytical experiments, while little attention is given to the sensory part (Greger and Schieberle, 2007, Guth, 1997, Kirchhoff and Schieberle, 2001, Schieberle and Hofmann, 1997). However, the use of instrumental analysis to create flavour models is limited, as only individual aroma qualities can be measured, and interactions within flavours are not taken into account. Furthermore, some volatiles with low OAVs can have an essential contribution to the flavour (Escudero et al., 2004).

In this context, sensory analyses becomes of major importance to assess and challenge the flavour models created instrumentally. Sensory omission experiments are performed to determine the key volatiles of flavour. Omission studies involve omitting one or a group of volatiles and comparing that omission sample to the original flavour model. More recently, sensory omission methods have been developed and have begun to occupy a more important place in the literature (House and Acree, 2002, Benkwitz et al., 2012). A review of the sensory methods used in sensory omission studies is presented below.

1.3.2.1 Qualitative methods

Several studies have used a qualitative method for omission experiments. This method can be used to describe the aroma impact of one volatile on the

whole model. Flavour profile with attribute rating appears to be a popular technique (House and Acree, 2002, Ito et al., 2002, Schieberle and Hofmann, 1997). It was used in association with triangle tests by Wagner and Grosch (1998) and with similarity rating by Reiners and Grosch (1998). Description of odour impressions has also been used in conjunction with similarity rating (Schieberle et al., 1993). Paravisini *et al.* (2014) used a sensory approach where assessors answered the question 'Is that a good or bad example of a caramel odour?' using a linear scale. Panels were usually trained on the attributes of interest (Wagner and Grosch, 1998, Reiners and Grosch, 1998, House and Acree, 2002, Schieberle et al., 1993) or, in case of wine for example, were constituted of experienced assessors (Benkwitz et al., 2012). Numbers of assessors varied from 19 (Lytra et al., 2012) to as low as 5 (Wagner and Grosch, 1998, Schieberle et al., 1993).

1.3.2.2 Quantitative methods

Quantitative methods are useful to assess the importance of a volatile within a flavour model. The most popular approach in sensory omission experiments is the approach using the triangle test. Of the 21 publications discovered that used sensory omission methods, 13 used the triangle approach (Table 2).

Table 2: Published sensory omission studies using the triangle approach

Flavour model	Number of assessors	Tests in duplicate	Source
Strawberry juice	6	No	(Schieberle and Hofmann, 1997)
French fries	5	No	(Wagner and Grosch, 1998)
Roasted coffee	10	Yes	(Czerny et al., 1999)
Coffee brew	10	Yes	(Mayer et al., 2000)
Beef, chicken	11	Not mentioned	(Kerschler and Grosch, 1999)
Bread crumbs	10	No	(Kirchhoff and Schieberle, 2001)
Wine	7 to 11	No	(Ferreira et al., 2002)
Wine	10 to 16	No	(Escudero et al., 2004)
Pineapple	15	No	(Tokitonio et al., 2005)
Morel mushroom	8	Not mentioned	(Rotzoll et al., 2006)
Apricot	16	YES	(Greger and Schieberle, 2007)
Peanut	13	No	(Chetschik et al., 2010)
Orange juice	10	No	(Averbeck and Schieberle, 2011)
Red wine	17 to 19	No	(Lytra et al., 2012)
Red wine	18	No	(Lytra et al., 2013)

The triangle approach was sometimes used in conjunction with a 0-3 intensity scale (Wagner and Grosch, 1998). Threshold testing (Reiners and Grosch, 1998, Wagner and Grosch, 1998, Schieberle et al., 1993), similarity rating (Guth, 1997, Guth and Grosch, 1994, Reiners and Grosch, 1998), duo-trio tests (Ferreira et al., 2002, Escudero et al., 2004) and paired comparisons (Czerny et al., 1999) have also been used occasionally.

1.3.2.3 Limits of the triangle approach

Although the triangle approach is the most popular approach in omission experiments, there are limits to this approach which should be discussed. First of all, some omission studies are underpowered, with only 5 to 19 assessors (Table 2). The minimum number of assessors set by the Internal Standard for the triangle test (ISO 4120: 2007) is 7 under the widest parameters ($\alpha = 0.20$, $\beta = 0.20$ and $P_d = 50\%$). Although the chance of guessing correctly is reduced in the triangle test (33 % chance), a correct answer by chance can have important effect on the significance level when such a small number of assessors is used. For example, in a study with only 10 assessors, the significant difference at $\alpha = 0.05$ is fixed at 7 correct answers. In this case, only a couple of correct guesses would dramatically change the observed significant difference between samples. In the current PhD study, the Thurstonian d' was used as a measure of the degree of sensory difference between two samples (this will be discussed in section 1.4.4.2). Using such a low number of assessors for triangle testing in this type of study would result in higher variance of d' , which would not always allow concluding for a significant difference between samples.

In some studies, the assessors carried out the discrimination tests in duplicate (Wagner and Grosch, 1998, Czerny et al., 1999, Mayer et al., 2000, Greger and Schieberle, 2007). Unless the data is treated accordingly, this does not respect the hypothesis of independence and biases the results (Bi et al., 1997). In

Mayer *et al.* (2000) and Czerny *et al.* (1999), the presentation designs were not balanced. Other publications do not mention if they have used a balance presentation design. It is recommended to balance the presentation design in a triangle test, as the sequence of stimuli can have an impact on the signal perceived (Meilgaard *et al.*, 2007, Lee and O'Mahony, 2007a). Indeed, 'contrast effect' and 'convergence effect' can occur, as a stimulus is perceived differently depending on the sample that preceded it (Chambers and Wolf, 1996). A final comment is that the triangle approach only concludes if the omission of one aroma volatile is detected or not by the panel, but it does not assess the relative importance of the aroma volatiles within the flavour.

As a conclusion, analysis of the literature highlights scope for improvement in sensory omission experiments, in terms of the number of assessors, statistical approach (balance presentation design and independence of the replicates) and analysis (relative importance of the individual aroma compounds). The next session examines different alternatives to the triangle test in omission experiments.

1.4 Discrimination testing

This section discusses the advantages of certain discrimination tests over others in the particular context of omission studies, as well as the interests of Thurstonian modelling. The cognitive strategy used to answer the tests is also discussed, as they are vital for Thurstonian modelling. A particular focus is

given to the triangle and same-different tests, as they are the discrimination tests used in this study.

1.4.1 Discrimination tests for omission testing

Discrimination tests are widely used in sensory, as they are rapid techniques and can be performed by naïve assessors (Kemp et al., 2011). The aim of a discrimination test is to determine if a sensory difference exists between two 'confusable' products. Discrimination tests can be used to test the stability of a product, to assess a change in formulation or for quality control. They can also be used for panel screening. A broad range of discrimination tests are available and the selection of the appropriate test is critical for the objectives of the study. Discrimination tests that would be appropriate for omission testing are presented below. Attribute specific tests such as 2-AFC (ASTM E2164) and 3-AFC (ASTM E1432) are not presented as they could not be used for discrimination testing in this study, where the nature of the difference was unknown.

1.4.1.1 Triangle test (ISO 4120: 2007)

The triangle test has been used extensively for omission experiments. It is a very simple test and intuitive for naïve assessors. Moreover, the triangle test is efficient statistically because the chance of guessing is only 1/3. Assessors are presented with three samples and told that two samples are the same and one is different. The assessors report which sample they believe to be different. The triangle test usually requires large sample size to be effective

(Ennis, 1993). Typically a minimum number of 50 assessors are needed to test for difference (at $\alpha = 0.05$ and $\beta = 0.1$) (ISO 4120: 2007).

1.4.1.2 Same-different test (ASTM E2139-05 2011)

The same-different test is a good candidate for omission testing, as it is simple and intuitive, and involves the assessment of only 2 samples. This test has been used previously for omission testing (O'Mahony, 2012). Assessors are presented with one of four possible pairs of samples (A/A, B/B, A/B or B/A) and asked to assess the samples to determine if they are the same or different. The total number of same pairs (A/A and B/B) given to the assessors usually equals the number of different pairs (A/B and B/A). Typically 100 assessors are needed to test for difference ($\alpha = 0.05$ and $\beta = 0.1$) (ASTM E2139-05 2011).

A longer version of the same-different test exists, where each assessor is presented with two pairs: one pair of the same samples (A/A or B/B) and one pair of different samples (A/B or B/A). In this version, the assessor is unaware that one pair is the same and the other different.

A sureness rating can be added to the same-different test, increasing the statistical power of the test (Bi et al., 2013). In the same-different test with a sureness rating, the assessors assess the two samples and state whether they think they are the same or different. Secondly, the assessors are asked to state the sureness level of their decision, represented by a four point surety scale ('very unsure', 'unsure', 'sure', 'very sure'). The same-different test with

a sureness rating can be regarded as a version of the DOD test proposed by Aust *et al.* (1985) (Bi *et al.*, 2013, Christensen *et al.*, 2012).

1.4.1.3 Degree Of Difference (DOD) test (ISO 8587:2006)

The DOD test is an extension of the same-different test when an m-point scale ($m > 2$) instead of a 2-point scale is used for responses. Assessors are presented with the control sample and a blind coded test sample. They must assess the two samples and state if a difference exists between the two samples. They record the magnitude of difference on a scale. The DOD test was recommended for heterogeneous products, as it takes into consideration production variation (Aust *et al.*, 1985).

1.4.1.4 A-Not A test (ASTM E253 - 13a, ISO 8588:1987)

The A-Not A test can be seen as another version of the same-different test (Santosa *et al.*, 2011). Assessors are trained to identify a reference control 'A' and a 'Not A' sample. They are then presented with blind coded samples, which are 'A' or 'Not A'. They are asked to assess the samples and determine if they are similar or different from the control 'A'. A scale is provided to record the magnitude of the difference. Typically 10 to 50 trained assessors are used for the test, and 20 to 50 individual presentations of equal numbers of both 'A' and 'Not A' are provided to each assessor.

1.4.1.5 Duo-trio test (ISO 1.399: 2010)

The duo-trio test is particularly relevant when samples are not homogenous as the question asked is which sample is the most similar to the reference. In the duo-trio test, three samples are presented to the assessor, two are blind coded and one labelled as the reference sample. Assessors are asked to assess the reference sample first, then the two coded samples. They must determine which of the two blind coded samples is the most similar to the reference. There are many variations of the duo-trio test, depending on the reference mode and the place of the reference (Kemp et al., 2011).

1.4.1.6 Tetrad test (ASTM WK32980)

Although not considered in this thesis, it should be noted that the tetrad test was recently restudied and promoted (Ennis and Jesionka, 2011, Ennis, 2012, Ennis et al., 2014). The tetrad test was presented as a suitable alternative to the classic triangle test in discrimination testing (Ennis, 2012). The tetrad test is a forced-choice method. In the unspecified tetrad test, assessors are presented with four samples and are instructed to group the samples into two groups of two.

1.4.2 Comparison of discrimination tests

The sensitivity of discrimination tests (i.e. their ability to discriminate between samples) has been compared in the literature. Three-stimulus protocols are usually less sensitive than two-stimulus protocols. For example, the 2-AFC test was more sensitive (i.e. yields higher d' values) compared to 3-

AFC test (Rousseau and O'Mahony, 1997, Dessirier and O'Mahony, 1999). Carry-over, fatigue and memory effects are more likely in three stimuli protocols, as more samples are assessed. Increasing the number of samples increases effects related to memory. In particular, in the duo-trio and triangle tests, the assessor needs to remember differences between samples that are not adjacent. Discrimination tests that require fewer samples per test are more appropriate when the samples are complex, or when it is crucial to avoid the fatigue or carry-over between samples. For example, in the case of aroma samples, the samples are particularly complex and the carry-over effect is important. Therefore, discriminations involving fewer samples are advantageous for omission studies.

The same-different test constitutes a good alternative to the triangle test in omission studies, as it is simple and does not require any specific training. The same-different test was chosen previously to develop a new approach in sensory omission studies, using an artificial strawberry flavour model (O'Mahony, 2012). The lower number of samples per test limits the carry-over between samples as well as the memory effects (Christensen and Brockhoff, 2009). The triangle and same-different tests have been compared previously in the literature. Some studies showed the higher sensitivity of the same-different test over the triangle test, while others did not. Rousseau *et al.* (1999) and Lau *et al.* (2004) showed that the same-different tests yielded higher d' values compared to the triangle tests. Although they found no

significant difference Rousseau *et al.* (2002) and Rousseau and O'Mahony (2001) showed a trend for the same-different test to yield a higher d' . This trend was also observed by Rousseau and O'Mahony (2000), when retasting was allowed. Stillman and Irwin (1995) did not find any significant difference in d' values between the same-different and triangle tests. Rousseau *et al.* (1998) showed that the long version of the same-different test yielded a higher d' compared to the triangle test. Kim *et al.* (2006) and Lau *et al.* (2004) also found that the long version of the same-different test yielded higher d' values when a warm-up procedure was used.

The A-Not A test is also interesting for omission testing as only one sample is assessed at a time. The 'A' sample would refer to the original flavour model and different 'Not A' samples could be used for each omission samples. However, there is a strong memory effect, as the stimulus 'A' can be forgotten or confused (Santosa *et al.*, 2011). Furthermore, The A-Not A test requires training on the 'A' and 'Not A' samples. Training on 'Not A' samples would be a major inconvenience in omission studies, as 'Not A' samples could each be omission samples, and are as many as the volatiles contained in the flavour model.

Recently more interest has been given to the unspecified tetrad test (Ennis, 2012). Mathematical modelling has shown it to be more powerful compared to the triangle test (Ennis, 2012, Ennis and Jesionka, 2011, Ennis *et al.*, 2014). The tetrad test could be a suitable alternative to the classic triangle test in

omission experiments. However, the tetrad test requires the evaluation of four samples, which could generate additional perceptual noise related to fatigue and memory effects.

1.4.3 Response bias

Response bias is a central problem in discrimination testing as it can affect the results of a sensory test. Response bias occurs when an assessors answer depends on where the assessor 'draws a line' when making a decision. It is the tendency to respond in a particular way irrespective of the sensory information (Ennis, 1993). The A-Not A and same-different tests are both subject to response bias (O'Mahony, 1995).

In the same-different test, when two samples are very similar, a second question is implied in the judgement: "How great does the difference have to be for the two stimuli to be called 'different'?". The cognitive criterion involved in this response bias is called the tau-criterion. The tau-criterion can be visualised as a sensory yardstick. The assessor responds 'same' if the difference between the stimuli is smaller than tau-criterion, and 'different' if it is larger (Figure 4).

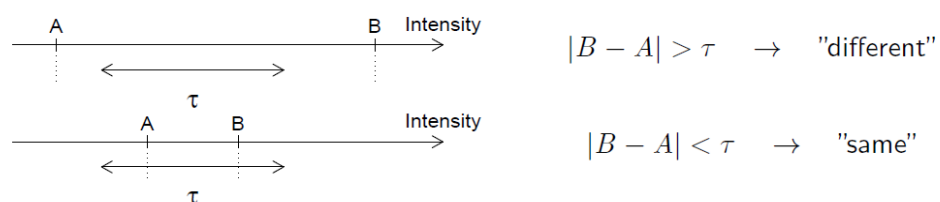


Figure 4: The tau-criterion in same-different tests. Source: Christensen et al. (2012).

The tau-criterion is a cognitive factor and does not depend on the assessor sensitivity. For example, lack of self-confidence or motivation can lead to a high tau-criterion: the naïve assessors answer 'same' when the samples are different. The tau-criterion is assumed to be constant for an assessor during a session (ASTM E2139-05 2011). However, the cognitive criterion can vary between assessors and among the same assessor over time.

The first solution to response bias is the use of forced choice methods, such as the triangle test, the 2-AFC or the 3-AFC (O'Mahony, 1992). These common forced procedures stabilise the response criterion. In the triangle test, the assessor is forced to set his criterion to a sufficient level of strictness so he can place the appropriate number of stimuli in each group: one odd sample and two similar samples (McBride, 1990).

Another solution to overcoming response biases associated with the A-Not A and same-different tests is the use of Thurstonian modelling which is described in the next session.

1.4.4 Signal detection theory

1.4.4.1 Perceptual variance

The theory of perception in sensory psychophysics is based on Signal Detection Theory (SDT), related to Thurstonian modelling (Rousseau, 1998). SDT was originally used for visual and auditory stimuli but its applications have then been expanded to a wide range of perceptual, cognitive, and psychological tasks. Thurstonian models were introduced by Louis Leon

Thurstone (1885-1955). In Thurstonian modelling, detection performance is based on two processes: a sensory process and a decision process (O'Mahony and Rousseau, 2002). The sensory process transforms the perceived stimulus into internal sensations and the decision process decides on a response, based on a cognitive strategy (Figure 5). The sensory process is characterised by a sensitivity parameter and the decision process by a response criterion parameter (Lewis and Harvey, 2004).

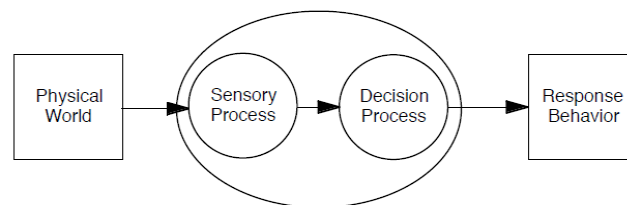


Figure 5: Internal processes involved in detection: sensory process and decision process. Source: Lewis (2004)

Thurstonian modelling is based on the fact that the response of the sensory nervous system to a sensory input is not constant. This perceptual variance (or noise) is particularly important for food products, due to the interactions occurring in the mouth (Lee and O'Mahony, 2007b) and sequence and adaptation effects (Rousseau et al., 1998). The Thurstonian law assumes that a stimulus can be seen as a perceptual distribution and the noise contributes to the variance of the distributions (Figure 6).

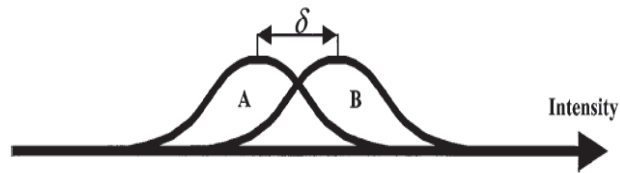


Figure 6: Thurstonian representation of the likelihood of intensity perception of two products A and B. Source: ASTM E2262-03

Discriminating between two stimuli is equivalent to establishing the distance between the two perceptual distributions. If two stimuli are more different, the distance between the perceptual distributions increases (Lee and O'Mahony, 2007b). Under Thurstonian standard assumptions, the two perceptual distributions of the two confusable stimuli have univariate normal distributions with equal variance (Kim et al., 2006), and are uncorrelated (Bi et al., 1997).

1.4.4.2 Definition of the Thurstonian distance d'

The Thurstonian distance d' is the difference between the means of the perceptual distribution of two products, measured in standard deviations (Figure 6). d' is a measure of the degree of sensory difference between two samples (Bi et al., 2013).

Different factors can affect the perceptual variance and thereby d' :

- Physiological and psychological factors related to the assessors (O'Mahony, 1992, Chambers and Wolf, 1996, Meilgaard et al., 2007)
- Cognitive factors such as response biases

- Experimental factors such as memory (Kim et al., 2006), sensory fatigue (O'Mahony and Rousseau, 2002), and the sequence of presentation of the stimuli (O'Mahony and Rousseau, 2002).

In the literature δ is sometimes used as a population parameter, and d' is used as its experimental estimate (Lee and O'Mahony, 2007b). In this thesis, d' will be used to refer to both a parameter and an estimate.

In theory, the Thurstonian measure d' should be positive. Negative values of d' can arise by chance. However, converting these negative values into zeros is not recommended as it would result in a loss of information (Macmillan and Creelman, 2005).

The Thurstonian d' has many interesting applications and is particularly interesting in sensory omission testing. Firstly, Thurstonian modelling provides a useful tool to assess the cognitive strategy used by assessors to answer a discrimination test (Lee and O'Mahony, 2007a). This will be discussed in the next session. Secondly, the Thurstonian d' allows comparison of the results obtained from different discrimination tests (Ennis, 1990, Jesionka et al., 2014). Irrespective of the discrimination test used, sensory results should lead to similar values of d' (Irwin et al., 1993). Therefore, the Thurstonian d' can be used to compare the sensitivities of different discrimination tests (O'Mahony and Rousseau, 2002, Rousseau et al., 1998) (ASTM E2262-03). This is particularly interesting in this study which aimed to compare the sensitivity of the triangle and same different tests.

Finally, the aim of Thurstonian modelling is not to determine whether or not the difference is perceived, but to estimate the size of the difference between samples (Jesionka et al., 2014). When used in omission testing, the Thurstonian measure d' reflects the relative importance of each individual volatile in a particular flavour. This is of major interest as omission experiments usually focus on identifying the key volatiles in flavours and do not measure the relative importance of individual volatiles in flavours.

1.4.4.3 Calculation of the Thurstonian distance d'

The calculation of the Thurstonian d' depends on the discrimination test used. ASTM E2262-03 groups the published tables of d' for the most common discrimination tests: the triangle, duo-trio, 3-AFC and 2-AFC tests (Ennis, 1993), the A-Not A test (Dorfman and Alf, 1969), and the same-different test (Ennis et al., 1988). Several software packages are also available to estimate d' : sensR (Brockhoff and Christensen, 2010), IFProgram (Ennis, 2003), and SDT assistant (Hautus, 2012).

The ROC curve is a commonly used tool in Thurstonian modelling, and in particular for the modelling of discrimination test with response bias (Macmillan and Creelman, 2005). The ROC curve is the plot of 'Hit' (H) proportion versus the 'False Alarm' (FA) proportions (Figure 7). 'Hit' referred to the proportion of assessors who give the answer 'same' for the same pairs and 'False Alarm' to the proportion of assessors who give the answer 'same'

for different pairs. The points on the curve correspond to the same sensitivity at different cognitive criteria.

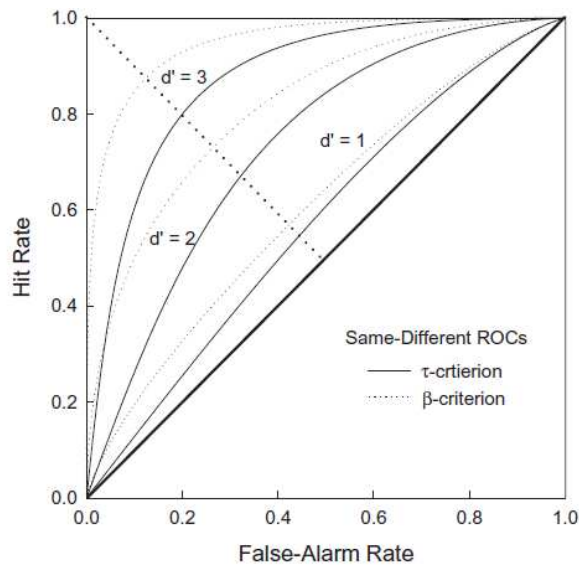


Figure 7: ROC curves obtained for the same-different test, using the model corresponding to a tau or a beta-strategy. Source: Hautus (2008)

ROC fitting software computes d' value using the degree to which the curve bows out: the more the curve arches up, the higher d' . The final estimate of d' is obtained by systematically adjusting the value to minimise the goodness of fit statistic chi-square corresponding to the normalised squared distance between each data point and the ROC curve. Different models can be used to estimate d' , depending on the cognitive strategy used by assessors to answer the discrimination test which are discussed in the next section.

1.4.4.4 Cognitive strategies used in discrimination testing

Each discrimination test is associated with one or more specific cognitive strategies. Knowing the cognitive strategies associated with a particular discrimination test is vital to analyse the results and build Thurstonian

models, as different strategies can lead to different levels of performance (Hautus et al., 2011). Based on the literature, Table 3 highlights the cognitive strategies associated with some popular discrimination tests.

Table 3: Cognitive strategies used in some discrimination tests

Discrimination test	Strategy usually assumed	Alternative strategy
A-Not A	Beta-strategy (O'Mahony et al., 1994)	
Same-different	Tau-strategy (Kim et al., 2006)	Beta-strategy (Rousseau, 2001)
Triangle	COD-strategy (Kim et al., 2006)	Beta-strategy (Versfeld et al., 1996)

The cognitive strategy adopted by assessors depends on the experimental design, the instructions given (for example if the dimension of difference is specified), the familiarity with the product, the degree of difference between the products, and the complexity of the stimuli (Rousseau, 2001). For the same-different test, a tau-strategy (see section 1.4.3) is usually assumed (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002).

In contrast, the Comparison Of Distances (COD) strategy is generally assumed for the triangle test (O'Mahony et al., 1994). When using a COD-strategy, the assessor compares the distances between the sensory perceptions of the three stimuli (Figure 8). The closest stimuli are paired, while the sample that is furthest from the other two is selected as the odd stimulus (Delwiche and O'Mahony, 1996, O'Mahony, 1995). In the case of Figure 8, product A is

identified incorrectly as the odd product, because products B and C appear to be closer to each other on the sweetness perception axis.

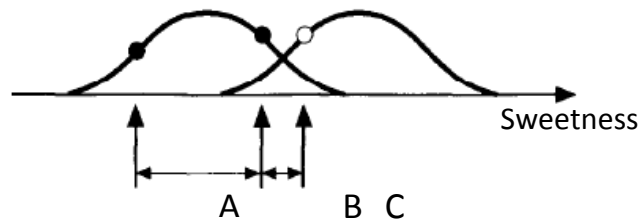


Figure 8: Comparison of distance strategy in the triangle test. Source: O'Mahony (1995)

Another cognitive strategy called the beta strategy can be used with the same-different test (Rousseau, 2001) and the triangle tests (Versfeld et al., 1996). The beta-strategy is commonly used with single stimulus presentation, such as A-Not A tests (Figure 9). When using a beta-strategy, the assessor draws an imaginary line between the two categories called 'reference sample' and 'different sample'. It is the position of the line that determines the beta-criterion, which corresponds to a level of sensory evidence. Each stimulus is evaluated independently and the likelihood that a sample falls into one category or another is compared to the beta-criterion.

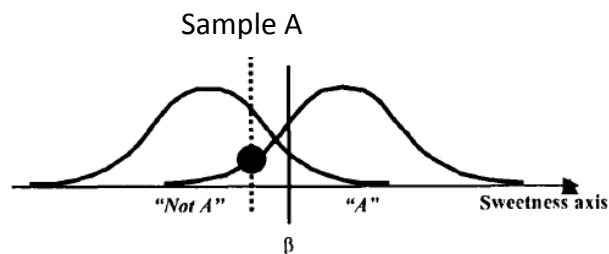


Figure 9. The beta-criterion in the A-Not A test. Source: Rousseau et al. (2001)

In a A-Not A test, the assessors answer depends on where the 'Not A' region stops and where the 'A' region starts on the intensity axis. In the case of Figure 9, the sample A is categorised as 'Not A' because its perceived intensity falls on the 'Not A' side of the beta-criterion. As with the tau-criterion, the beta-criterion is a psychological parameter and does not relate to the assessors sensitivity (Rousseau, 2001). Familiarisation with the reference product is commonly used to stabilise beta-criteria among assessors.

Some cognitive strategies are more efficient than others, and therefore assessors can perform better in some discrimination tests than others, even if the products are the same. The beta-strategy is also called the optimal decision rule, as it leads to better performance compared to the tau- and COD strategies (Rousseau, 2001, Noreen, 1981, Macmillan and Creelman, 2005). For this reason, some researchers have tried to induce the use of a beta strategy, using previous exposure (Santosa et al., 2011), prior sets of single stimulus judgments (Lee et al., 2007) or an affective approach (Lee et al., 2007, Chae et al., 2010).

Different methods can be used to determine the cognitive strategy adopted by assessors to answer a discrimination test. One method is to require the assessor to 'think aloud' and describe how he is are making his decision (Wong, 1997). However, this method assumes that assessors are actually using the cognitive strategy that they describe. A more sophisticated approach was used in this study and consists in fitting Thurstonian models

assuming different cognitive strategies to the data collected from the tests and to determine which model fits the best (Irwin et al., 1993, Hautus et al., 1994, Hautus and Irwin, 1995, Hautus et al., 2008). Both the location and the shape of the ROC curve depend on the assessor decision strategy (Figure 7) (Irwin et al., 1993, Macmillan and Creelman, 2005, Irwin et al., 1992, Hautus et al., 1994). A ROC curve asymmetrical about the negative diagonal indicates a tau-strategy, whereas a ROC curve symmetrical about the negative diagonal indicates a beta-strategy (Irwin et al., 1993, Macmillan and Creelman, 2005, Hautus et al., 2008, O'Mahony and Hautus, 2008).

A third method for the experimental confirmation of the cognitive strategy assumed is to require the same assessors to perform different types of discrimination tests. The computed d' values are expected to be the same if the assumed cognitive strategies are correct (Kim et al., 2006). This approach was used previously to confirm the cognitive strategies used for the triangle test and the 3-AFC (Stillman, 1993, Tedja et al., 1994, Rousseau and O'Mahony, 1997, Delwiche and O'Mahony, 1996) and the duo-trio test, the same-different test, and the 2-AFC (Kim et al., 2006).

1.4.4.5 R-index vs. d'

The R-index (Brown, 1974) can be used as an alternative to d' to measure the magnitude of sensory difference between samples (O'Mahony, 1992). The R-index corresponds to the probability of distinguishing between the two samples in a discrimination test and lies between 50 % and 100 % (Bi and

O'Mahony, 2007, O'Mahony, 1992). A critical value for the R-index can be used to determine if a significant difference exists between the samples (Bi and O'Mahony, 2007). O'Mahony (2012) used the R-index in omission testing to measure the relative importance of individual volatiles within a flavour model.

The R-index is an intuitive measure as it corresponds to the probability of distinguishing between the two samples in a discrimination test (O'Mahony, 1992, Bi et al., 2013). However, the R-index is a non-parametric index (Rousseau, 2011) and it is method-dependant, as it does not take into account the decision strategy used by the assessors to answer the discrimination test. For example, the same R-index obtained with the A-Not A and same-different tests does not correspond to the same underlying sensory difference (Ennis et al., 2014).

R-index and d' are related, as the R-index is equivalent to the area under the ROC curve (Rousseau, 2011, O'Mahony, 1992). Both d' and R-index remove the response bias related to the A-Not A and same-different tests (O'Mahony, 1992). The Thurstonian d' presents some advantages over the R-index, as it is independent of the discrimination test used and it takes into account the cognitive strategy. Furthermore, d' values can be analysed and compared using parametric statistics. In conclusion, the Thurstonian measure d' is a very useful tool to compare methods and performances (Ennis, 1990).

1.4.5 Statistical power and sensitivity of discrimination tests

The statistical power ($1 - \beta$) of a discrimination test is defined as the test ability to detect a difference when the difference exists (Rousseau and O'Mahony, 2000). It corresponds to the probability that the null hypothesis will be rejected when the null hypothesis is false. Some discrimination tests have higher statistical power than other. For example, the statistical power of the tetrad test is higher compared to the triangle test (Ennis and Jesionka, 2011, Ennis, 2012, Ennis et al., 2014). Modelling showed that the longer version of the same-different test (see section 1.4.1.3) was more powerful compared to the triangle and duo-trio tests (O'Mahony and Rousseau, 2002).

When the statistical power is the same, the discrimination tests should theoretically yield similar d' values. However, external factors can interfere with the assessors performance and add noise to the perceptual distribution (Rousseau et al., 1999, Van Hout et al., 2011, Kim and Lee, 2012).

The sensitivity of a discrimination test is characterised by the noise added to the perceptual distributions by external factors such as carry-over, memory effects, sensory adaptation, familiarisation and fatigue. Here, the term 'fatigue' is used to refer to the feeling of tiredness and lack of concentration of assessors when too many tasks are given in one session. A test will be described as more sensitive if it adds less noise to the perceptual distributions (Rousseau et al., 1999, Stocks et al., 2013).

The Thurstonian d' is a measure of the sensitivity of a discrimination test: a more sensitive test yields larger d' values (Rousseau et al., 1999). Since d' is measured in term of standard deviations of the perceptual distribution, the larger the amount of noise, the smaller the d' value, and the less likely an existing sensory difference will be detected (Rousseau and O'Mahony, 2000, Rousseau et al., 1999).

1.4.6 Determination of the number of assessors

BS ISO 4120:2004 and ASTM E2139-05 propose a statistical approach to determine the number of assessors that should be used in triangle and same-different tests, respectively. For the triangle test, BS ISO 4120:2004 recommends setting α , β and P_d (the proportion of true discriminators or maximum allowable proportion of distinguishers). A common value of P_d is between 0.25 and 0.35. For the same-different test, setting values for α , β , P_1 (the proportion of assessors in the population who would respond 'different' to a same pair) and Δ (the minimum difference in proportion that the researcher wants to detect) is recommended. Δ corresponds to the difference between P_2 (the probability of responding different to unmatched pair) and P_1 . Commonly, P_1 and Δ are both set at 0.3.

Although they have become widespread, Δ and P_d are not good standard measures of the underlying sensory difference. Firstly, as mentioned in ASTM E2139-05, the same value of Δ can correspond to different underlying measures of sensory difference, as P_1 varies. On the other hand, P_d is directly

related to the proportion of correct answers P_c , and thereby highly method specific (Ennis, 1993, Jesionka et al., 2014). Furthermore, the assumption required for the calculation of P_d that some assessors always make correct judgments while other are always guessing, is not valid (Ennis and Rousseau, 2013).

The Thurstonian approach can be used to determine the number of assessors, based on the size of the difference that the researcher wants to investigate (Ennis, 1993, Jesionka et al., 2014). In this approach, five related parameters: the type of discrimination test, the size of the difference of interest δ , the significance level α , the statistical power $(1 - \beta)$ and the number of assessors are involved. As all the parameters are related, if four parameters are set, the fifth parameter becomes fixed and can be calculated from published tables (Ennis and Jesionka, 2011).

The aim of sensory discrimination testing in a Thurstonian perspective is not to determine whether or not the difference is perceived, but to establish accurately the size of the difference (Jesionka et al., 2014). One major advantage of the Thurstonian approach is that it can be used to determine the number of assessors needed to compare the sensitivity of different discrimination tests (Ennis, 1990, Jesionka et al., 2014, O'Mahony and Rousseau, 2002, Rousseau et al., 1998).

1.5. Objectives and experimental approach

A new approach in sensory omission testing was developed at the University of Nottingham (O'Mahony, 2012), which uses the same-different test associated with a sureness rating. The main focus of this thesis was to fully define and evaluate the same-different approach, and to investigate its application to help gain a better understanding of flavour. The project had five main objectives:

1. The first objective was to identify a period of stability for both the strawberry and savoury flavour models. Any change that occurs over time in a flavour itself may affect perception. As this could confound the results of sensory experiments, it was crucial to assess the stability of the flavour models over the period required to complete omission studies, so that any perceived differences could be attributed to the omission tests. Chapter 3 investigates the stability of the strawberry and savoury flavour models over time to inform the design of the subsequent sensory studies.

2. The second objective (presented in chapter 4) was to evaluate the same-different approach. First, the cognitive strategy used by assessors to answer the same-different tests was investigated as different cognitive strategies can be adopted. In a second part, the same-different approach was compared to the triangle approach in terms of sensitivity.

3. The third objective was to apply the same-different approach to assess the relative importance of individual volatiles in the sweet and the savoury

flavours, delivered ortho- or retronasally. 'Fractional omission testing' was used to measure the effect of a decrease in volatile concentration on the flavour perceived orthonasally. Sensitivities via ortho- and retronasal routes were compared. This is discussed in chapter 5.

4. The fourth objective was to use the same-different approach to explore interactions between volatiles in flavour mixtures. In the first part of chapter 6, d' values obtained from omission tests were compared to OAVs of individual volatiles to determine if OAVs of the aroma compounds reflect the relative importance of individual volatiles in flavour mixtures. The second part of chapter 6 explored interactions between specific volatiles in the savoury flavour mixture delivered orthonasally. 'Group omission testing' was used: two or more volatiles were removed from the savoury flavour before comparing the new sample to the original flavour model.

5. The fifth objective was to employ the same-different approach to investigate interactions between volatiles and tastants in flavour at two different levels: the physico-chemical interactions in the food matrix before consumption; and the cross-modal interactions at a cognitive level after consumption. This is discussed in chapter 7.

The materials and methods used to meet the outlined objectives are discussed in their relevant chapters, but the following chapter (chapter 2) presents the general materials and methods used throughout this research project.

Chapter 2. Materials and methods

2.1 Materials

The experiments in this research project involved the use of two different flavour models: a strawberry flavour and a savoury flavour model.

The strawberry flavour model was based on a commercial product developed by a flavour company (Aromco, UK) which is used in a wide range of products.

The strawberry flavour was composed of 9 volatiles (all Sigma Aldrich, UK) (Table 4). The volatiles involved were grouped into 4 flavour blocks, buttery, fruity/floral, caramel and green, based on their particular sensory character.

Table 4: Concentration of the volatiles in the strawberry flavour.

Flavour block	Volatile	Concentration in PG (mg/kg)	Aroma
Fruity/ floral	Ethyl butanoate	5,000	Fruity
	Ethyl hexanoate	3,360	Green, pineapple
	Methyl dihydrojasmonate	3	Jasmine
Buttery	2,3-Butandione	5	Buttery
	Butanoic acid	920	Sweaty, rancid
	Gamma-decalactone	1,330	Fatty, peach-like
Caramel	4-Hydroxy-2,5-dimethyl-3-furanone (furaneol™)	10,700	Strawberry, caramel
	Methyl(E)-3-phenylprop-2-enoate	2,700	Balsamic, strawberry
Green	Cis-3-hexen-1-ol	10,800	Leaf-like

A savoury flavour model containing 9 volatiles (Table 5) was developed from the boiled beef flavour model published by Kerschler (Kerschler and Grosch, 1999). The 9 volatiles were grouped into 3 different flavour blocks: top note,

meaty block and fatty block. Indole was added to the savoury flavour as a control. As it was unlikely that indole would contribute to the sensory quality of the savoury flavour model, its removal in subsequent discrimination testing was not expected to be detected significantly. 12-methyltridecanal was supplied by Symrise (UK), and all other volatiles were purchased from Sigma-Aldrich (UK).

Table 5: Concentration of the volatiles in the savoury flavour.

Flavour block	Volatile	Concentration in PG (mg/kg)	Aroma
Top note	2-Methylpropanal	23.4	Green, pungent
Meaty block	2-Furfurylthiol	43.5	Roasty
	4-Hydroxy-2,5-dimethyl-3-furanone (furaneol™)	13,600	Caramel
	3-Mercapto-2-butanone	103	Meat, fried onion
	2-Methyl-3-furanthiol	36	Roast meat
	3-Methylthiopropional	54	Potato
Fatty block	E,E-2,4-Decadienal	27	Deep fried
	12-Methyltridecanal	962	Sweaty
	1-Octen-3-one	9.4	Mushroom
Control	Indole	70	Sweet, burnt

Propylene glycol (PG) (Sigma Aldrich, UK) was used as a solvent for the volatiles in the flavours as it is easily miscible with the related compounds and works effectively as a volatile carrier (Seidenfeld and Hanzlik, 1932). Other consumables included Evian™ mineral water (Danone Group, France) used as

a palate cleanser during sensory testing and as a solvent for any aqueous solutions. Plain, unsalted matzo crackers (Rakusens Limited, UK) were also used for palate cleansing.

2.2 Methods

2.2.1 Preparation of the flavour samples

2.2.1.1 Preparation of the flavours in PG

Strawberry and savoury flavours were prepared exactly to specification (Table 6 and Table 7, respectively) by pipetting the volatiles into Duran® GL 45 laboratory glass bottles (SCHOTT, USA) using a calibrated balance and allowing a 5 % error, as this is the method used by Mars (Waltham).

The strawberry flavour model was prepared as described in Table 6. Secondary bases were prepared for methyl dihydrojasmonate and 2,3-butandione, as these two volatiles were of very low concentration in the strawberry flavour model (3 and 5 mg/kg, respectively). The secondary base was then diluted into the primary bases, which was subsequently added to the strawberry flavour model. The strawberry flavour model was diluted in PG and mixed on a roller bed for 30 minutes and kept at 4° C.

Table 6: Specification for preparation of the strawberry flavour model

Volatile	Flavour model in PG (g)	Primary base (g)	Secondary base (g)
Ethyl butanoate	0.5		
Ethyl hexanoate	0.336		
Methyl dihydrojasmonate	1g primary base	1g secondary base	0.3
2,3-Butandione	1g primary base	1g secondary base	0.5
Butanoic acid	0.092		
Gamma-decalactone	0.133		
4-Hydroxy-2,5-dimethyl-3-furanone (15 % in PG)	7.15		
Methyl (E)-3-Phenylprop-2-enoate	0.27		
Cis-3-hexen-1-ol	1.08		
Total made with PG (g)	100	100	10

The savoury flavour model was prepared as described in Table 7. Secondary bases were prepared for 2-methylpropanal, 2-furfurylthiol, 3-mercapto-2-butanone, 2-methyl-3-furanthiol, 3-methylthiopropional and E,E-2,4-decadienal. A tertiary base was prepared for 1-octen-3-one. The savoury flavour model was diluted in PG and mixed on a roller bed for 30 minutes and kept at 4° C.

Table 7: Specification for preparation of the savoury flavour

Volatile	Flavour model in PG (g)	Primary base (g)	Secondary base (g)	Tertiary base (g)
2-Methylpropanal	1g secondary base		0.234	
2-Furfurylthiol	1g secondary base		0.435	
4-Hydroxy-2,5-dimethyl-3-furanone	1.36			
3-Mercapto-2-butanone	1g secondary base		1.035	
2-Methyl-3-furanthiol	1g secondary base		0.36	
3-Methylthiopropional	1g secondary base		0.54	
E,E-2,4-Decadienal	1g secondary base		0.27	
12-Methyltridecanal	10g primary base	0.962		
1-Octen-3-one	10g primary base	1g tertiary base		0.94
Indole	1g secondary base		0.7	
Total made with PG (g)	100	100	100	100

Omission samples (n - 1) were prepared as described above, by omitting one volatile from the original flavour model (n). 9 omission samples were prepared for the strawberry flavour and 10 for the savoury flavour, each omission sample omitting one volatile from the original flavour model.

In addition, 'fractional omission samples' (n - 0.5) were prepared by removing 50 % of a volatile from the savoury flavour model, and 'group omission samples' were prepared by removing 2 or more volatiles from the savoury flavour model.

2.2.1.2 Dilution of the flavour samples in water

Certain sets of experiments (straw4, straw5, straw6, straw7 and all the experiments on the savoury flavour) (Table 8) required the dilution of the flavour models with water before conducting the sensory sessions. The strawberry and savoury flavours (and corresponding omission samples) were diluted in mineral water at 0.75 % and 0.1 % w/w, respectively. Flavour samples in water were kept at 4° C and used within 24 hours.

2.2.1.3 Addition of tastants

To investigate the interactions between volatiles and tastants, congruent tastants were added to the flavours diluted in water.

Sucrose and citric acid (Sigma Aldrich, UK) were added to the strawberry flavour in water, alone or in combination, at 2 % and 0.05 % w/w, respectively. The concentrations of sucrose and citric acid in the strawberry flavour were determined by O'Mahony (2012) to give the right balance to the strawberry flavour.

Sodium chloride, Inosine Monophosphate (IMP), Monosodium Glutamate (MSG) and proline (Sigma-Aldrich, UK) were added to the savoury flavour in water, alone or in combination. The concentrations used were 3.6 %, 0.0526 %, 0.8 %, and 2.5 % w/w, respectively. The concentrations of tastants for the savoury flavour were developed at Mars (WALTHAM®) to give a sensible balance to the savoury flavour.

The tastants concentrations used in the flavour models were not equi-intense. According to the study of Green *et al.* (1996) a concentration of NaCl of 3.6% corresponds to a log perceived intensity of about 1.5, whereas a concentration of sucrose of 2% corresponds to a log perceived intensity below 0.6 on the Labelled magnitude Scale (LMS).

In the current PhD study, the volatile concentrations in the strawberry flavour model in water varied from 2.25 E^{-6} to $8.1 \text{ E}^{-3} \%$ (w/w). According to Green *et al.* (1996), the perceived intensity of Phenyl Ethyl Alcohol PEA (floral smell) concentrations between 2.25 E^{-6} and $8.1 \text{ E}^{-3} \%$ (w/w) were equivalent to sucrose concentrations between 0 and 0.12% (w/w). These sucrose concentrations are much lower than the concentration used in this study (2% w/w). This will be discussed in section 7.3.2.3.

2.2.2 pH measurements

The pH of flavour samples in PG or water were measured using an inoLab® pH Meter Level 1 (WTW, Germany) and a Sentinex® 82 pH electrode (WTW, Germany). pH measurements were conducted at room temperature ($20^\circ \text{ C} \pm 2^\circ \text{ C}$).

2.2.3 Omission experiments

Omission testing measures the impact of removing (i) one volatile completely, (ii) a fraction of a volatile, or (iii) several volatiles at a time, on perceived flavour. Various sets of omission experiments were carried out throughout the duration of this research project to meet the objective outlined in the introduction.

An omission test refers to a discrimination test comparing one omission sample ($n - 1$), with the original flavour model (n). Nine and ten omission tests were carried out with the strawberry and savoury flavours, respectively, to compare each omission sample with the original flavour model.

'Fractional omission testing' refers to a discrimination test comparing 'fractional omission samples' ($n - 0.5$) with the original flavour model.

'Fractional omission testing' was carried out to measure the effect of a decrease in volatile concentration on the flavour perceived.

'Group omission testing' refers to a discrimination test comparing a 'group omission sample' with the original flavour model. 'Group omission testing' can be carried out to investigate interactions between specific volatiles in flavour mixture.

2.2.4 Sensory sessions

All sensory sessions were carried out in isolated booths. Naïve assessors (~ 80 % females and 20 % males, aged between 18 and 25) were recruited from the students of the University of Nottingham. 'Naïve assessors' refers to the fact that there was no screening and no training for the assessors for the current study. New assessors were recruited for each sensory session although assessors were allowed to take part in as many sessions as they wanted. Measurement of detection thresholds and checking for anosmia were not carried out in the current study, due to organisation constraints related to the high number of volunteers. Literature values were used to estimate ortho- and retronasal detection thresholds.

All assessors signed to indicate that they had given informed consent to participate in the study. Assessors were instructed to fast (except water) at least one hour prior to the sessions. FIZZ software (Biosystèmes, France) was used to design the sensory sessions. The order of presentation for the samples was randomised over each test, and discrimination tests were randomised over each session.

The different omission experiments performed as part of this research project are discussed in detail in their relevant chapters but are summarised in Table 8. Sessions Straw1 to Straw7 were conducted on the strawberry flavour, while sessions Sav1 to Sav6 were conducted on the savoury flavour.

Table 8: Omission experiments used throughout the research project

Flavour model	Session	Delivery	Dilution	Discrimination test	Number of assessors	Purpose	Chapter
Strawberry flavour	Straw1 †	orthonasal	PG	same-different	50	To assess $P_{s/s}$ * To determine the cognitive strategy	Chapter 4
	Straw2 †	orthonasal	PG	same-different	50	To compare the same-different and triangle tests To determine the cognitive strategy	Chapter 4
	Straw3	orthonasal	PG	triangle	72	To compare the same-different and triangle tests	Chapter 4
	Straw4 †	retronasal	water	same-different	100	To compare ortho and retronasal sensitivities To determine the cognitive strategy	Chapter 4, 5 and 6
	Straw5	orthonasal	water	same-different	100	To compare ortho and retronasal sensitivities	Chapter 5 and 6
	Straw6 †	retronasal	water + sucrose	same-different	100	To assess the effect of sucrose	Chapter 7
	Straw7 †	retronasal	water + citric acid	same-different	100	To assess the effect of citric acid	Chapter 7
Savoury flavour	Sav1	orthonasal	water	same-different	100	To compare ortho and retronasal sensitivities	Chapter 5 and 6
	Sav2	retronasal	water	same-different	100	To compare ortho and retronasal sensitivities	Chapter 5 and 6
	Sav3	orthonasal	water	same-different	100	To conduct 'fractional omission testing'	Chapter 5
	Sav4	orthonasal	water	same-different	100	To conduct 'group omission testing'	Chapter 6
	Sav5	orthonasal	water	same-different	100	To conduct 'group omission testing'	Chapter 6
	Sav6	retronasal	water + tastants	same-different	100	To assess the effects of the savoury tastant mixture	Chapter 7

* $P_{s/s}$: Proportion of assessors who answered 'same' for the same pair of samples

† Raw data from O'Mahony (2012)

Table 8 describes, for each omission experiment, the mode of delivery (ortho- vs. retronasal delivery), the matrix of the flavour sample (in PG or in water, with or without tastants), the discrimination test (triangle or same-different tests), the number of assessors, the purpose of the experiment and the chapter(s) where the experiment will be mostly used. Raw data obtained from previous omission experiments on the strawberry flavour (O'Mahony, 2012) were incorporated into this research project in order to conduct further data analysis.

Sessions Straw1, Straw2 and Straw4 were used to determine the cognitive strategy used by the assessors to answer the same-different tests conducted in the current study, either ortho- or retronasally. Straw 1 was used to calculate $P_{s/s}$ (proportion of assessors who answered 'same' for the same pair of samples), in order to determine the number of assessors required to compare the triangle and same-different approaches.

Session Straw3 was conducted to allow subsequent comparison with Straw2, in order to compare the triangle and same-different approaches.

Session Straw4 was carried out orthonasally to determine the relative importance of individual volatiles in the strawberry flavour diluted in water.

Session Straw5 was carried out retronasally to allow subsequent comparison with session Straw4, in order to compare ortho- and retronasal sensitivities.

Session Sav1 was conducted orthonasally to determine the relative importance of individual volatiles in the savoury flavour. Session Sav2 was

conducted retronasally to allow subsequent comparison with session Sav1, in order to compare ortho- and retronasal sensitivities.

Session Sav3 involved 'fractional omission testing' on the savoury flavour to determine the effect of a change in volatile concentration on orthonasal perception of flavour.

Sessions Sav4 and Sav5 involved conducting 'group omission testing' to investigate interactions between specific volatiles in the savoury flavour delivered orthonasally.

Sessions Straw6 and Straw7 were conducted and subsequently compared to session Straw4 to determine the effect of the addition of sucrose or citric acid on the assessors sensitivity to the removal of individual volatiles.

Session Sav6 was carried out and subsequently compared with session Sav2 to determine the effect of the addition of the savoury tastant mixture on the assessors sensitivity to the removal of individual volatiles.

2.2.5 Sample presentation

All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure flavour samples were at room temperature (20° C \pm 2° C).

As it was shown previously that the instructions given can affect the cognitive strategy used by assessors to answer a discrimination test (Rousseau, 2001), it is important to mention that during the recruitment process and the sensory

sessions, the strawberry flavour was referred to as a 'strawberry flavour', and the savoury flavour was referred to as a 'savory flavour' for the assessors.

For orthonasal delivery, screw top 20 ml glass bottles containing 10 ml of the sample were presented to the assessors. Assessors were instructed to sniff the samples and replace the lid immediately to prevent aroma dispersing throughout the test area.

For retronasal delivery, assessors were instructed to sip from a 20 ml sample through the straw of a lidded pot (thus avoiding orthonasal detection) (Figure 10). Mineral water and crackers were provided as a palate cleanser between samples to minimize carry-over effect.



Figure 10: Retronasal sampling pots labelled with a random three digit code.

2.2.6 Discrimination testing

All sensory testing was carried out in the Sensory Science Centre testing booths at the University of Nottingham (ISO 8589:2007) under Northern Hemisphere daylight. A 5 minute break was allocated after every 2
80

discrimination tests to limit sensory fatigue and carry-over effects. For retronasal delivery, assessors were instructed to use water and crackers as a palate cleanser between samples to minimize carry-over effect.

2.2.6.1 Triangle tests

For each triangle test (ISO 4120: 2007), assessors were given three samples simultaneously and told that two were the same and one was different. They were instructed to assess the samples from left to right and indicate which was the odd sample. They were allowed to re-evaluate the samples if necessary. A complete randomised balanced design was used for sample presentation.

2.2.6.2 Same-different tests

The protocol used throughout this research project was an extension of the same-different test (ASTM E2139-05 2011) with a sureness rating (Irwin et al., 1993). Assessors were presented simultaneously with 2 samples. They were instructed to assess the samples from left to right and to state whether they thought they were the same or different. Secondly, the assessors were asked to state the sureness level of their decision, represented by a four point surety scale ('very unsure', 'unsure', 'sure', 'very sure'). Assessors were allowed to re-evaluate the samples if necessary.

A complete randomised balanced design was used for the sample presentation with half of the assessors presented with a 'same pair' and the other half presented with a 'different pair'. The data from the same-different

tests with sureness rating were organised as shown in Table 9 to facilitate calculation of d' values. A separate table was compiled for each same-difference test.

Table 9: Data obtained from the assessors answers to a same-different test with a sureness rating

	Answered 'same'			Answered 'different'				
	Very sure	sure	unsure	Very unsure	Very sure	sure	unsure	Very unsure
Same pair	a	b	c	d	e	f	g	h
Different pair	i	j	k	l	m	n	o	p

2.2.7 Data analysis

2.2.7.1 Estimation of the Thurstonian d'

2.2.7.1.1 Triangle test

Two different cognitive strategies, the COD and the beta-strategy, can be used to answer the triangle test (see section 1.4.4.4). Using signal detection theory, Versfeld (1996) established a relationship between the probability of a correct response P_c and d' (psychometric function) for the triangle test. This psychometric function was derived in the case of different degrees of correlation among observations: 1) highly correlated observations, which corresponds to the COD strategy and 2) independent observations, which corresponds to the beta-strategy. The model associated with the beta strategy generates lower d' values compared to the model associated with

the COD strategy. For example, a proportion of correct answers (P_c) of 0.45 corresponds to a d' value of 1 when the beta strategy is assumed, whereas the same P_c corresponds to a d' of 1.2 when the COD strategy is assumed.

When the COD-strategy was assumed (COD-triangle), d' values were estimated using the proportion of correct answers P_c and published tables (ASTM E2262-03). Equation 1 was used to estimate the variance of d' (Bi et al., 1997), where n was the number of assessors and S was the standard deviation. Values for the coefficient B were taken from the table in ASTM E2262-03.

$$S^2 = B/n$$

Equation 1: Calculation of the variance of d' for triangle tests

When the beta-strategy was assumed (beta-triangle), the d' values were estimated using P_c and published tables (Versfeld et al., 1996).

2.2.7.1.2 Same-different test

For each omission test, the maximum likelihood estimate d' and its variance were obtained using ROC fitting (see section 1.4.4.3). ROC curves were modelled from data obtained from omission tests (as presented in Table 9), using ROC fitting software (SDT Assistant version 1.0, available from <http://hautus.org>). Two different models based on equal-variance perceptual distributions were used: the differencing model, associated with the tau-strategy, and the likelihood-ratio (LR) model, associated with the beta-

strategy. The best fitting ROC curve was obtained by systematically adjusting the value to minimise the goodness of fit statistic (chi-square). The chi-square corresponds to the normalised squared distance between each data point and the estimated ROC curve (M. Hautus, personal communication).

The LR model generated lower d' values compared to the differencing model (see section 4.3.1.1). The difference in d' varied from 0.27 units (19.7 %) for 2,3-butandione to 0.51 units (20.8 %) for Methyl(E)-3-phenylprop-2-enoate.

2.2.7.2 Confidence intervals for d'

95 % confidence intervals were built for d' using R software version 3.1.0 (R development Core Team, 2014), based on likelihood statistics. The 'discrim' function was used for the triangle test and the 'dod' function was used for the same-different test (Christensen, 2014). For same-different tests, confidence intervals based on the likelihood root statistic are more appropriate compared to the Wald statistic (Christensen and Brockhoff, 2009, Christensen, 2014). This is because Wald-based confidence intervals are only appropriate for large sample sizes and when d' is around 2-3 (Christensen and Brockhoff, 2009).

2.2.7.3 Testing for a significant difference between the samples

R software version 3.1.0 (R development Core Team, 2014) was used along with the sensR package (Christensen, 2014) to determine if significant differences were perceived between the original flavour model and omission samples (at $\alpha = 0.05$).

For the triangle test, binomial tests were computed using R software.

For the same-different tests, the signed square root of the Pearson statistic was used to test for a difference, using the 'dod' function in sensR (Christensen, 2014). The signed square root of the Pearson statistic corresponds to the relative difference between the frequencies of Hit, False Alarm, Miss and Correct rejections (Table 10) at the maximum likelihood estimate and under the null model (e.g. at $d' = 0$) (R.H.B. Christensen, personal communication). This method is relevant when the tau-strategy is assumed for the same-different test (R.H.B. Christensen, personal communication). Results on cognitive strategy (discussed later in section 4.3.1) showed that the hypothesis of the tau-strategy was appropriate in this study.

Table 10: Frequencies obtained for the same-different test

	Answered 'same'	Answered 'different'
Same pair	Hit	Miss
Different pair	False Alarm	Correct Rejection

Chapter 3. Determining the stability of the strawberry and savoury flavours

3.1 Introduction

3.1.1 Understanding flavour stability

The stability of a flavour ensures the sensory properties of the product remain constant from manufacture to the time of consumption. Flavour stability is a critical issue in the food and flavour industry, as instability can result in the decrease and/or disappearance of important volatiles, as well as the creation of volatiles generating new aromas (Grab, 1994). The majority of the published work on flavour stability is related to microbiology, pharmaceuticals, or naturally occurring flavour in food and there is only limited information in the literature on the stability of flavourings that are added to food or beverage.

Flavour stability is well known as the key factor determining the shelf life of flavours but it should also be considered when carrying out sensory studies on model flavour systems. In particular, some sensory studies require large numbers of assessors and may be carried out in multiple sessions over several days. It is therefore important that the flavour being tested remains stable for the duration of the sensory test so the results can be attributed to the experimental treatment rather than flavour instability. As such, sensory assessments of model flavours are necessary to determine when flavour

instability is detectable by a consumer panel to define the shelf life of model flavours.

3.1.2 Objectives of this chapter

The objective of this study was to determine the shelf life of two model flavours to determine if a model flavour would be stable for a future sensory test involving multiple sessions over several days. Two model flavours were used: a sweet strawberry flavour containing nine volatiles and a savoury beef stock flavour containing ten volatiles.

First, an analytical approach, using gas chromatographic methods (GC-MS and GC-FID), was used to measure the chemical changes in the strawberry and savoury flavours over time.

Sensory discrimination tests were carried out to determine if a panel of assessors was able to detect the changes in the flavours with time. Sensory results were compared with instrumental analysis to determine if the chemical changes in the flavours could be detected by the assessors.

In addition, a 2 week old and a 4 week old savoury flavour sample were compared, to investigate if the flavour had stabilised over time.

As significant changes were measured in the savoury flavour, both sensorially and analytically, GC-O was used to gain deeper insight into the savoury flavour and to compare the sensory perception of the fresh and the 4 weeks aged savoury flavour.

3.2 Materials and methods

Gas chromatographic methods and sensory experiments were used to measure the changes in the strawberry and savoury flavours over time. For the strawberry flavour, 1 and 8 day old samples were compared as the maximum length of any future study was to be a week. Fresh and aged (1, 4, or 7 days old) savoury flavours were compared. In addition, a two week old and a four week old savoury flavour were compared to investigate if flavour stabilisation occurred over time.

3.2.1 Instrumental analysis

3.2.1.1 Samples preparation

3.2.1.1.1 Preparation of the flavours

The strawberry flavour in PG was prepared as described in section 2.2.1.1. 2,3-Butandione and methyl dihydrojasmonate were tested using the primary base (Table 6) due to their low concentration in the flavour. The strawberry flavour in PG and the primary base in PG were stored at 4° C and sampled after 1 and 8 days.

As pH can have an effect on the stability of 4-hydroxy-2,5-dimethyl-3-furanone (Hirvi et al., 1980), the pH of the fresh strawberry flavour in PG was measured as described in section 2.2.2.

The savoury flavour was prepared as described in section 2.2.1.1. Because of their low concentrations in the flavour, 2-methyl-3-furanthiol, E,E-2,4-decadienal, and 3-methylthiopropional were tested using the secondary base

(table 7). The savoury flavour and secondary base were stored at 4° C and sampled at 0, 1, 4, 8, 14 and 28 days.

3.2.1.1.2 Preparation of the internal standard

An internal standard (5 g/L ethyl vanillin in diethyl ether) was used to take into account variations involved in the analysis procedure. The internal standard was prepared by diluting 0.5 g of ethyl vanillin into 100 ml of diethyl ether. The internal standard was prepared fresh to avoid evaporation of diethyl ether and concentration of ethyl vanillin in the solution.

3.2.1.1.3 Preparation of the samples for GC-MS, GC-FID and GC-O

Three chromatographic methods, GC-MS, GC-FID and GC-O, were used in this study. GC-MS was used to assess the purity of the standard volatile samples. GC-FID was used to measure the volatile concentrations in the flavour mixtures at different age points. As results showed major changes over time within the savoury flavour, GC-O was used to compare the fresh and the 4 weeks aged savoury flavours in order to gain a deeper insight into the savoury flavour.

The flavour, primary or secondary base in PG (0.2 g) was diluted in 2 ml of diethyl ether, and 50 µL of the internal standard was added to the solution. The samples were shaken and aliquoted into 2 ml amber vials. The vials were capped immediately to prevent any loss from evaporation. The sampling procedure was carried out 12 times for each sample, as this is the method used by Mars, WALTHAM (Jones, L. personal communication).

3.2.1.2 GC analysis

3.2.1.2.1 GC-MS analysis

An Agilent system comprising of a 7890A Gas Chromatograph with G4513A autosampler and 5975C Mass Spectrometer Detector (MSD) was used (Agilent, US). The analysis was performed on a polar Phenomenex FFAP 30 m x 0.25 mm x 0.25 μ m column. The samples (injection volume 1 μ l) were applied to the column using a cold-on-column inlet that was programmed to track oven temperature. The flow rate of the carrier gas, helium, was held at a constant 1.75 ml/min. The temperature of the GC oven was initially 30° C, with the first ramp of 60° C at 3° C/min with no hold time. The second ramp was to a temperature of 180° C at 8° C/min with no hold time. The final ramp was to 250° C at 60° C/min and a hold time of 5 min. The inlet initial temperature was 30° C with a ramp of 5° C/s with a hold time of 5 min.

3.2.1.2.2 GC-FID analysis

An Agilent 7890A Gas Chromatograph with a standard FID was used to analyse the flavour. The column used was a polar Phenomenex FFAP (30 m x 0.32 mm, 0.25 μ m film thickness). The samples (injection volume 1 μ l) were applied to the column using a cool-on-column inlet that was programmed to track the oven temperature. The flow rate of the carrier gas, Helium, was held constant at 2.5 ml/min. The GC oven and inlet initial temperature was held at 35° C for 1 min, with a ramp of 60° C/min for 1 min, then 6° C/min to a final temperature of 250° C, holding for 10 min. The total run time was 42.18

min. The FID temperature was held at 250° C, with hydrogen flow of 40 ml/min, airflow at 450 ml/min and make-up gas (nitrogen) at 45 ml/min flow.

For the experiments on GC-MS and GC-FID, the same column was used for both the strawberry and the savoury flavour mixture. A good clear separation was obtained for each volatile compound in both the strawberry and the savoury flavours. Butanoic acid in the strawberry flavour was not detected on the GC-FID as it co-eluted with the PG peak. 12-Methyltridecanal was not detected due to its low concentration in the savoury flavour mixture.

3.2.1.2.3 GC-O analysis

The instrumentation used to analyse these samples was an Agilent GC and MS fitted with a Gerstel Multi Purpose Sampler (MPS). The column used was a ZB-FFAP 30 m x 0.25 mm x 0.25 µm and the injection volume was 1 µl. The initial oven temperature was 35° C with a hold time of 2 min. The oven was ramped at 8° C per min to 250° C and held for 5 min. The inlet temperature was 250° C. Post column eluent was split 1:1 to MS and a Gerstel ODP 3 odour detection port. The sample was incubated for 45 min at 35° C, extracted for 15 min and the desorption time was 5 min.

In this study, the olfactometry panel consisted of two experienced assessors, 1 male (assessor A) and 1 female (assessor B), between 25 and 35 years old. Each assessor carried out GC-O on the same flavour sample. Assessor A assessed each sample in duplicate, while assessor B assessed each sample 5 times. Delahunty *et al.* (2006) recommended using 6 to 12 assessors for GC-O

analysis. Here, the risk of using only 2 assessors is that specific anosmia and differences in sensitivity could impact the results.

The frequency of a volatile detection at a particular retention time was calculated from the 7 GC-O runs (2 replicates for assessor A and 5 replicates for assessor B). This method is commonly used to determine whether individual volatiles are perceived in flavour (Stevens, 1961, Delahunty et al., 2006). The volatiles that are detected more frequently are concluded to have a greater importance in the perceived flavour. Peaks detected at least 70 % of the time were included in this study, as this is the method used by Mars (WALTHAM®) (J. Addison, personal communication).

3.2.1.3 Data analysis

A five point calibration standard was prepared for analysis on GC-FID. The concentrations in diethyl ether were 0.2 %, 0.15 %, 0.1 %, 0.05 %, and 0.03 % (m/v) for the strawberry flavour; and 0.01 %, 0.005 %, 0.002 %, 0.001 %, 0.0005 % and 0.0002 % (m/v) for the savoury flavour.

3.2.1.3.1 Calculation of the Response Factors

Linearity graphs were obtained from the calibration results on the GC-FID.

The Response Factor (RF) was calculated for each volatile compound based on

Equation 2:

$$RF_v = \frac{Area_v}{Area_{is}} \times \frac{Amount_v}{Amount_{is}}$$

Equation 2: Calculation of the Response Factor for a volatile (RF_v). Amount_v: amount of volatile, Amount_{is}: amount of the internal standard, Area_v: peak area of the volatile of interest, Area_{is}: peak area of the internal standard.

3.2.1.3.2 Calculation of the volatile concentration

The concentration of the volatiles in the flavour mixture was calculated using Equation 2 and the calculated RF. The difference in peak areas was used to estimate the rate of degradation of volatile compounds. Student t-tests ($\alpha = 0.05$) (Excel 2010, Microsoft, USA) were used to determine if the compounds decreased significantly over the time period studied.

3.2.1.3.3 Identification of new volatiles created over shelf life

Compounds were identified using their mass spectrum, RI (Retention Index), and their aroma descriptors (on GC-O). Mass spectral matches were made by comparison of mass spectral libraries. The Kovats linear RI of the volatiles created over shelf life was calculated using their retention time on GC-MS and Equation 3.

$$RI = 100 \times \left(N + \frac{RT_V - RT_N}{RT_{N+1} - RT_N} \right)$$

Equation 3: Calculation of the Kovats Linear RI. RT: Retention Time, V: Volatile compound of interest, N: Number of carbon atoms in the preceding n-alkane, N+1: Number of carbon atoms in the subsequent n-alkane.

Alkane standards were run on the GC-MS to calculate the retention times RT_N and RT_{N+1} . RI available from online libraries (<http://www.pherobase.com/> and <http://flavornet.org/>) were used for preliminary identifications. Identifications were then confirmed by comparing mass spectrum and RI to those of authentic standards.

3.2.2 Sensory analysis

3.2.2.1 Preparation of the flavours

The strawberry and savoury flavours were prepared as described in section 2.2.1.1.

To compare the strawberry flavour at different age points, the flavour was prepared 8 days and 1 day before conducting sensory experiments and stored at 4° C.

To compare the savoury flavour at different age points, the flavour was prepared at 28, 14, 7, 4, 1 days, and fresh, before conducting omission experiments. The savoury flavour was diluted in mineral water at 0.1 % w/w (as described in section 2.2.1.2) before conducting omission experiments, then stored in sealed plastic bottles kept in the dark at 4° C.

3.2.2.2 Sensory sessions

The sensory sessions were conducted as described in section 2.2.4. Samples were delivered orthonasally (as described in section 2.2.5), as orthonasal perception is usually more sensitive compared to retronasal perception (Hummel et al., 2006, Bojanowski and Hummel, 2012). This assumption was confirmed later (see chapter 5) for both the strawberry and the savoury flavours.

Fifty naïve assessors carried out a series of triangle tests to compare the flavours at different age points. Sessions were designed using Fizz software and triangle tests were presented to the assessors according to a randomised balanced design. Up to 10 triangle tests were carried out in one session and a break of 5 minutes was allocated after every 2 tests to limit sensory fatigue and carry-over effects.

For the strawberry flavour, a 1 and 8 day old sample were compared as the maximum length of any future study was to be a week. For the savoury flavour, fresh and aged samples (1, 4 and 7 days old) were compared. Savoury samples were also compared at 14 and 28 days, to investigate if the savoury flavour had stabilised.

3.2.2.3 Data analysis

The objective of this section was to determine if assessors could perceive a significant difference between fresh and aged flavours. Binomial tests were computed using R software version 3.1.0 (R development Core Team, 2014) to

determine if significant differences were perceived between the two samples (at $\alpha = 0.05$).

3.3 Results and discussion

3.3.1 Stability of the strawberry flavour

3.3.1.1 Instrumental analysis

3.3.1.1.1 Results

Table 11 shows the evolution of the volatile concentrations in the strawberry flavour after 8 and 14 days, measured using GC-FID. No measurement could be carried out for butanoic acid, as it co-eluted with the PG peak in the GC-FID chromatogram. Instrumental analysis indicates that the changes in the volatile concentrations were within an acceptable range (below 8 %)

Table 11: Evolution of the volatile concentrations in the strawberry flavour. Data measurement for 1 vs. 14 day old flavour were carried out by Chu (2013)

Volatile	Decrease (%) 1 vs. 8 days old	Decrease (%) 1 vs. 14 day old (Chu, 2013)
Ethyl butanoate	- 7.43*	- 5.93*
Ethyl hexanoate	- 5.48*	- 2.69
Methyl dihydrojasmonate	0.04	- 4.96*
2,3-Butandione	- 3.43	1.84
Gamma Decalactone	- 4.76	- 1.46
4-hydroxy-2,5-dimethyl-3-furanone	4.04*	34.5*
Methyl(E)-3-phenylprop-2-enoate	- 2.84*	4.1*
Cis-3-hexen-1-ol	5.23*	0.04

* $p < 0.05$

The main change observed in the strawberry flavour was the decrease in concentration of 4-hydroxy-2,5-dimethyl-3-furanone after 14 days.

3.3.1.1.2 Discussion

Instrumental analysis indicates that the changes in the strawberry flavour were relatively small over the first 8 days. The main change observed in the strawberry flavour was the decrease in concentration of 4-hydroxy-2,5-dimethyl-3-furanone. 4-Hydroxy-2,5-dimethyl-3-furanone is present in many fruits such as strawberry, mango, and pineapple (Larsen et al., 1992). It is widely used as a flavouring agent for food and beverages due to its pleasant aroma and low detection threshold (0.6-60 µg/kg in water) (Schieberle, 1992, Rychlik et al., 1999). 4-Hydroxy-2,5-dimethyl-3-furanone is known to be sensitive to oxidation. Roscher *et al.* (1997) showed that 4-hydroxy-2,5-dimethyl-3-furanone strongly decomposed at all pH values after 32 days, when stored in capsulated vials kept in the dark at 23°C. 4-Hydroxy-2,5-dimethyl-3-furanone is particularly unstable at a pH below 3 or above 5 (Roscher et al., 1997, Hirvi et al., 1980). In this study, the low pH measured for the strawberry flavour in PG (2.39 ± 0.19) could explain the decrease in the concentration of 4-hydroxy-2,5-dimethyl-3-furanone over 14 days. One way to limit the oxidation of hydroxy-2,5-dimethyl-3-furanone would be to use nitrogen for the storage of the flavour samples.

The significant increase observed for ethyl butanoate, ethyl hexanoate, and methyl(E)-3-phenylprop-2-enoate over the first 8 days could be due to

general fluctuations of the GC-FID and changes in detector response. An increase in the concentrations of ethyl butanoate and ethyl hexanoate was previously observed in a study conducted on the same instrument (Chu, 2013). Ducruet *et al.* (2001) observed an increase in concentration of ethyl butyrate over a 50 days period of storage of a strawberry flavour. This result could not be explained.

3.3.1.2 Sensory analysis

Sensory experiments showed no perceived difference orthonasally between the 1 and 8 days old savoury flavour in PG ($P_c = 0.44$, $p = 0.076$). Although *cis*-3-hexen-1-ol and 4-hydroxy-2,5-dimethyl-3-furanone decreased significantly after 8 days ($p < 0.001$ and $p = 0.04$ respectively), the decrease remained below 6 % and was unlikely to affect the sensory perception of the strawberry flavour.

In conclusion, the sensory results corroborate instrumental analysis, and no major change was observed in the strawberry flavour between 1 and 8 days aging. This result shows that the strawberry flavour is stable for 1 week and gives flexibility for the strawberry flavour samples to be used in sensory studies over a period of one week.

3.3.2. Stability of the savoury flavour

3.3.2.1. Instrumental analysis

3.3.2.1.1 Results

Table 12 shows the decrease in the volatile concentrations between day 0 and day 1, and between day 0 and day 4. GC-MS results showed that 2-methyl-3-furanthiol (MFT) formed dimers bis(2-methyl-3-furyl) disulphide (MFT-MFT) in the net standard even before being added to the flavour. 12-methyltridecanal was not detected on GC-FID due to its low concentration in the flavour.

Table 12: Evolution of the volatile concentration within the savoury flavour

Volatile	Decrease (%) Fresh vs. 1 day old	Decrease (%) Fresh vs. 4 days old
2-Methylpropanal	-0.79	-16.17*
2-Furfurylthiol	3.39	15.37*
4-Hydroxy-2,5-dimethyl-3-furanone	9.44*	9.95*
3-Mercapto-2-butanone	7.17*	17.04*
2-Methyl-3-furanthiol (in form of MFT-MFT)	6.41*	14.05*
3-Methylthiopropional	9.02	4.84
E,E-2,4-decadienal	20.38*	37.87*
1-Octen-3-one	1.10	22.83*
Indole	4.25	4.06*

* $p < 0.05$

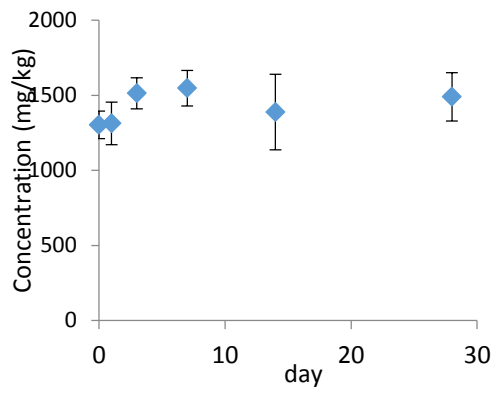
Major changes were measured in the savoury flavour after only a short period of storage. The concentration of 4-hydroxy-2,5-dimethyl-3-furanone, 3-mercapto-2-butanone, MFT-MFT and E,E-2,4-decadienal decreased

significantly after 1 day. After 4 days, all concentrations except for 3-methylthiopropional showed a significant change.

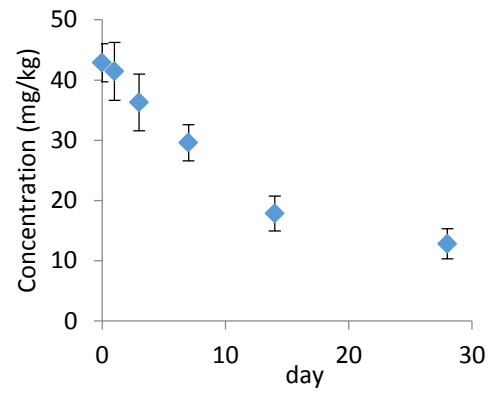
The most important change after 1 day was observed for E,E-2,4-decadienal, which decreased by more than 20 %. This is not surprising as this compound is highly reactive and reacts with PG to form acetals (Heydanek and Min, 1976). The aldehydes E,E-2,4-decadienal, 12-methyltridecanal, 2-methylpropanal and 3-methylthiopropional are highly susceptible to oxidation. They undergo condensation with other carbonyl compounds and alcohols to give aldols and acetals, respectively. 3-Mercapto-2-butanone and 2-furfurylthiol showed a dramatic change in the savoury flavour as they decreased by more than 15 % over 4 days. Sulfur compounds are known to be unstable and thiols can oxidise to give disulfides or mixed disulfides (Hofmann et al., 1996).

Figure 11 presents the volatile concentrations measured in the savoury flavour over a period of 4 weeks. The concentration of 2-methylpropanal, 4-hydroxy-2,5-dimethyl-3-furanone and indole remained relatively constant over the period of study. The concentration of 2-furfurylthiol, 3-mercapto-2-butanone, 2-methyl-3-furanthiol, E,E-2,4-decadienal decreased remarkably over the first 2 weeks, and seemed to reach a plateau after 2 weeks. 1-Octen-3-one could not be measured in the flavour after 2 weeks of aging.

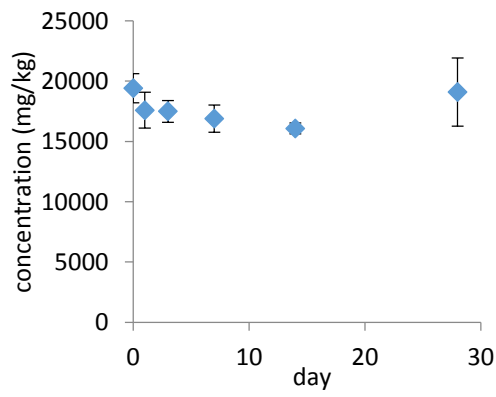
2-methylpropanal



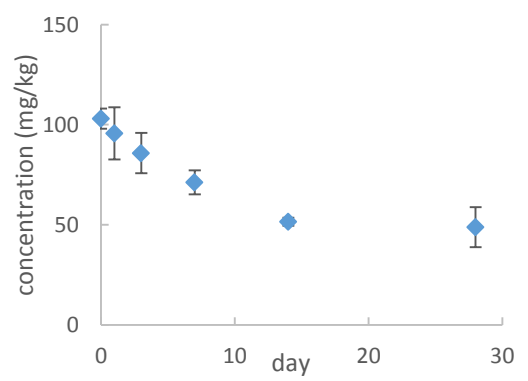
2-furfurylthiol



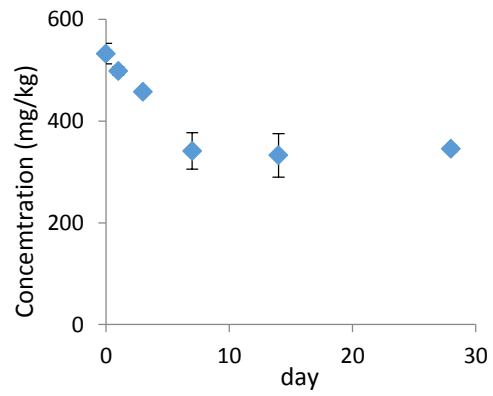
4-hydroxy-2,5-dimethyl-3-furanone



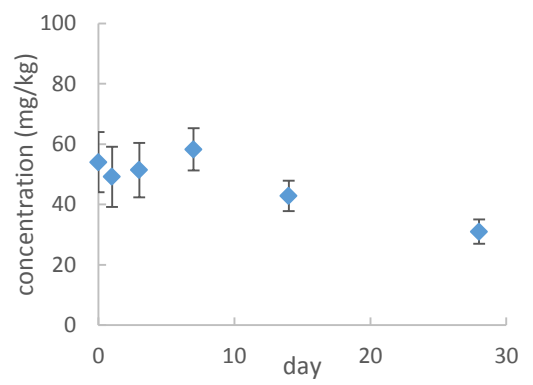
3-mercapto-2-butanone



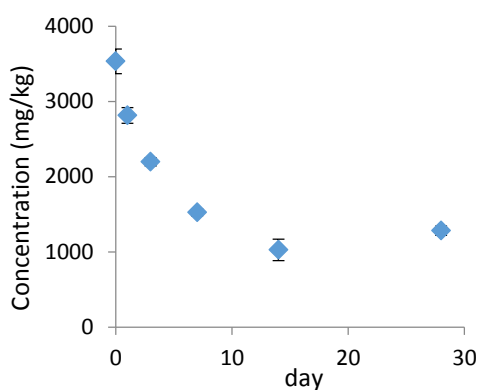
2-methyl-3-furanthiol



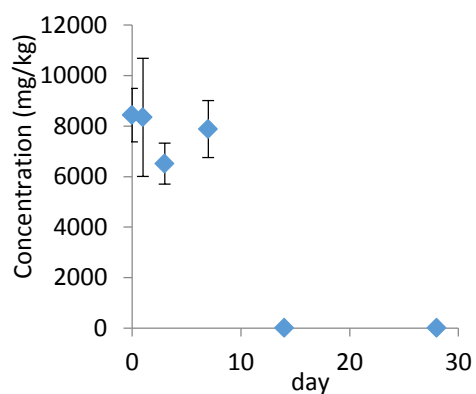
3-methylthiopropional



E,E-2,4-decadienal



1-octen-3-one



Indole

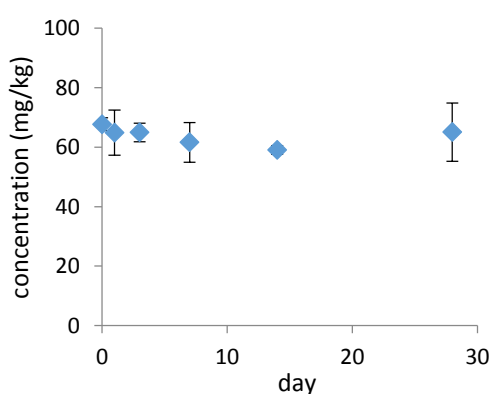
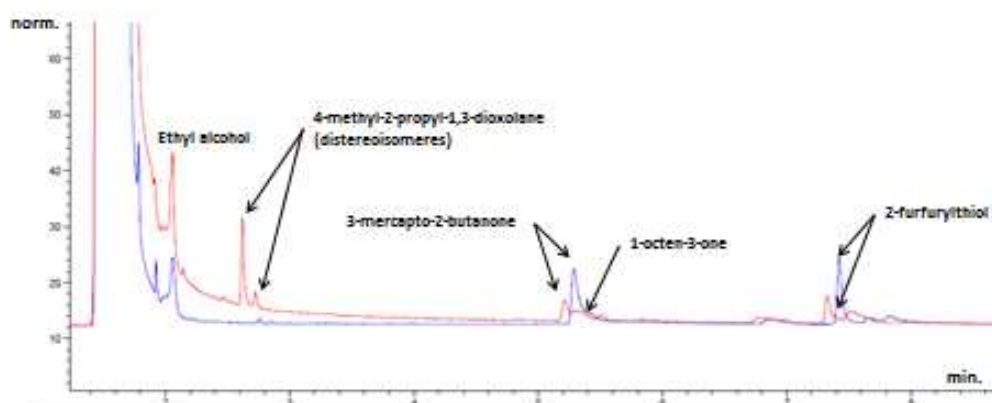


Figure 11: Evolution of the volatile concentration in the savoury flavour over a period of 4 weeks storage. Error bars correspond to standard deviation

A second manifestation of the instability of a flavour is the formation of new volatiles, which can generate new aromas. Figure 12a and Figure 12b show the GC-FID chromatograms obtained for the fresh and 2 week old savoury flavour. Analysis of the chromatograms shows the formation of new volatiles during storage.

a



b

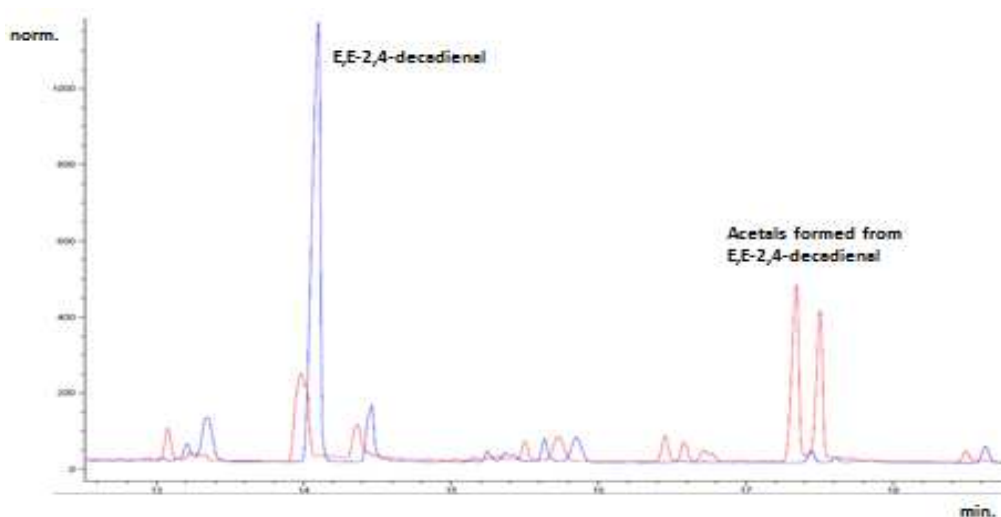


Figure 12: GC-FID chromatograms; blue: fresh flavour; red: after 2 weeks; a. savoury flavour 0-8 min b. secondary base 13-18 min

Different PG-acetal diastereoisomers were identified on the GC-FID chromatograms. The reaction of PG with aldehydes to form the corresponding acetals has been widely reported (Heydanek and Min, 1976). PG-acetals were formed from (i) E,E-2,4-decadienal (Figure 13), (ii) propanal (Figure 14) and (iii) 2-Methylpropanal (Figure 15). The transformation of aldehydes into PG-acetals can render them stable. This reaction can be

partially reversed in water: PG-acetals are hydrolysed to regenerate the original aldehydes (de Roos, 2007, Sharma et al., 1998).



Figure 13: reaction between E,E-2,4-decadienal and PG forming the corresponding acetal

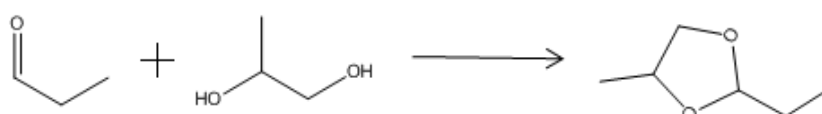


Figure 14: reaction between propanal and PG forming 4-methyl-2-propyl-1,3-dioxolane

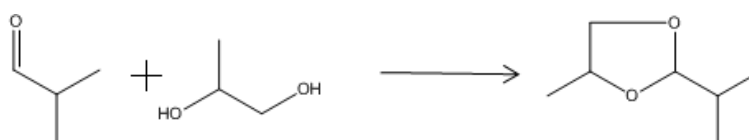


Figure 15: reaction between 2-methylpropanal and PG forming isobutyraldehyde PG-acetal

3.3.2.1.2 Discussion

The savoury flavour was highly unstable and showed major chemical changes after only one day of storage. After 4 days, all concentrations except for 3-methylthiopropional showed a significant change. The decrease of the volatile concentrations in the savoury flavour was the result of chemical reactions occurring during storage, between the volatiles themselves, and between volatiles and PG. Unlike results observed for the strawberry flavour, 4-

hydroxy-2,5-dimethyl-3-furanone remained relatively constant in the savoury flavour over the period of study.

The changes occurring during storage were a concern for the sensory properties of the savoury flavour, as they could contribute to the loss in desirable meaty flavour. In particular, the alteration of the concentrations of sulfur compounds and aldehydes could have a major impact on the sensory properties of the savoury flavour. Sulfur compounds are of high importance for the sensory properties of savoury flavour (Chang and Peterson, 1977) and aldehydes play an important role in meat flavour, as they give a characteristic fatty aromas to cooked meat (Mottram, 1998). Furthermore, chemical reactions such as oxidations can lead to the formation of undesirable flavours.

3.3.2.2 Sensory perception

3.3.2.2.1 Results

Results of the sensory study on the stability of the savoury flavour are summarised in Table 13. No significant differences were observed between the fresh flavour and the one day aged flavour ($p = 0.513$), but a significant difference was perceived between the fresh and 4 days aged savoury flavour ($p = 0.022$). This change became even more evident after 7 days, as the proportion of correct answers (P_c) to the triangle test increased from 48 % to 56 %. No significant differences were detected between the 2 weeks aged and 4 weeks aged flavour ($p = 0.47$).

Table 13: Results of the triangle tests on the savoury flavour at different age points

Triangle test	P _c (%)	<i>p</i> -value
1 day old vs. fresh	34	0.51
4 days old vs. fresh	48	0.02*
7 days old vs. 1 day old	56	<0.001*
2 weeks old vs. 4 weeks old	35	0.4

* *p* < 0.05

2.3.2.2.2 Discussion

Results from sensory studies and instrumental results were compared to determine when the chemical changes were detected by the sensory panel. No significant differences were perceived between the fresh and 1 day old savoury flavour. This indicates that the perception of the savoury flavour was not affected by the decrease in volatile concentration after 1 day. In particular, the 20 % decrease in E,E-2,4-decadienal observed after 1 day of storage was not perceived by assessors. This is in accordance with later results on omission testing showing that the removal of E,E-2,4-decadienal was not significantly detected in the savoury flavour (chapter 5).

The significant differences perceived between the fresh and 4 days aged flavour could be due to the changes in the concentrations of E,E-2,4-decadienal, 1-octen-3-one, 3-mercapto-2-butanone and 2-furfurylthiol, as the human nose can be very sensitive to a change in the concentration ratio of a flavour (Pineau et al., 2009, Le Berre et al., 2008a).

No significant differences were detected by assessors between the 2 and 4 weeks aged savoury flavour. This was in accordance with instrumental results and showed that the savoury flavour stabilised after 2 weeks. During informal conversations, assessors described the 2 weeks aged savoury flavour as more 'rich' and 'rounded' compared to the fresh flavour. The increase in the proportion of 4-hydroxy-2,5-dimethyl-3-furanone and the formation of new compounds such as PG-acetals could have a positive effect and give nuance to the savoury flavour. The process of maturation and stabilisation of flavour is commonly used in the food industry but not well published in the literature.

The low stability of the savoury flavour was a challenge for sensory analysis in this PhD study. For the rest of the study, the savoury flavour was prepared freshly every day preceding the sensory sessions. One alternative would have been to age the flavour for 2 weeks prior to sensory testing, as the mature savoury flavour appears to become stable.

3.3.2.3 GC-O analysis

GC-O was used to gain a deeper insight into the savoury flavour and to compare the sensory perception of the fresh and the 4 weeks aged savoury flavour. Table 14 presents the GC-O analysis of the fresh savoury flavour. All 10 of the volatiles added to the savoury flavour were detected at day 0 in the fresh flavour.

Comparison between GC-FID and GC-O results highlights the high sensitivity of the human nose. Most of the volatiles perceived by the GC-O panel were

not observed on the GC chromatogram. 2-Methyl-3-furanthiol and 12-methyltridecal were not detected by the GC-FID but were detected by the sensory panel. One reason is the extremely low sensory threshold of sulfur compounds; so low is the threshold that the human nose can detect these volatiles under the sensitivity limit of a FID or a MS (Golovnja and Rothe, 1980).

Table 14: GC-O analysis and identification of the volatiles in the fresh savoury flavour

Measured Retention Index (GC-O)	Aroma	Identified volatile	Retention Index (literature values)
984	nutty	2-methylpropanal	
1097	fruity	unknown	
1179	musty	unknown	
1280	damp	3-mercapto-2-butanone	1309
1287	mushroom	1-octen-3-one	1295
1299	nutty	2-methyl-3-furanthiol	1307
1361	herbal	(Z)-1,5-octadien-3-one (tentative)	1372
1375	roasted	unknown	
1419	meaty/roasted	unknown	
1436	roasted	2-furfurylthiol	1432
1439	green	Acetic Acid (tentative) or 1-octen-3-ol (tentative)	1451
1446	potato	3-methylthiopropional	1449
1514	popcorn	unknown	
1524	biscuit	unknown	
1555	brown	unknown	
1700	fusty	Contamination 3-mercapto-2-butanone	1702
1778	nutty	unknown	
1805	tarragon	E,E-2,4-decadienal	1804
1816	meaty	Unknown	
1871	catty	12-methyltridecanal	1863
1906	caramel	Unknown	
1987	candyfloss		
2021	candyfloss	4-hydroxy-2,5-dimethyl-3-furanone	2031
2042	burnt		
2123	burnt spices	Unknown	
2136	sweet	bis(2-methyl-3-furyl)disulphide	2179
2162	curry	Unknown	
2266	praline	Unknown	
2293	bacon	Unknown	
2345	brown	Unknown	
2412	orange	Indole	2450
2526	coffee	Unknown	
2582	urine	Unknown	
2656	grilled meat	bis(furfuryl)disulfide (tentative)	2624

Aromas that disappeared after 4 weeks.

A high number of extra aromas were identified in the savoury flavour. One explanation for the presence of extra aromas is the contamination of the net standards. The composition of the volatile standards used in the savoury flavour, as provided by the suppliers, is presented in Table 15. 3-Mercapto-2-butanone, 2-methylpropanal, and 1-octen-3-one samples all showed signs of contamination on GC-MS. For example, the 'fusty' aroma observed at RI 1700 (Table 14) corresponds to the peak of contamination observed in the 3-mercapto-2-butanone standard.

Table 15: Composition of the standards used for the preparation of the savoury flavour model

Net standard	Concentration in the standard as indicated by the supplier (%)
2-Methylpropanal	> 96
2-Furfurylthiol	98
4-Hydroxy-2,5-dimethyl-3-furanone	> 98
3-Mercapto-2-butanone	> 80
2-Methyl-3-furanthiol	95
3-Methylthiopropional	> 97
E,E-2,4-Decadienal	Not stated
12-Methyltridecanal	10
1-Octen-3-one	50 in 1-octen-3-ol
Indole	> 99

Another hypothesis to explain the presence of extra aromas in the savoury flavour is the formation of new volatiles by chemical reactions as soon as volatiles and PG are mixed together, as the savoury flavour was shown to be

highly instable. For example, the oxidation of thiols into disulfides generated MFT-MFT and bis(furfuryl)disulfide (tentatively). Finally, artefact aromas can be generated during the GC-O analysis, due to the high instability of sulfur compounds (Block, 2011).

GC-O analysis of the 4 weeks aged savoury flavour is presented in Table 16. The sensory properties of the savoury flavour changed significantly after 4 weeks of storage compared to the fresh flavour. Only 13 of the 30 aromas smelled in the fresh savoury flavour were still detected after 4 weeks of storage. Among the volatiles added to the flavour, 2-methylpropanal, 3-mercapto-2-butanone, and 12-methyltridecanal were no longer detectable after 4 weeks. Furthermore, some aromas such as 'roasted' (RI 1375), 'popcorn' (RI 1514), and 'bacon' (RI 2293) were not detected by GC-O in the 4 weeks aged flavour.

New aromas appeared during storage, such as 'gravy' (RI 1579), 'malty' (RI 1947) and 'smoky' (RI 2256) aromas. The unpleasant aromas 'urine' (RI 2136), 'sweaty' (RI 1828) and 'skunky' (RI 1562) could be due to the oxidation of some volatiles, which is often perceived as off note (Taylor and Linforth, 2010). Differences observed between the fresh and 4 weeks aged savoury flavour could also be due to volatiles having different aromas at different concentrations (Taylor and Linforth, 2010).

Table 16: GC-O analysis and identification of volatiles in the 4 weeks aged savoury flavour

Measured Retention index (GCO)	Aroma	Identified volatile	Retention index (literature values)
1037	roasted	unknown	
1063	fruity	unknown	
1198	nutty	unknown	
1287	mushroom	1-octen-3-one	1295
1299	nutty	2-methyl-3-furanthiol	1307
1368	geranium	(Z)-1,5-octadien-3-one (tentative)	1372
1375	geranium	unknown	
1419	meaty	2-furfurylthiol	1432
1443	green	Acetic Acid (tentative) or 1-octen-3-ol (tentative)	1451
1453	potato	3-methylthiopropional	1449
1497	fatty	Unknown	
1562	skunky	Unknown	
1579	gravy	Unknown	
1805	tarragon	E,E-2,4-decadienal	1804
1828	sweaty	Unknown	
1947	malty	Unknown	
1967	white flower	Unknown	
1979	candyfloss		
2025	candyfloss	4-hydroxy-2,5-dimethyl-3-furanone	2031
2046	burnt sugar		
2080	brown/meaty	4-hydroxy-2(5)-ethyl-5(2)-methyl-3-furanone (tentative)	2087
2136	urine	bis(2-methyl-3-furyl)disulphide	2179
2175	celery	Unknown	
2256	smoky	Unknown	
2354	brown	Unknown	
2412	orange	Indole	2450
2526	weak, roasted	Unknown	
2582	urine	Unknown	
Aromas that appears after 4 weeks			

3.4 Conclusions

The objective of this chapter was to determine how long the two flavour models used within this research project remained stable, in order to plan future research studies on flavour perception. This study underlines the importance of assessing flavour stability and provides crucial guidance to help in the design of future sensory sessions. The differential results obtained with the strawberry and savoury flavours highlight the need to conduct stability tests on each particular model flavour before proceeding with sensory experiments of this nature.

The process of stabilisation and maturation observed for the savoury flavour is commonly used in the food industry but not well published in the literature. The fact that the flavour was described as more 'rich' and 'rounded' by the assessors shows the impact of the new volatiles formed during storage. These volatiles could enhance the perceived complexity of the savoury flavour and thereby increase its hedonic properties.

Sensory studies on flavour stability coupled with instrumental analysis provides new insight into the perception of flavour, as this facilitates determination of when changes in the flavour are detectable by a consumer panel. The results also highlight the importance of assessing the stability of a flavour sensorially as well as instrumentally, as some changes that are not detectable instrumentally could be perceived by a sensory panel. On the

contrary, some changes observed instrumentally were not detected by the sensory panel.

The results obtained in this part of the PhD study were crucial for the subsequent chapters. First of all, as changes in flavour components over time were likely to affect sensory results, it was important to compare flavours of the same age in discrimination testing, so that any perceived difference could not be attributed to the instability of the flavour. Results also showed that the strawberry flavour was stable for 1 week and gave flexibility for the same sample to be used in sensory studies over a period of one week. On the contrary, the low stability of the savoury flavour was a challenge for sensory analysis and implied that such a flavour needed preparing freshly every day preceding the sensory sessions. The alternatives would have been to freeze the savoury flavour samples to increase their stability over time, or to age the savoury flavour prior to sensory testing, as the flavour mixture became more stable after 2 weeks.

Chapter 4. Evaluating the same-different approach in sensory omission testing

4.1 Introduction

4.1.1 Use of the Thurstonian distance d' in omission studies

The analysis of the literature concludes that there is scope to improve the approach used in sensory omission studies. The same-different approach was developed at the University of Nottingham and used to identify the key volatiles in a strawberry flavour (O'Mahony, 2012). In this thesis, the Thurstonian d' was used for the first time in association with the same-different approach. One major interest of Thurstonian measure d' is that it allows comparison of the results obtained from different discrimination tests (Ennis, 1990, Jesionka et al., 2014, O'Mahony and Rousseau, 2002, Rousseau et al., 1998). Therefore, the Thurstonian d' can be used in this study to compare the sensitivity of the triangle and same-different approaches in omission testing.

The sensitivity of the triangle and same-different tests has been compared previously in the literature. Some studies showed the higher sensitivity of the same-different test over the triangle test (Lau et al., 2004, Rousseau et al., 1999), while others did not (Rousseau et al., 1998, Stillman and Irwin, 1995). However, this is the first study that compares the triangle and same-different tests in the context of omission testing. It can be hypothesised that lower carry-over, memory effects and fatigue would give an advantage to the same-

different test over the triangle test, as three-stimulus protocols are usually less discriminating than two-stimulus protocols (Dessirier and O'Mahony, 1999, Rousseau and O'Mahony, 1997).

Another major interest of the Thurstonian d' is that its aim is not to determine whether or not the difference is perceived, but to estimate the size of the difference between samples (Jesionka et al., 2014). When used in omission testing, the Thurstonian d' reflects the relative importance of each individual volatile in a particular flavour. This is of major interest as omission experiments usually focus on identifying the key volatiles in flavours and does not allow measuring of the relative importance of individual volatiles in flavours.

4.1.2 Different cognitive strategies in discrimination testing

Assumption on the cognitive strategy is crucial in obtaining a sensitive estimate of d' (Hautus et al., 2011) (see section 1.4.4.4). For the same-different test, the tau-strategy is usually assumed (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002). The tau-strategy relies upon a tau-criterion (see section 1.4.3). In contrast, the COD-strategy is generally assumed for the triangle test (Kim et al., 2006, O'Mahony et al., 1994). When using a COD-strategy, the assessors compare the distances between the sensory perceptions of the three stimuli, in order to determine which stimulus is furthest away from the other two stimuli (O'Mahony, 1995)(see section 1.4.4.4).

Another cognitive strategy called the beta strategy can be used with same-different tests (Rousseau, 2001) and triangle tests (Versfeld et al., 1996). When using a beta-strategy, the assessors draw an imaginary line between two categories: 'reference sample' and 'different sample'. The position of the line determines the beta-criterion, which corresponds to a level of sensory evidence (O'Mahony and Rousseau, 2002)(see section 1.4.4.4).

One approach to investigate the cognitive strategy used by assessors to answer the same-different test consists of fitting Thurstonian models assuming different cognitive strategies to the data collected from the tests to determine which model fits the data better (Irwin et al., 1993, Hautus et al., 2008, Lee and O'Mahony, 2007a). Two models can be used for the same-different test: the differencing model corresponds to a tau-strategy, whereas the Likelihood-Ratio (LR) model assumes a beta-strategy.

4.1.3 Objectives of this chapter

This chapter focuses on using the Thurstonian d' as a tool to evaluate the same-different approach in omission experiments. The strawberry flavour was used to this end as this was the flavour used to develop the same-different approach in omission testing (O'Mahony, 2012).

In the first part, Thurstonian modelling was used to investigate the cognitive strategy adopted by the assessors to answer the same-different tests. As the tau-strategy is usually assumed for the same-different tests, it was

hypothesised that the assessors primarily used a tau-strategy to answer the same-different tests in this study.

In the second part, the Thurstonian d' was used to compare both the triangle and same-different approaches with respect to their sensitivity. Thurstonian d' were estimated using (i) the triangle approach and (ii) the same-different approach.

4.2 Materials and methods

In the first part of this study, ROC fitting was used to determine the cognitive strategy used by the assessors to answer the same-different omission tests on the strawberry flavour delivered ortho- or retronasally (sessions Straw1 + Straw2, and session Straw4, respectively). In the second part, data from the triangle and same-different approaches were compared (Sessions Straw1 + Straw2, and session Straw3, respectively).

4.2.1 Part 1: Determining the cognitive strategy used to answer same-different tests

An assumption on the cognitive strategy used to answer the same-different tests in this study was vital in order to make a valid estimation of d' (Hautus et al., 2011).

Data from omission experiments were used to determine the cognitive strategy used by assessors. To evaluate the strategy for the same-different approach data collected orthonasally on the strawberry flavour in PG were pooled from sessions Straw1 and Straw2 in order to conduct the subsequent

analysis on 100 assessors. To determine the cognitive strategy used during the same-different approach when the flavour was delivered retronasally, data from session Straw4 (conducted on 100 assessors) were used.

The cognitive strategy was determined using Thurstonian modelling (Hautus et al., 2008). ROC fitting software (SDT Assistant version 1.0, available from <http://hautus.org>) was used to model ROC curves. Two different models, the differencing model, associated with a tau-strategy, and the LR model, associated with a beta-strategy, were fitted to the ROC curves. The best fitting model was determined by Wilcoxon signed-rank tests (O'Mahony, 1986) on chi-squares.

4.2.2 Part 2: Comparison between the triangle and same-different approaches

4.2.2.1 Preparation of the strawberry flavour samples

The strawberry flavour and corresponding omission samples were prepared as described in section 2.2.1.1 to conduct session Straw3. The strawberry flavour in PG was kept at 4° C and used up to 8 days after preparation. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure the flavour samples were at room temperature (20° C ±2° C).

4.2.2.2 Omission tests using the triangle approach

Session Straw3 was conducted on the strawberry flavour in PG delivered orthonasally. Session Straw3 involved 72 assessors carrying out 9 triangle tests to compare each one of the 9 omission samples (n - 1) with the original

strawberry flavour model (n). To limit sensory fatigue and carry-over effects assessors were allocated a 5 minute break after every 2 tests. Values of d' calculated from this session were subsequently compared with d' values determined using the same-different tests.

4.2.2.3 Determining the number of assessors

To compare discrimination tests with respect to their relative sensitivity, it is necessary to compare them under the same assumptions about the number of assessors (Ennis, 1993). The numbers of assessors were set so that the same-different and triangle tests had the same statistical power. Therefore, theoretical d' values would be predicted to be the same for the same-different and triangle tests (see section 1.4.5). Any difference in d' values could then be attributed to extra noise related to external factors such as familiarisation, memory effects and carry-over (Rousseau et al., 1999, Van Hout et al., 2011, Kim and Lee, 2012).

The IFProgram software (Institute for Perception, Richmond, VA) was used to determine the number of assessors. For the same-different test, the probability of answering 'same' for the same pair of samples ($P_{s/s}$) is required to determine the number of assessors. Preliminary omission tests on the strawberry flavour (session Straw1 using 50 assessors) showed that $P_{s/s}$ was 57 % in the same-different test.

The parameters α , β and δ must also be defined prior to the test to determine the number of assessors (Ennis and Jesionka, 2011). The size of the difference

of interest δ can be determined using standard or established values. For example, industry standard recommends $\alpha = 0.05$, $\beta = 0.2$ and $\delta = 1$ for the triangle test (Worch and Delcher, 2013).

Psychometric functions relate d' and P_c taking into account the type of discrimination test and the cognitive strategy (Ennis, 1993, Ennis et al., 1998, Ennis and Jesionka, 2011). Jesionka *et al.* (2014) built a transition function to transit from P_d to d' , through P_c , using psychometric functions of the Thurstonian model. Using the transition function of Jesionka *et al.* (2014), a P_d of 0.3 corresponds to a δ of just above 1.5 in the triangle test (Worch and Delcher, 2013).

In this study, δ was set at 1.55 in order to have a manageable number of assessors. Using $\alpha = 0.05$, $\beta = 0.10$ and $\delta = 1.55$, the required number of assessors was 72 for the triangle test (Straw3) and 50 for the same-different test (Straw2).

4.2.2.4 Comparison of d' values estimated using the triangle and same-different approaches

The triangle and same-different approaches were compared using the strawberry flavour in PG delivered orthonasally. Results from session Straw3, where 72 assessors carried out 9 triangle tests, were compared with results from session Straw2, where 50 assessors carried out 9 same-different tests.

The triangle and same-different approaches were first compared assuming the conventional cognitive strategies. The tau-strategy was assumed for the

same-different tests (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002) and the COD-strategy was assumed for the triangle test (COD-triangle) (Kim et al., 2006, O'Mahony et al., 1994).

As discussed later in section 4.3.1, results suggested that some assessors might have used the beta-strategy to answer the same-different tests in this study. Given this result, it is also possible that some assessors might have used a beta-strategy to answer the triangle tests (beta-triangle). Consequently, further analyses were performed to compare the triangle and same-different approaches when a beta-strategy was assumed.

The d' values were estimated as described in sections 2.2.7.1. Student t-tests ($\alpha = 0.05$) (Excel 2010, Microsoft, USA) were used to compare (1) the overall d' values obtained using both the triangle and the same-different approaches and (2) for each volatile, d' values obtained from both the triangle and the same-different approaches.

R software version 3.1.0 (R development Core Team, 2014) was used to determine if significant differences were perceived between the original flavour model and each omission sample (at $\alpha = 0.05$) (see section 2.2.7.3). For the triangle test, binomial tests were computed using R software. For the same-different tests, the signed square root of the Pearson statistic was used.

95 % confidence intervals were built for d' using R software version 3.1.0 (R development Core Team, 2014), as described in section 2.2.7.2.

4.3 Results and discussion

4.3.1 Part 1: Determining the cognitive strategy used to answer the same-different tests

4.3.1.1 Results

Both the tau- and the beta-strategy can be employed by assessors to answer same-different tests and an assumption on the cognitive strategy is vital in order to make a valid estimation of d' (Hautus et al., 2011) (see section 1.4.4.4). Figure 16 illustrates the ROC curves obtained from pooled data on 100 assessors (Straw1 and Straw2) comparing the original strawberry flavour with the flavour omitting (i) methyl(E)-3-phenylprop-2-enoate (Figure 16a) and (ii) cis-3-hexen-1-ol (Figure 16b). A 'Hit' was defined as answering 'same' when the samples were the same and a 'False Alarm' was defined as answering 'same' when the samples were different.

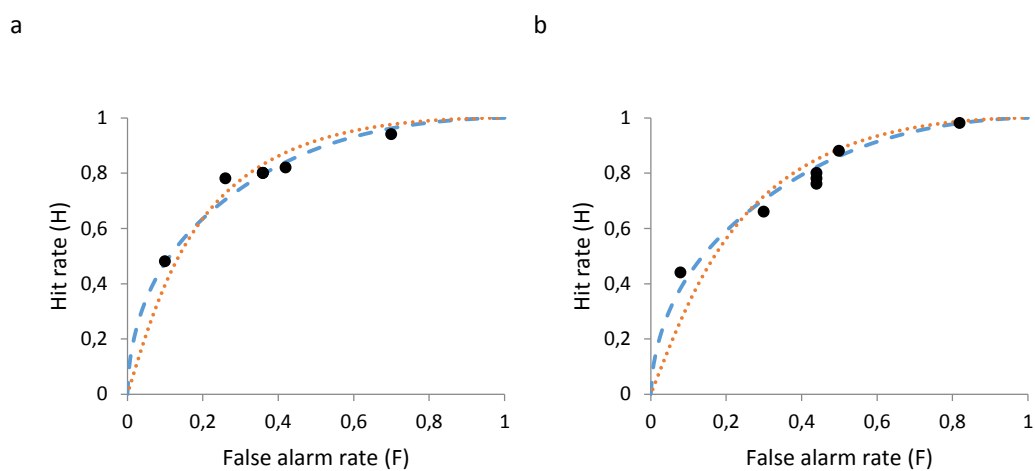


Figure 16: ROC curves obtained from omission testing on a. Methyl(E)-3-phenylprop-2-enoate, and b. Cis-3-hexen-1-ol. The best-fitting ROC curves were estimated based on (1) the differencing model (---), and (2) the LR model (—).

The smooth curves in Figure 16 depict the best fit of the data for (1) the differencing model associated with a tau-strategy and (2) the LR model associated with a beta-strategy as calculated by the software. Visual inspection of the ROC curves showed that the data points seemed closer to the curve generated with the LR model compared to the differencing model. However, the ROC curves lay close to the major diagonal of the ROC space, and it was difficult in this region to determine which of the two models fitted the data better, as observed by Hautus *et al.* (1995).

Here, a four-point sureness rating was used; resulting in 8 response categories and seven data points to fit the ROC curve. For ROC fitting, the more data points, the more accurate the estimation of d' . Examination of the frequency distributions over the response categories of the sureness ratings revealed that the majority of assessors tended to avoid certain degrees of certainty such as the 'very sure' and 'very unsure' categories. This leads to fewer data points for the ROC curve or very close points, which can affect the fit of the model. Encouraging the assessors to use all the available sureness rating categories could help obtaining a well-determined ROC curve and improve the fitness of the model (Irwin *et al.*, 1993).

Table 17 lists the d' values obtained from omission testing on the strawberry flavour in PG delivered orthonasally. Data were pooled from 100 assessors (Straw1 and Straw2). Both the differencing and LR models were used to estimate d' . The chi-square values related to the model and associated p -

values are also given in Table 17. The smaller the chi-square, the better the model fits the data. For ethyl butanoate, the ROC curve departed significantly from the differencing model ($p = 0.01$), and the p -value obtained from the LR model was close to significance ($p = 0.076$). The orthonasal investigation showed that the chi-squares obtained with the LR model were significantly smaller compared to the differencing model (Wilcoxon signed-rank test, $p < 0.05$), indicating that the LR model may fit the data better. However, for 8 out of 9 volatiles, the differencing model could not be rejected as the chi-square was not significant ($p > 0.05$).

Table 17: d' values and chi-squares obtained for the strawberry flavour in PG delivered orthonasally using (1) the differencing model and (2) the Likelihood-Ratio (LR) model.

Volatile	Differencing model (tau strategy)			LR model (beta strategy)		
	d'	χ^2 (DF)	p (χ^2)	d'	χ^2 (DF)	p (χ^2)
Ethyl butanoate	2.28	16.7 (6)	0.01*	1.80	11.4 (6)	0.076
Ethyl hexanoate	1.75	6.95 (6)	0.325	1.38	5.02 (6)	0.541
Methyl dihydrojasmonate	1.83	4.41 (5)	0.492	1.46	3.00 (5)	0.701
2,3-Butandione	1.37	1.93 (5)	0.859	1.10	1.66 (5)	0.894
Butanoic acid	1.73	3.81 (6)	0.702	1.39	1.89 (6)	0.93
Gamma decalactone	1.77	7.14 (6)	0.308	1.45	4.32 (6)	0.633
4-Hydroxy-2,5-dimethyl-3-furanone	1.95	5.44 (6)	0.489	1.62	1.90 (6)	0.929
Methyl(E)-3-phenylprop-2-enoate	2.45	2.75 (4)	0.6	1.94	3.56 (4)	0.468
Cis-3-hexen-1-ol	2.24	7.02 (6)	0.319	1.82	3.64 (6)	0.725

DF: degree of freedom * Indicates that the ROC curve departed significantly from the model ($p < 0.05$)

Table 18 shows the retronasal d' values estimated using both the differencing and LR models, along with the chi-squares and associated p -values. Looking at the retronasal data, the Wilcoxon signed-rank test did not show significant differences between the LR and differencing models ($p > 0.2$). The ROC curve obtained for 4-hydroxy-2,5-dimethyl-3-furanone departed significantly from both the differencing and the LR models ($p = 0.033$ and 0.037 , respectively).

No specific conclusion could be made in this study regarding which cognitive strategy was dominant, as chi-squares and p -values indicated that the tau- and the beta-strategies were both equally likely.

Table 18: d' values and chi-squares obtained for the strawberry flavour delivered retronasally using (1) the differencing model and (2) the LR model

Volatile	Differencing model (tau strategy)			LR model (beta strategy)		
	d'	χ^2 (DF)	$p(\chi^2)$	d'	χ^2 (DF)	$p(\chi^2)$
Ethyl butanoate	-0.71	1.1 (4)	0.894	-0.3	1.56 (4)	0.815
Ethyl hexanoate	0	6.45 (5)	0.265	-0.49	4.93 (5)	0.424
Methyl dihydrojasmonate	0.69	4.24 (4)	0.375	-0.44	4.54 (4)	0.338
2,3-Butandione	0.82	4.23 (5)	0.516	0.46	4.29 (5)	0.509
Butanoic acid	-0.86	3.27 (5)	0.659	-0.59	3.23 (5)	0.664
Gamma decalactone	-0.57	1.84 (4)	0.765	0.18	2.07 (4)	0.723
4-Hydroxy-2,5-dimethyl-3-furanone	-0.52	12.1 (5)	0.033*	0.1	11.8 (5)	0.037*
Methyl(E)-3-phenylprop-2-enoate	-1.11	6.31 (5)	0.277	-0.55	7.88 (5)	0.163
Cis-3-hexen-1-ol	0.92	4.11 (5)	0.534	0.65	4.28 (5)	0.51

DF: degree of freedom * Indicates that the ROC curve departed significantly from the model (at $p < 0.05$)

4.3.1.2 Discussion

4.3.1.2.1 Cognitive strategy

Two cognitive strategies can be used to answer the same-different test: the tau-strategy and the beta-strategy. The tau-strategy relies on the cognitive tau-criterion. The assessor responds 'same' if the difference between the two stimuli is smaller than the tau-criterion, and 'different' if it is larger. On the contrary, the beta-strategy is based on absolute judgements: each stimulus is evaluated independently. The assessors draw an imaginary line between two categories - 'reference sample' and 'different sample' – and the position of the line determines the beta-criterion.

In this PhD study, it was not possible to conclude if one particular strategy was adopted here over the other. Looking at the orthonasal data, although the LR model assuming a beta-strategy provided a more satisfactory account of the data, neither strategy was rejected for 8 of the 9 volatiles. Furthermore, the retronasal investigation did not show differences between the LR and differencing model.

Although results did not allow the rejection of the tau-strategy hypothesis, they suggested that some assessors might have used the beta-strategy to answer the same-different tests. This was surprising as it is conventionally assumed that a tau-strategy is used for the same-different tests both in food sciences and psychology (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002). Assessors adopted the tau-strategy to answer same-different tests on

auditory stimuli (Hautus et al., 1994) orange drinks (Irwin et al., 1993), raspberry drinks (Stillman and Irwin, 1995) and milk (Hautus and Irwin, 1995). The use of the beta-strategy has been shown occasionally, for example for visual stimuli (Irwin and Francis, 1995) or food stimuli (Lee et al., 2007, Chae et al., 2010). Several studies have reported that a beta-strategy can also be used for same-different tests for visual stimuli, word semantics (Irwin and Francis, 1995), and food discrimination (Santosa et al., 2011).

The cognitive strategy depends on the experimental design, the instructions given (for example, if the dimension of difference is specified), the familiarity with the product, the degree of difference between the products, and the complexity of the stimuli (Rousseau, 2001).

Three hypotheses can be suggested to explain why some assessors might have used the beta-strategy in this study. First, the fact that consumers were exposed to the product previously can induce the use of a beta strategy, as assessors learn to recognise and categorise the stimuli (Chae et al., 2010, Lee et al., 2007). The design of the experiment in this study involved repeated exposure to the strawberry flavour. For each of the 9 discrimination tests presented in one session, the assessors had a 75 % chance of smelling the strawberry flavour at least once. Some assessors, as they attended several sessions, would also have become familiar with the flavour through repeated exposure. However, the hypothesis that experience causes assessors to use a beta-strategy can be disputed, as Santosa *et al.* (2011) showed that three out

of four assessors were still using a tau-strategy even after 2,000 same-different tests.

A second hypothesis is based on the complexity of the samples (a complex flavour mixture delivered orthonasally). Assessors tend to adopt a beta-strategy when the samples are more complex (Rousseau, 2001). The complexity of a stimulus can be defined as the number of aspects of the stimulus that can be varied independently. Irwin and Francis (1995) showed that increasing the complexity of visual stimuli led to the use of the beta-strategy by assessors. Irwin and Francis (1995) hypothesised that increasing the complexity of the stimulus allowed absolute judgement to be made, which corresponds to the beta-strategy. In this PhD study, the relative complexity of the flavour samples (9 aroma molecules) could have led assessors to make independent judgements about the two stimuli.

A third hypothesis is the use of a 'fixed' experimental design (as opposed to a 'roving' design) (Rousseau, 2001). In a 'fixed' experimental design, the same two samples A and B are compared over several trials. The use of a 'fixed' experimental design can result in the use of a beta-strategy (Dai et al., 1996, Versfeld et al., 1996, Macmillan and Creelman, 2005). In a 'roving' experimental design, the nature of the stimulus varies from trial to trial and the assessors cannot form a hypothesis regarding the size of the difference between the samples. The experimental design used in this study is very similar to a 'fixed' experimental design, as each sensory session involved 9

discrimination tests comparing the complete strawberry flavour with each of the 9 omission samples, all were very close to the strawberry flavour in terms of sensory properties. Assessors may have been able to form a hypothesis regarding the dimension of difference between the strawberry flavour and omission samples, resulting in the use of a beta-criterion.

Given the results from the same-different test, it is also possible that some assessors might have used a beta-strategy to answer the triangle tests, instead of the usual COD-strategy. To the author's knowledge, there is no published systematic procedure/software to determine if the assessors were using a COD- or a beta-strategy to answer the triangle test. A way of determining this strategy could be to compare the performance in the triangle test to the 3-AFC (Kim et al., 2006). Given that the sensory sequences and memory effects are the same between the triangle and the 3-AFC, a difference in d' values could infer the use of a beta-strategy in the triangle test (B. Rousseau, personal communication).

4.3.1.2.2 Further work on determining the cognitive strategy

Results suggest that different assessors used different cognitive strategies to answer same-different tests in this study. Some assessors could have used the tau-strategy, while others used the beta-strategy. A way to confirm this hypothesis would be to determine the cognitive strategy used by individual assessors, using ROC fitting. Such types of study have used a small number of assessors (4 to 10) to carry out a large number of discrimination tests (60 to

2,000 tests per assessors) (Santosa et al., 2011, Hautus et al., 1994, Hautus et al., 2011).

Further experimental work is required to determine the cognitive strategy used by assessors to answer the same-different tests in omission testing. One way to investigate the cognitive strategy would be to use a smaller number of assessors (up to 10) to carry out a large number of same-different tests. The ROC curves could then be drawn, and ROC fitting could be used to determine the cognitive strategy used by each individual assessor to answer the discrimination tests. This type of study is ambitious as it requires assessors to carry out a large number of discrimination tests (up to 2,000 in Santosa *et al.* (2011)).

In this study, each assessor was presented with only one pair of samples for each omission test (either a same pair or a different pair). The fact that different assessors provided the proportions of hits and false alarms could induce a distortion of the ROC curve (B. Rousseau, personal communication). The long version of the same-different test, where each assessor would test two pairs of stimuli, one pair the same and one pair different, could be used instead. This approach would produce a more meaningful ROC curve as the proportion of hits and false alarms would come from the same assessors but would increase the time and number of samples involved.

4.3.2 Part 2: Comparison between the triangle and same-different approaches

4.3.2.1 Results

4.3.2.1.1 Assuming the conventional cognitive strategies

Table 19 shows the d' values obtained using the same-different and triangle approaches for omission testing on the strawberry flavour. The tau-strategy was assumed for the same-different tests (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002), while the COD-strategy was assumed for the triangle-test (Kim et al., 2006). Each row of Table 19 corresponds to the omission test comparing the original strawberry flavour with the omission sample omitting one volatile. Thus, d' reflects the relative importance of each individual volatile in the strawberry flavour.

Interpreting the same-different approach data, every volatile contributes to the quality of the aroma, as the omission of each individual compound could be significantly detected. This is not surprising as these volatiles have all previously been shown to make an important contribution to strawberry aroma (Larsen et al., 1992, Pyysalo et al., 1979). Furthermore, the strawberry flavour model used in this research is a commercial flavour recipe (Aromco, UK) which has been developed to contain only the key volatiles.

Table 19: d' values estimated assuming a tau-strategy for the same-different tests and a COD-strategy for the triangle tests

Volatile	Triangle approach (COD-strategy)		Same-different approach (tau-strategy)	
	d' [CI]	S	d' [CI]	S
Ethyl butanoate	1.69* [1.44;3.14]	0.35	2.30*† [1.44;3.14]	0.14
Ethyl hexanoate	1.24* [0.38;1.84]	0.33	1.53* [0;2.39]	0.22
Methyl dihydrojasmonate	0.99 [0;1.63]	0.37	1.53* [0;2.4]	0.18
2,3-Butandione	0.79 [0;1.49]	0.44	1.82*† [0.72;2.7]	0.2
Butanoic acid	0 [0;0.9]	Not available ¹	1.49* [0;2.39]	0.15
Gamma decalactone	0.79 [0;1.49]	0.44	1.7* [0.5;2.55]	0.19
4-Hydroxy-2,5-dimethyl-3-furanone	0.55 [0;1.34]	0.59	1.45* [0;2.33]	0.23
Methyl(E)-3-phenylprop-2-enoate	1.11* [0;1.74]	0.35	2.32*† [1.41;3.16]	0.19
Cis-3-hexen-1-ol	0.68 [0;1.41]	0.49	2.36*† [1.46;3.2]	0.18

The strawberry flavour in PG was delivered orthonasally. S: standard deviation, CI: 95 % confidence interval ¹ A d' of zero does not allow estimation of variance.* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$) † Indicates a significant difference with the d' measured using the triangle approach (Student t-test, $p < 0.05$)

Looking at the triangle approach, the removal of only 3 individual volatiles, ethyl butanoate, ethyl hexanoate and methyl(E)-3-phenylprop-2-enoate, was significantly detected ($p < 0.001$, = 0.018 and = 0.045, respectively). The d' of zero for the test omitting butanoic acid corresponds to very similar samples that could not be differentiated by assessors.

For each individual volatile, the same-different approach generated higher d' values compared to the triangle approach. A Student t-tests on the overall d' values showed that d' values were significantly higher using the same-different approach compared to the triangle approach ($p = 0.001$). Looking at

the individual volatile d' values, the same-different approach generated a significantly higher d' for 2,3-butandione, ethyl butanoate, cis-3-hexen-1-ol, and methyl(E)-3-phenylprop-2-enoate (Student t-tests, $p = 0.016, 0.002, < 0.001, \text{ and } 0.001$, respectively).

4.3.2.1.1 Assuming a beta-strategy

The results on model fitting showed that some assessors might have used the beta-strategy to answer the same-different tests. Given this result, some assessors might also have used a beta-strategy to answer the triangle tests (Versfeld et al., 1996). Table 20 shows the d' values estimated when a beta-strategy was assumed for both the same-different and triangle tests. As observed in the previous section, for each individual volatile, the same-different approach generated higher d' values compared to the triangle approach. A Student t-test on the overall d' values showed that the same-different approach generated significant higher d' values compared to the triangle approach ($p < 0.001$).

Table 20: d' values estimated assuming a beta-strategy for both the triangle and the same-different tests.

Volatile	d' Triangle approach (beta-strategy)	d' Same-different approach (beta-strategy)
Ethyl butanoate	1.4	1.82
Ethyl hexanoate	1.1	1.29
Methyl dihydrojasmonate	0.8	1.17
2,3-Butandione	0.7	1.39
Butanoic acid	0	1.27
Gamma decalactone	0.7	1.35
4-Hydroxy-2,5-dimethyl-3-furanone	0.5	1.29
Methyl(E)-3-phenylprop-2-enoate	1.0	1.84
Cis-3-hexen-1-ol	0.6	1.92

The strawberry flavour in PG was delivered orthonasally

4.3.2.2 Discussion

4.3.2.2.1 Superiority of the same-different approach over the triangle approach

In this study, it was evident that the same-different approach was more sensitive than the triangle approach: the same-different approach allowed the significant detection of every individual volatile within the strawberry flavour and generated significantly higher d' values compared to the triangle approach. Using the Thurstonian d' as a measure of the sensitivity, results showed that the same-different approach was more sensitive compared to the triangle approach: the d' values obtained using the same-different test were 1.2 to 3.5 times higher than the d' values obtained using the triangle test. Results on the superiority of the same-different test were in accordance

with previous results from Rousseau *et al.* (1999) and Lau *et al.* (2004). Although they found no significant difference Rousseau *et al.* (2002), Rousseau and O'Mahony (2000), and Rousseau and O'Mahony (2001) showed a trend for the same-different test to yield a higher d' .

When the same-different test was found to be more sensitive, this phenomenon was attributed to memory effects (Rousseau *et al.*, 1998, Rousseau and O'Mahony, 2000, Lau *et al.*, 2004) or fatigue, such as the irritation associated with mustard samples (Rousseau *et al.*, 1999). It must be noted that studies in the literature focused on retronasal stimuli, such as aqueous solutions of tastants (Lau *et al.*, 2004, Kim *et al.*, 2006), flavoured beverages (Rousseau *et al.*, 2002, Rousseau and O'Mahony, 2001, Rousseau and O'Mahony, 2000, Stillman and Irwin, 1995), flavoured yoghourts (Rousseau *et al.*, 1998) or mustards (Rousseau *et al.*, 1999).

In the present study, the superiority of the same-different approach over the triangle approach was more extreme than in prior studies. This could be due to stronger carry-over and memory effects for orthonasal stimuli compared to retronasal stimuli. Avoiding carry-over is particularly important with volatile samples as the smell can persist in the air or in the nasal cavity of the assessors. Memory effects could also play a major role, as assessors commented that they found it difficult to remember the first stimulus after assessing the third sample in the triangle test. Furthermore, the studies mentioned above used the same number of assessors to compare different

discrimination tests. The number of assessors needs to be adjusted to compare discrimination tests with respect to their relative sensitivity (Ennis, 1993), as using the same number of assessors would give an advantage to the discrimination test that is statistically more powerful (discussed in 4.2.2.3).

4.3.2.2.2 Limitations of the study

The first limitation of this study was that the data from each sensory session were pooled from assessors with various sensitivities and biases. It has been shown that pooling data obtained from individual assessors can add noise to the system and lead to underestimation of d' (Hautus, 1997, Macmillan and Kaplan, 1985, Rousseau and O'Mahony, 2000). Furthermore, different groups of assessors were used for each sensory session, and the differences observed between the triangle and same-different approaches could be due to variation in sensitivities between assessors. However, the assessors were recruited from the same environment and age class and it is unlikely that the difference in sensitivity can explain such large differences between d' values.

Another limitation of this study was the calculation of the number of assessors to compare the triangle and same-different approaches. Such calculations assume a specific cognitive strategy. For example, the tau-strategy was assumed for the same-different test, while the COD-strategy was assumed for the triangle test. In this study, the calculation of the number of assessors assumed a same-different test without a sureness rating. Adding a sureness rating should have been taken into account in determining the

number of assessors (B. Rousseau, personal communication). To the author's knowledge, there is no published systematic procedure/software to determine the number of assessors required for the same-different test with a sureness rating. As adding a sureness rating to the same-different test increases its statistical power (Bi et al., 2013), it can be anticipated that the number of assessors required for the same-different test with a sureness rating would decrease compared to the same-different test without a sureness rating (B. Rousseau, personal communication).

4.4 Conclusions

This chapter provides vital information for the estimation of the Thurstonian d' when using the same-different approach. Although some assessors might have used a beta-strategy when the flavours were delivered orthonasally, the hypothesis of a tau-strategy could not be rejected. Furthermore, results on retronasal flavours did not allow conclusions on the cognitive strategy. As the results on cognitive strategy were inconclusive, and as the tau-strategy is usually assumed (Lee and O'Mahony, 2004, O'Mahony and Rousseau, 2002), the differencing model associated with the tau-strategy will be used in the rest of the study.

Although the same-difference and triangle tests have been compared previously, it was the first time that both the approaches were compared in omission experiments. It was evident that the same-different approach was more sensitive than the triangle approach, as the d' values obtained using the

same-different test were 1.2 to 3.5 times higher than the d' values obtained using the triangle test. Carry-over, memory effects and fatigue were suspected to play a major role in this observation.

This study addresses a number of areas in omission research in which improvements can be made, in terms of sensory methodology and analysis of the data. First, the same-different approach constitutes a relevant alternative to the triangle approach in omission testing. Secondly, the Thurstonian measure d' proved to be a very useful tool as it allows the relative importance of the different volatiles within a flavour to be assessed. The Thurstonian d' is widely used in psychology and other fields such as electrical engineering (Wichchukit and O'Mahony, 2010), but is still rarely used in sensory testing.

Chapter 5. Determining key volatiles in flavours, and a comparison of ortho- and retronasal sensitivities

5.1. Introduction

5.1.1 Determining key volatiles in flavour

Instrumental studies have shown that only a small fraction of volatiles contribute to the overall flavour of food (Grosch, 2001). From a commercial perspective, it is important for the food and flavour industries to identify the key compounds of flavour, in order to develop flavourings with a minimum number of components necessary to represent the target flavour. Sensory omission experiments can be used to identify key volatiles in flavour mixtures (Ito et al., 2002, Tokitonio et al., 2005, Greger and Schieberle, 2007) (see section 1.3.2). The same-different approach presented in this study, using the same-different test and Thurstonian d' , could be used to identify the key volatiles in flavour and measure the relative contribution of individual volatiles within a flavour model.

Volatiles can reach the olfactory epithelium via the ortho- and retronasal routes (Goldstein, 2010) (see section 1.2.2.2). Omission studies often concentrate on orthonasal delivery, and only a few omission studies have considered the retronasal delivery of flavour (House and Acree, 2002). As flavours are predominantly sensed by the retronasal olfactory system (Chen and Engelen, 2012, Shepherd, 2006), this type of delivery is particularly important for food and beverage products; and, therefore, there is a clear

need to investigate the relative impact of odorants in flavour perception retronasally.

5.1.2 Ortho- and retronasal detection thresholds

Detection thresholds are determined to assess the sensitivity of assessors to a specific volatile. Table 21 and Table 22 present the detection thresholds of volatiles in the strawberry and savoury flavours, respectively, along with their air/water partition coefficients (K_{aw}).

Table 21: Ortho- and retronasal detection thresholds and K_{aw} of the volatiles in the strawberry flavour

Volatile	Orthonasal threshold ¹ ($\mu\text{g}/\text{kg}$)	Retronasal threshold ¹ ($\mu\text{g}/\text{kg}$)	K_{aw}
Ethyl butanoate	0.005-13.6	0.1	$1.63 \cdot 10^{-2} \sim$
Ethyl hexanoate	5	11	$2.96 \cdot 10^{-2}$
Methyl dihydrojasmonate	240-15,360 (Leffingwell)	Not available	$2.05 \cdot 10^{-5}$
2,3-Butandione	4-15	0.2-5.4	$5.44 \cdot 10^{-4} \sim$
Butanoic acid	50-1000	1,000-6,800	$2.19 \cdot 10^{-5} \sim$
Gamma-decalactone	5-11	88	$2.3 \cdot 10^{-2}$
4-Hydroxy-2,5-dimethyl-3-furanone	0.6-60	30	$6.01 \cdot 10^{-4}$
Methyl(E)-3-phenylprop-2-enoate	Not available	11	$1.69 \cdot 10^{-4}$
Cis-3-hexen-1-ol	39	30	$6.34 \cdot 10^{-4}$

¹ Values were taken from Rychlik et al. (1999). K_{aw} were estimated by Estimation Programs Interface (EPI) suiteTM (version 4.1). Experimental values of K_{aw} were used when available. \sim Indicates experimental values of K_{aw}

Table 22: Ortho- and retronasal detection thresholds and K_{aw} of the volatiles in the savoury flavour

Volatile	Orthonasal threshold ¹ ($\mu\text{g}/\text{kg}$)	Retronasal threshold ¹ ($\mu\text{g}/\text{kg}$)	K_{aw}
2-Methylpropanal	0.006-2.3	28.0-125	$7.36 \cdot 10^{-3} \sim$
2-Furfurylthiol	0.005-0.12	0.005	$6.42 \cdot 10^{-3}$
4-Hydroxy-2,5-dimethyl-3-furanone	0.6-60	30	$6.01 \cdot 10^{-4}$
3-Mercapto-2-butanone	3 (Belitz et al., 2004)	Not available	Not available
2-Methyl-3-furanthiol	0.007-0.0004	Not available	Not available
3-Methylthiopropional	0.2-1.8	0.04-10	$3.93 \cdot 10^{-5}$
E,E-2,4-Decadienal	0.07-0.2	0.001-0.009	$8.99 \cdot 10^{-3}$
12-Methyltridecanal	0.1	Not available	Not available
1-Octen-3-one	0.005-0.05	0.01	$3.32 \cdot 10^{-3}$
Indole	90	Not available	Not available

¹Values were taken from Rychlik et al. (1999). K_{aw} were estimated by Estimation Programs Interface (EPI) suite™ (version 4.1). Experimental values of K_{aw} were used when available. \sim Indicates experimental values of K_{aw}

The detection threshold of a volatile depends on its physico-chemical properties, such as K_{aw} and its availability in the sample (free or entrapped).

The K_{aw} of a volatile is the ratio between the concentration of a volatile in the air phase and the concentration of the volatile in the water phase at equilibrium. It has been shown previously that the orthonasal detection threshold correlates to K_{aw} (Guyot et al., 1996): the higher the K_{aw} of a volatile, the lower its detection threshold. This observation can be explained

by the higher concentrations in the headspace at equilibrium for volatiles with a higher K_{aw} . In this case, a high number of the volatile molecules reaching the olfactory mucosa at the same time and activating the olfactory receptors could result in higher sensitivity to the volatiles.

The ortho- and retronasal detection thresholds presented in Table 21 and Table 22 are given as volatile concentrations in water (Rychlik et al., 1999). Using this method, the differential headspace concentrations between ortho- and retronasal delivery (Linforth et al., 2002) is taken into account. Rychlik *et al.* (1999) compiled detection thresholds from different sources. As a result, the estimated detection thresholds vary greatly with the methods used. For example, Schieberle *et al.* (1991) obtained 1 $\mu\text{g/L}$ for the detection threshold of ethyl butanoate using triangle tests, whereas Larsen *et al.* (1992) found a detection threshold of 0.005 $\mu\text{g/L}$ using duo-trio tests.

The variation in detection thresholds in the literature can be attributed to different factors. First, individual variation among assessors, as well as the number of assessors used, can influence the detection threshold measured (Vuilleumier et al., 2002, Meilgaard et al., 2007, Plotto et al., 2004, Brown et al., 1978). Secondly, the difficulty to deliver consistent aroma stimuli (temperature, sample composition...) can result in variation of the measured thresholds (Taylor and Linforth, 2010, Walker et al., 2003, Vuilleumier et al., 2002). A third factor is the use of different methodologies to measure the detection threshold (Meilgaard et al., 2007, Taylor and Linforth, 2010).

Different methods are available to estimate the detection threshold of volatiles or tastants (Walker et al., 2003). The forced-choice ascending method of limits (ASTM E679) is commonly used (Jaeger et al., 2014). In this method, a small group of assessors receive a set of 3-AFC tests. Each test contains one sample of diluted stimulus and 2 samples of water. The tests are presented in order of increasing concentrations. Even within this method, the estimated detection threshold can be biased by the small number of assessors, the concentrations chosen, or the number of 3-AFC tests in a set.

The detection thresholds used in this study for the volatiles in the strawberry and savoury flavour models were taken directly from the literature. To overcome the large variation and inaccuracy of these detection thresholds, further experiments could measure the sensitivity of the assessors that conducted the discrimination tests in this PhD study. This would be an ambitious study as it would involve measuring the individual detection threshold of hundreds of assessors, for the 9 volatiles in the strawberry flavour and the 10 volatiles in the savoury flavour model.

5.1.3 Objectives of this chapter

The first part of this chapter focuses on determining the key volatiles in the strawberry and savoury flavours delivered orthonasally. Omission testing was carried out on the strawberry and savoury flavours diluted in water and delivered orthonasally (Straw4 and Sav1, respectively). The same-different

approach was used along with the Thurstonian d' to determine the relative importance of each individual volatile in the flavour.

In the second part, 'fractional omission testing' was conducted on the savoury flavour to measure the effect of removing only 50 % of a volatile on the flavour perceived orthonasally (Sav3).

In the third part, ortho- and retronasal sensitivities were compared, for both the strawberry and savoury flavour. For the strawberry flavour, data collected from previous omission experiments were used (Straw4 and Straw5). A new series of omission tests (Sav2) were carried out on the savoury flavour delivered retronasally and subsequently compared with previous data collected on the orthonasal savoury flavour (Sav1).

5.2. Materials and methods

5.2.1 Part 1: Determining key volatiles in flavours delivered orthonasally

5.2.1.1 Preparation of the flavour samples

The strawberry and savoury flavours and their corresponding omission samples were prepared as described in section 2.2.1.1. The strawberry flavour and corresponding omission samples in PG was kept at 4° C and used up to 8 days after preparation. The savoury flavour and corresponding omission samples were prepared freshly on the day preceding the sensory sessions.

The strawberry and savoury flavours (and corresponding omission samples) were diluted in mineral water at 0.75 % and 0.1 % w/w, respectively, as

described in section 2.2.1.2. Flavour samples diluted in water were kept at 4° C and used within 24 hours. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure flavour samples were at room temperature (20° C ±2° C).

5.2.1.2 Assessing the effect of removing individual volatiles

The sensory sessions were carried out as described in section 2.2.4. Orthonasal omission tests (session Straw5) were carried out on the strawberry flavour diluted in water to assess the effect of removing individual volatiles on the perceived flavour. One hundred assessors carried out 9 same-different tests to compare each of the 9 omission samples with the original flavour model.

Orthonasal omission tests (session Sav1) were carried out on the savoury flavour diluted in water to determine the relative importance of each individual volatile within the flavour. One hundred assessors carried out 10 same-different tests to compare each of the 10 omission samples with the original flavour model.

5.2.1.3 Data analysis

Thurstonian d' values were estimated to determine the relative importance of individual volatiles in the flavour. d' values were estimated using the differencing model as described in section 2.2.7.1.

Pearson signed square root statistic was used to test for a significant difference between the original flavour model and omission samples (at $\alpha = 0.05$) (see section 2.2.7.3).

5.2.2 Part 2: Assessing the effect of removing a fraction of a volatile

5.2.2.1 Preparation of the 'fractional omission samples'

'Fractional omission samples' were prepared as described in section 2.2.1.1, by omitting 50 % of a volatile from the original savoury flavour model. The savoury flavour and 'fractional omission samples' were prepared freshly on the day preceding the sensory sessions.

The flavour samples were diluted in mineral water at 0.1 % w/w, as described in section 2.2.1.2. Flavour samples diluted in water were kept at 4° C and used within 24 hours. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure flavour samples were at room temperature (20° C \pm 2° C).

5.2.2.2 Fractional omission testing

Where an omission test in Sav1 indicated that the complete removal of a volatile was perceived significantly, further samples were prepared by removing 50 % of that volatile. Session Sav1 indicated that the removal of 2-methylpropanal, 2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3-furanone, 3-mercapto-2-butanone and 2-methyl-3-furanthiol were all significantly detected ($p < 0.05$) and so session Sav3 involved 5 omission tests to investigate the effect of the individual removal of 50 % of these volatiles.

5.2.2.3 Data analysis

Thurstonian d' values were estimated to measure the effect of removing 50 % of a volatile on the perceived flavour. d' values were estimated using the differencing model as described in section 2.2.7.1.

Pearson signed square root statistic was used to test for a significant difference between the original flavour model and each 'fractional omission sample' (at $\alpha = 0.05$) (see section 2.2.7.3).

5.2.3 Part 3: Comparing ortho- and retronasal perceptions

5.2.3.1 Preparation of the savoury flavour samples

Savoury flavours and the corresponding omission samples were prepared freshly on the day preceding the sensory sessions, as described in section 2.2.1.1.

The flavour samples in PG were diluted in mineral water at 0.1 % w/w, as described in section 2.2.1.2. Flavour samples diluted in water were kept at 4° C and used within 24 hours. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure flavour samples were at room temperature (20° C \pm 2° C).

5.2.3.2 Assessing the effect of removing individual volatiles on the savoury flavour delivered retronasally

Session Sav2 was carried out retronasally using the savoury flavour diluted in water to assess the effect of removing individual volatiles on the perceived flavour. Session Sav2 involved 100 assessors carrying out 10 same-different

tests to compare each one of the 10 omission samples with the original flavour model. Session Sav2 was split over two sub-sessions. Within each sub-session, assessors were allocated a 5 minute break after every 2 tests.

5.2.3.3 Data analysis

Thurstonian d' values were estimated to measure the effect of removing individual volatiles on the flavour perceived retronasally. d' values were estimated using the differencing model as described in section 2.2.7.1.

Pearson signed square root statistic was used to test for a significant difference between the original flavour model and each omission sample (at $\alpha = 0.05$) (see section 2.2.7.3).

Sessions Straw5 and Straw6 and sessions Sav1 and Sav2 were used to compare ortho- and retronasal perceptions in sweet and savoury flavours, respectively. Student t-tests ($\alpha = 0.05$) were used to compare (1) the d' values obtained ortho- and retronasally and (2) d' values obtained ortho- and retronasally for each individual volatile.

5.3 Results and discussion

5.3.1 Part 1: Determining key volatiles in flavours delivered orthonasally

5.3.1.1 Strawberry flavour

Table 23 presents the d' values obtained from the orthonasal omission tests.

Note that the d' values measured for methyl dihydrojasmonate, butanoic acid and methyl(E)-3-phenylprop-2-enoate were negative. This phenomenon is

called a ‘floor effect’: the assessors are not able to discriminate between the samples and the negative value of d' are due to sampling variability (Hautus, 1997). This has been observed previously (Kim et al., 2012, Stocks et al., 2013).

Table 23: d' values obtained from omission testing on the strawberry flavour diluted in water and delivered orthonasally

Flavour block	Volatile	d'
Fruity/floral	Ethyl butanoate	0
	Ethyl hexanoate	1.22*
	Methyl dihydrojasmonate	- 1.13
Buttery	2,3-Butandione	0.4
	Butanoic acid	- 0.71
	Gamma-decalactone	0.51
Caramel	4-Hydroxy-2,5-dimethyl-3-furanone	0.96
	Methyl(E)-3-phenylprop-2-enoate	- 0.65
Green	Cis-3-hexen-1-ol	1.22*

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$)

Cis-3-hexen-1-ol and ethyl hexanoate exhibited the highest d' values (both $d' = 1.22$), and their removal was significantly detected ($p = 0.028$ and 0.037 , respectively). The d' values measured for 4-hydroxy-2,5-dimethyl-3-furanone was relatively high ($d' = 0.96$), although the difference between the original flavour model and omission sample cannot be claimed to be perceived significantly by assessors ($p = 0.127$).

Results are in accordance with results from Schieberle *et al.* (1997), who showed that 4-hydroxy-2,5-dimethyl-3-furanone ('strawberry', 'caramel' aroma) and cis-3-hexenal ('green', 'leaf-like' aroma) played a key role in the strawberry flavour. In particular, 4-hydroxy-2,5-dimethyl-3-furanone is regarded as the most important volatile in strawberry due to its high concentration (Larsen *et al.*, 1992) and low detection threshold (0.6-60 µg/kg). Ethyl butanoate and ethyl hexanoate are amongst the most abundant esters in strawberries (Pysalo *et al.*, 1979). The high d' obtained for ethyl hexanoate ($d' = 1.22$) highlighted the importance of the fruity/floral block in the strawberry flavour. The green flavour block constituted of cis-3-hexen-1-ol also played a major role in the perception of the strawberry flavour.

5.3.1.2 Savoury flavour

Results from orthonasal omission experiments on the savoury flavour are presented in Table 24. Each row of the table corresponds to the omission test comparing the whole flavour with flavour omitting a 100 or 50 % fraction of a volatile. Table 24 shows that the complete removal of the top note, 2-methylpropanal, was detected significantly ($p = 0.022$). The complete removal of 4 out of 5 individual volatiles from the meaty block (2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3-furanone, 3-mercapto-2-butanone and 2-methyl-3-furanthiol) was detected significantly ($p = 0.031, 0.029, 0.002$ and 0.016 , respectively).

Table 24: d' values obtained from omission testing and fractional omission testing on the savoury flavour delivered orthonasally

Flavour block	Volatile	d'	
		Complete removal	50 % removal
Top note	2-Methylpropanal	1.27*	0
	2-Furfurylthiol	1.23*	0.88
	4-Hydroxy-2,5-dimethyl-3-furanone	1.25*	0.58
Meaty block	3-Mercapto-2-butanone	1.63*	0.49
	2-Methyl-3-furanthiol	1.32*	- 0.71
	3-Methylthiopropional	0.81	
	E,E-2,4-Decadienal	- 1.04	
Fatty block	12-Methyltridecanal	0.6	
	1-Octen-3-one	- 0.59	
Control	Indole	1.08	

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$)

These results are in accordance with previous results from omission experiments on boiled beef (Grosch, 2001, Kerschler and Grosch, 1999). Sulfur compounds are a major contributor to meat flavours (Mottram, 1998, Mottram and Madruga, 1994, Shahidi, 1989, Golovnja and Rothe, 1980, Chang and Peterson, 1977, Gasser and Grosch, 1988, Gasser and Grosch, 1990). Although these sulfur compounds are generally present in low concentrations, they have a high impact on the flavour because of their very low detection thresholds (Golovnja and Rothe, 1980).

5.3.2 Part 2: Assessing the effect of removing a fraction of a volatile

Results from the 'fractional omission testing' on the savoury flavour are also presented in Table 24. Although the removal of 50 % of 2-furfurylthiol was not detected significantly ($p = 0.138$), the d' measured ($d' = 0.88$) was higher compared to the d' values obtained with the complete removal of other volatiles. The role of 2-furfurylthiol in the flavour of cooked meat has been reported previously (Gasser and Grosch, 1988, Gasser and Grosch, 1990, Farmer and Patterson, 1991, Kerscher and Grosch, 1997, Guth and Grosch, 1993, Kerscher and Grosch, 1999, Grosch, 2001).

Results from 'fractional omission testing' highlight the importance of the volatile concentrations on the perceived flavour delivered orthonasally. The results showed that the human nose can be very sensitive to a change in the volatile concentration of a mixture. It has been shown that the volatile concentration ratio is crucial in blending volatile mixtures. Pineau *et al.* (2009) showed that very small variations in the concentration of certain ethyl esters significantly affected the perceived aroma of red wine. Furthermore, variations in concentration under just noticeable difference were able to induce a significant decrease in pineapple odour of the ternary mixture (Le Berre *et al.*, 2008a). Le Berre *et al.* (2008a) compared this phenomenon with listeners who could detect a change in the chord of an orchestra, but were unable to say which chord had been modified (Acker and Pastore, 1996).

5.3.3 Part 3: Comparing ortho- and retronasal perceptions

5.3.3.1 Strawberry flavour

Table 25 shows d' values estimated for the same solution of strawberry flavour delivered either ortho- or retronasally. Cis-3-hexen-1-ol exhibited the highest d' ($d' = 0.92$) when the strawberry flavour was delivered retronasally. This result supports the key role of cis-3-hexen-1-ol in the strawberry flavour. The results highlight large differences in sensitivity to the removal of volatiles between the ortho- and retronasal routes. Assessors could not perceive the removal of any of the individual volatiles when samples were delivered retronasally ($p > 0.05$).

Table 25: d' values obtained from omission testing on the strawberry flavour delivered ortho- or retronasally

Volatile	d'	
	Orthonasal delivery	Retronasal delivery
Ethyl butanoate	0	- 0.71
Ethyl hexanoate	1.22*	0
Methyl dihydrojasmonate	- 1.13	0.69 †
2,3-Butandione	0.4	0.82
Butanoic acid	- 0.71	- 0.86
Gamma-decalactone	0.51	- 0.57 †
4-Hydroxy-2,5-dimethyl-3-furanone	0.96	- 0.52 †
Methyl(E)-3-phenylprop-2-enoate	- 0.65	- 1.11
Cis-3-hexen-1-ol	1.22*	0.92

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$) † Indicates a significant difference with the d' measured orthonasally (Student t-test, $p < 0.05$)

Although the overall comparison between orthonasal and retronasal d' values was not significant (Student t-test, $p = 0.26$), the omission of all individual volatiles in the flavour mixture, except 2,3-butanedione and methyl dihydrojasmonate, was better detected orthonasally (higher d' values). Student t-tests showed that the d' measured orthonasally was significantly higher for 4-hydroxy-2,5-dimethyl-3-furanone ($p < 0.001$) and gamma-decalactone ($p = 0.018$), compared to the d' measured retronasally.

Retronasal thresholds were higher than orthonasal thresholds for every volatile in the strawberry flavour except for cis-3-hexenol and ethyl butanoate (Table 21). For 2,3-butanedione, the lower retronasal detection threshold compared to the orthonasal thresholds could explain the higher retronasal sensitivity to the removal of this volatile.

5.3.3.2 Savoury flavour

Table 26 presents the d' values measured for the savoury flavour delivered retronasally. The removal of 2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3-furanone and 3-mercapto-2-butanone was significantly detected ($p = 0.007$, 0.015 and 0.012, respectively), which confirms the importance of the volatiles from the meaty flavour block in the savoury flavour. Although the removal of 2-methyl-3-furanthiol and 2-methylpropanal was perceived significantly orthonasally ($p = 0.016$ and 0.022, respectively), it was not perceived retronasally ($p = 0.376$ and 0.124, respectively).

Table 26: d' values obtained from omission tests on the savoury flavour delivered ortho- or retronasally

Volatile	d'	
	Orthonasal delivery	Retronasal delivery
2-Methylpropanal	1.27*	0.9
2-Furfurylthiol	1.23*	1.43*
4-Hydroxy-2,5-dimethyl-3-furanone	1.25*	1.33*
3-Mercapto-2-butanone	1.63*	1.36*
2-Methyl-3-furanthiol	1.32*	0.45 †
3-Methylthiopropional	0.81	- 0.2
E,E-2,4-Decadienal	- 1.04	- 0.26
12-Methyltridecanal	0.86	- 0.67 †
1-Octen-3-one	- 0.59	0.76 †
Indole	1.08	0.63

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$) † Indicates a significant difference with the d' measured orthonasally (Student t-test, $p < 0.05$)

Here again, the results highlighted the large differences between ortho- and retronasal sensitivities. Although the overall comparison between ortho- and retronasal d' values was not significant ($p = 0.46$), the d' values measured orthonasally were higher compared to d' values measured retronasally, except for 4-hydroxy-2,5-dimethyl-3-furanone, E,E-2,4-decadienal and 1-octen-3-one. Results indicate that a higher number of assessors might have perceived the removal of 12-methyltridecanal and 2-methyl-3-furanthiol orthonasally (Student t-tests, $p < 0.001$ and 0.011 , respectively).

On the contrary, a higher number of assessors perceived the removal of 1-octen-3-one retronasally ($p < 0.001$). Looking at the ortho- and retronasal detection thresholds for the volatiles in the savoury flavour, most intervals overlapped (Table 22). For E,E-2,4-decadienal, the lower retronasal detection threshold compared to the orthonasal thresholds could explain the higher retronasal sensitivity to the removal of this volatile.

5.3.3.3 Discussion

5.3.3.3.1 Orthonasal olfaction is more sensitive

In this study, assessors were generally more sensitive to the removal of volatiles when the flavour was presented orthonasally, compared to retronasal delivery. Although it is the retronasal olfactory system which is responsible for our ability to identify the flavour of food (Shepherd, 2006), it has been shown previously that orthonasal olfaction is more sensitive at the threshold and supra-threshold levels (Sun and Halpern, 2005, Bojanowski and Hummel, 2012, Hummel et al., 2006, Negoias et al., 2008). Orthonasal olfaction was shown to be more sensitive for detecting (Hummel et al., 2006, Voirol and Daget, 1986) and identifying aromas (Heilmann and Hummel, 2004, Sun and Halpern, 2005, Pierce and Halpern, 1996). Heilmann *et al.* (2004) found that both food and non-food aromas showed lower thresholds via the orthonasal route and suggested that the lower sensitivity retronasally is compatible with the higher concentrations experienced while eating.

However, other studies showed no difference between ortho- and retronasal sensitivities (Small et al., 2005, Kuo et al., 1993).

5.3.3.3.2 Possible mechanisms

To explain the differences observed between ortho- and retronasal perceptions, Rozin (1982) proposed that olfaction is a dual modality, as it can perceive objects in the outside world as well as food in the mouth. Psychophysical, electrophysiological, and imaging data support this theory, as they can identify clear differences in the perception and processing of ortho- and retronasal olfactory information (Bojanowski and Hummel, 2012, Negoias et al., 2008, Sun and Halpern, 2005, Small et al., 2005).

Rozin (1982) suggested three possible mechanisms for the olfactory duality. The first mechanism is the existence of a sensory gate that leads to different sensations depending whether the stimulus is perceived ortho- or retronasally. The gate could be controlled by the presence of a substance in the mouth, or by the direction of air flow through the olfactory mucosa (Mozell, 1964, Negoias et al., 2008).

The second mechanism is the combination of oral and olfactory stimuli that cause referral of the olfactory stimulus to the mouth (Hummel et al., 2006, Lim and Johnson, 2011, Small and Prescott, 2005) and give rise to the retronasal perception different from the orthonasal perception.

The mechanism based on the different volatile concentrations delivered to the olfactory epithelium via the ortho- and retronasal routes (Figure 17) was

the most likely hypothesis to explain the lower retronasal sensitivity observed in this study. Volatile concentration in the breath during the consumption of food appeared to be much lower than the concentration in the headspace above a sample solution (Deibler et al., 2001, Linforth et al., 2002). This is because aqueous systems are orally consumed within a few seconds and there is no time to reach equilibrium between the liquid and the gas phase in the mouth (Linforth et al., 2002). It was shown that volatiles in water produce breath concentrations of only 10 % of the concentration expected based on the K_{aw} (Taylor and Linforth, 2010). The liquid and gas dilution in the oral cavity (Taylor and Linforth, 2010), or the adsorption of volatiles on the oral surfaces could also lower the volatile concentration delivered to the olfactory receptors in the case of retronasal delivery (Wilkes et al., 2009, Linforth et al., 2002).

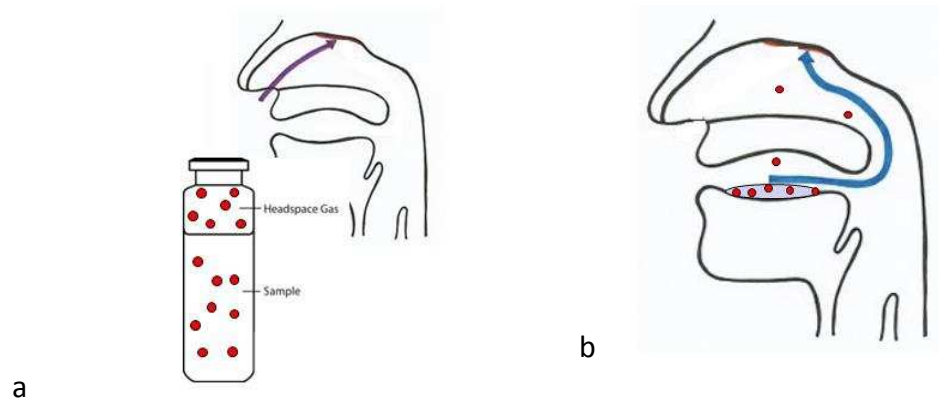


Figure 17: Volatile concentration reaching the olfactory receptors via a. the orthonasal route (sniffing) and b. the retronasal route (oral consumption)

In the case of retronasal delivery, the chemical properties of the volatiles, such as their polarity or molecular weight, could have a strong effect on the way they are delivered to the olfactory receptors. Less polar odorants are more persistent in the mouth, due to their adsorption into the oral, throat and nasal mucosa (Buettner and Schieberle, 2000). The polarity of the volatiles used in this PhD study could influence their delivery to the olfactory receptors. The more polar volatiles are less adsorbed into the oral, throat and nasal mucosa, and reach the olfactory mucosa faster, and at higher concentrations. On the contrary, the less polar volatiles are more adsorbed into the mucosa and could act as an aroma reservoir: they are released continuously and are responsible for the persistence of the flavour.

Another hypothesis to explain the higher orthonasal sensitivity was the different processing between ortho- and retronasal information. In the current study, the assessment of the flavours retronasally may have been more complicated as assessors were likely to expect the specific taste-aroma profile of the strawberry or the savoury flavour. The absence of congruent tastants, which would enhance flavour perception (Green et al., 2012) may have confused assessors and resulted in poor discrimination. The effects of congruent tastants on retronasal sensitivities will be discussed in the next chapter.

5.3.3.3.3 Importance of the experimental protocol

A limitation of this study was the unknown volatile concentration in the oral and nasal cavities, as the volatile concentration delivered to the olfactory receptors is difficult to control (and monitor), especially with 100 assessors. Furthermore, ortho- and retronasal routes could not be compared directly, as gustatory, thermal, and mechanical stimuli associated with the presence of the liquid in the mouth produced can interact with olfactory perception (Welge-Lussen et al., 2005, Bojanowski and Hummel, 2012, Negoias et al., 2008). Therefore, it was impossible to determine whether the differences observed via the ortho- and retronasal routes were related to the concentration delivered to the olfactory receptors or to the oral stimulation.

The presentation of volatile samples is a critical issue when comparing ortho- and retronasal sensitivities. The inconsistency of the studies comparing ortho- and retronasal perceptions in the literature could be due to the difficulty to deliver the same concentration to the olfactory receptors via the ortho- and retronasal routes. Vuilleumier *et al.* (2002) showed that when the volatile concentrations delivered to the olfactory receptors were the same, the perceived intensity of aromas was the same ortho- and retronasally. They suggested that, in order to compare ortho- and retronasal perception, volatiles should be delivered in the gas phase via both ortho- and retronasal routes using a special device to control the concentrations delivered to the olfactory mucosa (Heilmann and Hummel, 2004).

MS-nose could also be used in-vivo to monitor the volatile concentrations close to the olfactory receptors when the sample is delivered retronasally. MS-nose is very sensitive and can measure odours at concentrations around 10 parts per billion. The results can help understand the delivery of volatiles to the olfactory receptors, as they reflect the perceived odour when the samples are delivered retronasally.

5.4. Conclusions

In this chapter, the same-different approach was successfully applied to identify the key volatiles in the strawberry and savoury flavour. Cis-3-hexen-1-ol played a major role in the strawberry flavour, as it exhibited the highest d' both ortho- ($d' = 1.22$), and retronasally ($d' = 0.92$). In the savoury flavour, 3 volatiles from the meaty block, 2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3-furanone and 3-mercapto-2-butanone, appeared to play a major role, as their individual removal was significantly detected both ortho- and retronasally.

The current study successfully demonstrated the application of the same-different approach to fractional omission testing which enabled the effect of decreasing certain volatile concentrations on the perceived flavour to be assessed. Results from fractional omission testing confirmed the key role of 2-furfurylthiol in the savoury flavour. It was the first time that the approach using the same-different test was used for fractional omission testing. Results show the importance of using very precise volatile concentrations in flavour mixture for the food flavour and the perfume industry.

The new approach allowed the comparison of flavour perception via the ortho- and retronasal routes. An extensive literature search revealed no other studies using omission testing to compare ortho- and retronasal sensitivities. These results confirm that studies on orthonasal flavour do not represent perception retronasally. This finding has implications for the analysis of flavour mixtures used in food and beverage products which are consumed, rather than simply sniffed. In this case, it is recommended that retronasal analysis is carried out as well as orthonasal analysis, as the perception of the flavour can vary significantly between the two delivery routes.

Chapter 6. Comparing d' and OAVs, and investigating interactions between volatiles in flavour

6.1 Introduction

6.1.1 Odour Activity Values (OAVs)

The selection of the key volatiles contributing to a food flavour is often based on the idea that the higher the perceived intensity of a volatile, the higher its contribution to the flavour. Odour Activity Values (OAVs) or dilution techniques such as Aroma Extract Dilution Analysis (AEDA) or Charm analysis (Acree et al., 1984) are commonly used to determine key volatiles to the flavour of food products (Grosch, 1994). These instrumental analyses measure the individual qualities of aromas, and interactions within flavours are not taken into account. Furthermore, the 'OAV concept' assumes that the perceived intensity is proportional to the concentration of a volatile, instead of fitting Stevens' law which represents sensory perception (Stevens, 1961, Delahunty et al., 2006, Berglund et al., 1971).

Omission experiments have shown that OAVs cannot be used to assign a ranking of importance to volatiles in a specific flavour (Taylor and Mottram, 1996, Grosch, 2001). For example, volatiles with low OAVs can become essential for a flavour, such as guaiacol in olive oil (Reiners and Grosch, 1998, Grosch, 1999) or linalool and α -terpineol in Sauvignon blanc wine (Benkowitz et al., 2012), whereas volatiles with high OAVs can become of only minor importance, such as acetaldehyde in oil (Reiners and Grosch, 1998). However,

these studies did not allow comparing OAVs with the relative importance of individual volatiles in flavour mixtures. The new approach presented in this thesis allows the direct comparison of OAVs with the relative importance of individual volatiles in flavour mixtures as determined by d' values

6.1.2 Interactions between volatiles in mixture

Interactions between volatiles could explain why OAVs cannot be used to determine the individual contribution of volatiles to a flavour (Livermore and Laing, 1998). It is important to understand interactions between volatiles in a mixture, as it could help design better flavour models based on perceptual interactions between volatiles.

Interactions between volatiles can be qualitative, with effects on the aroma quality, or quantitative, with effects on the aroma intensity (Laing et al., 1984). The different types of interactions have been summarised by Breslin (1996) (see appendix 1). Suppressive interactions are the most common effect observed in volatile mixtures (Laing and Jinks, 2001). Suppressive interactions can cause certain volatiles to lose their intensity or even their individual aroma in a flavour mixture (Atanasova et al., 2005). For example, the woody aroma of wine dominates the fruity aroma in binary mixtures (Atanasova et al., 2005), and 3-methylthiopropional was suppressed in French fries flavour (Wagner and Grosch, 1998).

Suppressive interactions can also result in the perception of a mixture as a single unit, for example coffee or chocolate (Le Berre et al., 2008a, Livermore

and Laing, 1998). This phenomenon is called perceptual blending. Due to perceptual blending, humans are very poor at detecting or identifying volatiles in mixtures (Laing et al., 2002, Marshall et al., 2006, Weiss et al., 2012, Cain et al., 1998). It was shown that humans are only able to identify up to 3 or 4 aromas in complex mixtures (Le Berre et al., 2008b, Livermore and Laing, 1998, Laing and Francis, 1989).

Synergistic interactions can enhance the perceived intensity of a volatile in a particular mixture (Chaput et al., 2012, Benkwitz et al., 2012). Although synergistic interactions are quite rare in olfaction (Laing and Jinks, 2001), they have been observed at threshold and subthreshold levels (Ito and Kubota, 2005, Labbe et al., 2007, Miyazawa et al., 2008). For example, synergistic interactions increased the intensity of guaiacol in olive oil (Reiners and Grosch, 1998, Grosch, 1999) or linalool and α -terpineol in Sauvignon Blanc wine (Benkwitz et al., 2012).

6.1.3 Mechanisms of interactions between volatiles

Interactions between volatiles can occur at different levels, from the physico-chemical level (Walker et al., 2003) to the peripheral (receptor level) and central levels (Laing and Jinks, 2001, Chaput et al., 2012, Berglund et al., 1976) (see section 1.2.3.1). In particular, interactions at the receptor level are thought to play a major role in the processing of volatiles in mixtures (Oka et al., 2004, Brodin et al., 2009).

Different mechanisms have been proposed to explain suppressive interactions between volatiles in mixture. Suppression can occur at the receptor level when two volatiles compete for the same receptor sites (Laing and Jinks, 2001, Bell et al., 1987) (see section 1.2.3.1.2). Increasing the number of volatiles in a mixture increases the chance of competition between volatiles for the same receptor sites (Jinks and Laing, 1999). Suppression can also occur via lateral inhibition: the signal triggered by one volatile can inhibit or reduce the input of another volatile via neural connections between glomeruli or between mitral cells (Laing and Jinks, 2001, Valova et al., 2007) (see section 1.2.2.2).

Laing and Jinks (2001) proposed that synergistic interactions were due to a change in the headspace concentration of a volatile induced by the addition of other compounds. Interactions at the peripheral or central level could also induce synergistic effects (Miyazawa et al., 2008). At a cognitive level, odour processing is modulated based on memory, experience, emotions, and behavioural states (Chaput et al., 2012, Ishii et al., 2008, Grossman et al., 2008, Wilson et al., 2006). Therefore, factors such as previous experience, learned congruency and affective factors could affect interactions between aromas, in the same way as they affect taste-aroma interactions (see section 1.2.3.1.3). Grabenhorst *et al.* (2007) showed that interactions between pleasant (jasmine) and unpleasant (indole) aromas in specific regions of the

brain depended on whether the regions were associated with pleasant or unpleasantness aroma stimuli.

6.1.4 Investigating interactions in volatile mixtures

It is important to understand the interactions between volatiles in mixture, as interactions can modify the perception of flavour. Different approaches have been used to investigate interactions between volatiles. Early studies focused on qualitative and quantitative qualities of simple mixtures such as binary mixtures (Laing et al., 1984, Ferreira, 2012). It was shown that the perception of the intensity of a volatile mixture was higher or lower than the sum of the perceived intensity of each volatile (Laing and Jinks, 2001, Atanasova et al., 2005).

Studies on more complex mixtures often focused on identification of single aromas in mixtures (Jinks and Laing, 1999, Cashion et al., 2006, Marshall et al., 2006). Only a few omission experiments have been conducted to investigate the interactions between volatiles in complex mixtures (Lytra et al., 2013, Lytra et al., 2012, Benkwitz et al., 2012, Paravisini et al., 2014). Using omission experiments, Benkwitz *et al.* (2012) showed that the presence of β -damascenone enhanced the impact of varietal thiols on Sauvignon blanc wine. Paravisini *et al.* (2014) used a fractional factorial design to investigate the interactions between four odour notes ('vegetable', 'sharp', 'fruity' and 'nutty') in a caramel flavour, and highlighted high-order, complex interactions between 'vegetable', 'sharp' and 'nutty' odour notes.

Omission experiments appear to be a relevant approach to investigate interactions between volatiles, and the same-different approach used in this study offers an innovative approach to assess interactions between volatiles in complex mixtures. Furthermore, the Thurstonian d' can be used to measure the effect of the presence of a volatile on the assessor sensitivity to the removal of other volatiles in flavour mixture.

6.1.5 Objectives of this chapter

The first objective of this chapter was to determine if OAVs of the aroma compounds could predict the relative importance of individual volatiles in the strawberry and savoury flavour models, as measured by d' . For each individual volatile in the strawberry and savoury flavours, OAVs were compared to d' values obtained from omission testing. Analyses were carried out for both ortho- and retronasal delivery.

In the second part of this chapter, the same-different approach was used to investigate interactions between specific volatiles within the savoury flavour delivered orthonasally. 'Group omission testing' was used to this end: two or more volatiles were removed from the savoury flavour, before comparing the new sample to the original flavour model.

6.2 Materials and Methods

In the first part of this chapter, OAVs and d' were compared using data collected from previous omission experiments. In the second part, 'group omission testing' was carried out on the savoury flavour delivered orthonasally.

Firstly, as previous results showed that assessors could not significantly detect the removal of individual volatiles from the fatty flavour block (E,E-2,4-decadienal, 12-methyltridecanal and 1-octen-3-one), interactions between these volatiles were assessed.

Secondly, interactions between 4-hydroxy-2,5-dimethyl-3-furanone and other volatiles within the savoury flavour were investigated. 4-Hydroxy-2,5-dimethyl-3-furanone was chosen because it is widely used in the food flavour industry due to its characteristic properties to 'round' the character of savoury flavour mixtures (L. Jones, personal communication).

6.2.1 Part 1: Comparing d' values and OAVs

6.2.1.1 Calculation of Odour Activity Values (OAVs)

OAV refers to the ratio of the odorant concentration in the mixture to its odour threshold. Orthonasal Odour Activity Values (oOAVs) and retronasal Odour Activity Values (rOAVs) were calculated using Equation 4 and orthonasal and retronasal detection thresholds, respectively (Table 21 and Table 22). The purity of volatiles in the savoury flavour (see Table 15) was taken into account to calculate the OAVs.

$$\text{OAV} = \frac{\text{Concentration}_{\text{volatile}}}{\text{Detection threshold}_{\text{volatile}}}$$

Equation 4: Calculation of OAV

Ortho- and retronasal detection thresholds in Rychlik *et al.* (1999) are given as volatile concentrations in water. Using this method, the differential headspace concentrations between ortho- and retronasal delivery (Linthorpe *et al.*, 2002) is taken into account. Therefore, detection thresholds could be used directly to calculate oOAVs and rOAVs for the volatiles in the strawberry and savoury flavours in water. As the detection thresholds are variable in the literature, OAVs are presented as intervals.

6.2.1.2 Comparison between OAVs and d' values

It has been suggested that volatiles with higher OAVs contribute significantly more to a flavour (Acree *et al.*, 1984). Therefore, it was of interest in this study to compare the relative impact of volatiles in flavour and their respective OAV, for both ortho- and retronasal delivery.

Sessions Straw5 and Straw4 were used to compare d' values and OAVs for the strawberry flavour delivered ortho- and retronasally, respectively. Sessions Sav1 and Sav2 were used to compare d' values and OAVs for the savoury flavour delivered ortho- and retronasally, respectively.

6.2.2 Part 2: Investigating interactions between volatiles within the savoury flavour

6.2.2.1 Preparation of the savoury flavour

The savoury flavour was prepared as described in section 2.2.1.1. 'Group omission samples' were prepared by removing 2 or more volatiles from the savoury flavour model (as described in section 2.2.1.1). The savoury flavour and 'group omission samples' were prepared freshly on the day preceding the sensory sessions. The flavour samples were diluted at 0.1 % w/w in mineral water, as described in section 2.2.1.2. Flavour samples diluted in water were kept at 4° C and used within 24 hours.

6.2.2.2 'Group omission testing'

Sensory sessions were carried out as described in section 2.2.4. The savoury flavour samples were delivered orthonasally, as described in section 2.2.5.

Session Sav4 was carried out on the savoury flavour to investigate interactions between volatiles within the fatty flavour block. Session Sav4 involved 100 assessors carrying out 4 same-different tests. One omission test compared the original flavour model with the sample omitting the whole fatty block. The other three tests compared the original flavour model with omission samples omitting a pair of the volatiles from the fatty block: E,E-2,4-decadienal and 12-methyltridecanal (pair 1), E,E-2,4-decadienal and 1-octen-3-one (pair 2), and 12-methyltridecanal and 1-octen-3-one (pair 3). Assessors were allocated a 5 minute break after every 2 tests.

Session Sav5 was carried out to investigate interactions between 4-hydroxy-2,5-dimethyl-3-furanone and other volatiles from the savoury flavour. Here 4-hydroxy-2,5-dimethyl-3-furanone was removed from the original flavour, giving a new reference flavour, (r). The new reference (r) was then compared with the 9 omission samples (r - 1) in a new series of omission tests. The 9 omission tests (same-different tests) were carried out orthonasally by 100 assessors. Assessors were allocated a 5 minute break after every 2 tests.

6.2.2.3 Data analysis

d' values were estimated using the differencing model as described in section 2.2.7.1.

Student t-tests ($\alpha = 0.05$) (Excel 2010, Microsoft, USA) on d' values were used to determine (1) if the presence of 4-hydroxy-2,5-dimethyl-3-furanone had an overall effect on the d' values, and (2) if the presence of 4-hydroxy-2,5-dimethyl-3-furanone had a significant effect on individual d' values.

Pearson signed square root statistic was used to test for a significant difference between the original flavour model and each omission sample (at $\alpha = 0.05$) (see section 2.2.7.3).

6.3 Results and discussion

6.3.1 Part 1: Comparing OAVs and d' values

6.3.1.1 Results

OAVs and d' values obtained from omission tests on the strawberry and savoury flavours are presented in Table 27 and Table 28, respectively. An OAV above 1 indicates that a volatile can be perceived significantly when presented alone.

Table 27: Estimated d' values and OAVs for the volatiles in the strawberry flavour in water, delivered ortho- or retronasally.

Omitted volatile	Orthonasal delivery		Retronasal delivery	
	d'	oOAV ¹	d'	rOAV ¹
Ethyl butanoate	0	2,760-7,500,000	- 0.71	375,000
Ethyl hexanoate	1.22*	5,040	0	2,290
Methyl dihydrojasmonate	- 1.13	0.0015-0.094	0.69	Not available
2,3-Butandione	0.4	2.5-9.4	0.82	7-188
Butanoic acid	- 0.71	7-138	- 0.86	1.11-6.9
Gamma-decalactone	0.51	907-2000	- 0.57	113
4-Hydroxy-2,5-dimethyl-3-furanone	0.96	1,340-134,000	- 0.52	2,700
Methyl(E)-3-phenylprop-2-enoate	- 0.65	Not available	- 1.11	1,840
Cis-3-hexen-1-ol	1.22*	2,080	0.92	2,700

¹oOAVs and rOAV were calculated from the detection thresholds in Rychlik *et al.* (1999)

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$)

Table 28: Estimated d' values and OAVs for the volatiles in the savoury flavour, delivered ortho- or retronasally.

Omitted volatile	Orthonasal delivery		Retronasal delivery	
	d'	oOAV ¹	d'	rOAV ¹
2-Methylpropanal	1.27*	10.0-3,740	0.9	0.180-0.800
2-Furfurylthiol	1.23*	355-8,530	1.43*	8,530
4-Hydroxy-2,5-dimethyl-3-furanone	1.25*	222-22,200	1.33*	445
3-Mercapto-2-butanone	1.63*	28.0	1.36*	
2-Methyl-3-furanthiol	1.32*	4,890-85,500	0.45	Not available
3-Methylthiopropional	0.81	29.0-261	-0.2	5.20-1,310
E,E-2,4-decadienal	-1.04	135-388	-0.26	125-3,000
12-Methyltridecanal	0.86	962	-0.67	Not available
1-Octen-3-one	-0.59	94.0-940	0.76	470
Indole	1.08	0.770	0.63	Not available

¹oOAVs and rOAV were calculated from the detection thresholds in Rychlik *et al.* (1999)

* Indicates a significant difference between the original flavour model and omission sample (Pearson signed square root statistic, $p < 0.05$)

Looking at the orthonasal data, all the volatiles in the strawberry flavour exhibited oOAVs above 1, except for methyl dihydrojasmonate (oOAV = 0.0015 - 0.094). However, the removal of 7 out of 9 individual volatiles was not perceived orthonasally ($p > 0.05$).

In the savoury flavour, all oOAVs were above 1, except for Indole (oOAV = 0.77). However, the assessors could not significantly detect the removal of 3-methylpropional, 12-methyltridecanal, E,E-2,4-decadienal, and 1-octen-3-one ($p > 0.05$). In particular, despite a relatively high OAV (OAV = 962), the removal of 12-methyltridecanal was not significantly detected ($p = 0.156$). Suppression of 12-methyltridecanal in boiled beef has been shown previously using omission experiments (Grosch, 1999).

Looking at the retronasal data on the strawberry flavour, all rOAVs were above 1. However the removal of individual volatile was not detected retronasally ($p > 0.05$). For the savoury flavour, despite rOAVs above 1, assessors could not significantly detect the removal of 2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3-furanone, 3-methylthiopropional, E,E-2,4-decadienal and 1-octen-3-one ($p > 0.05$).

Figure 18 shows, for each volatile in the strawberry flavour, d' measured orthonasally as a function of oOAV. Ethyl butanoate and 4-hydroxy-2,5-dimethyl-3-furanone were not represented on the graph due to the high variability of their respective OAVs ([2,760-7,500,000] and [1,340-134,000], respectively). Visual observation of Figure 18 shows that d' values tend to increase with increasing oOAVs. Highest d' values measured for cis-3-hexen-1-ol and ethyl hexanoate corresponded to higher oOAVs. On the contrary, the lowest d' values measured for methyl dihydrojasmonate, butanoic acid and 2,3-butandione corresponded to lower oOAVs.

It must be noticed that despite their relatively high OAVs in the strawberry flavour, the individual removal of ethyl butanoate and 4-hydroxy-2,5-dimethyl-3-furanone were not detected by the assessors ($d' = 0$ and 0.96, respectively). The high oOAV of ethyl butanoate [2,760-7,500,000] suggested that this volatile would be perceived as intense, when presented alone at this concentration. Yet, a d' value of zero was measured for ethyl butanoate when the flavour was delivered orthonasally. This suggests the presence of

suppressive interactions between volatiles, which decrease the perceived intensity of ethyl butanoate in the strawberry flavour. Blending phenomenon could also suppress ethyl butanoate in the strawberry flavour. An alternative hypothesis was that the oOAV of ethyl butanoate was overestimated, due to the high variation of detection threshold found in the literature (discussed in section 5.1.2).

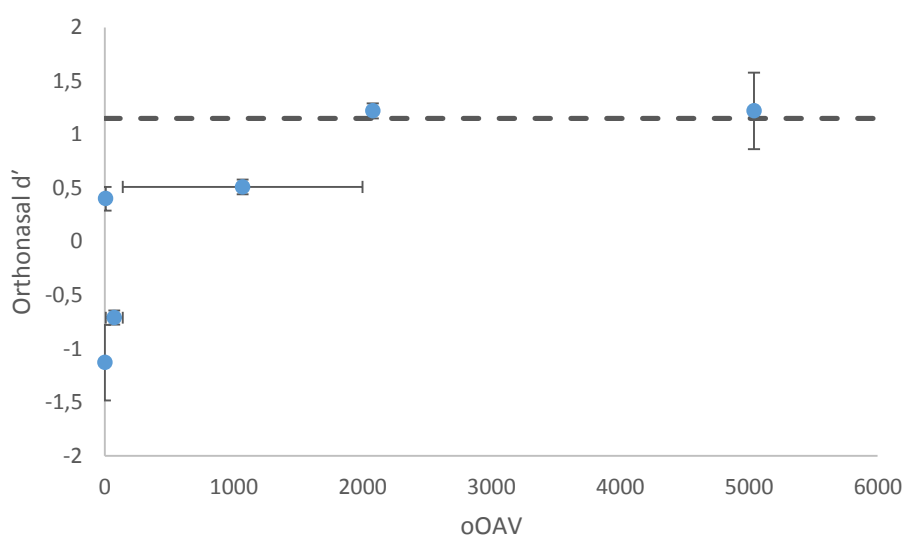


Figure 18: Orthonasal d' as a function of oOAVs for individual volatiles in the strawberry flavour. Vertical error bars correspond to the standard deviation of d' (S). Horizontal error bars correspond to the calculated intervals for oOAV. The dotted line indicates the limit value for a significant d' ($p < 0.05$).

Figure 19 shows, for each volatile in the savoury flavour, d' measured orthonasally as a function of oOAV. 3-Mercapto-2-butanone exhibited the highest d' in the savoury flavour ($d' = 1.63$). However, the low oOAV of 3-mercapto-2-butanone (oOAV = 28) suggested that the perceived intensity of this volatile would be quite low when it is presented alone at this

concentration. Synergistic interactions could have enhanced the detection of 3-mercapto-2-butanone in the savoury flavour.

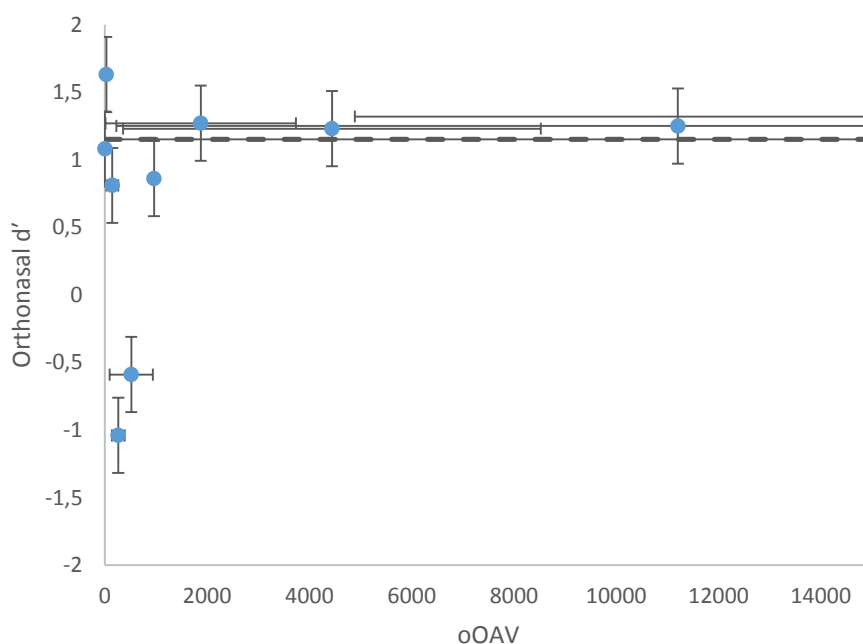


Figure 19: Orthonasal d' as a function of oOAV for individual volatiles in the savoury flavour. Error bars correspond to the standard deviation (S) Vertical error bars correspond to the standard deviation of d' (S). Horizontal error bars correspond to the calculated intervals for oOAV. The dotted line indicates the limit value for a significant d' ($p < 0.05$).

6.3.1.2 Discussion

6.3.1.2.1 Interactions between volatiles in mixtures

Comparison between oOAVs and orthonasal d' values showed that oOAVs do not always reflect the relative importance of individual volatiles in flavour models, as measured by d' . Interactions between volatiles can affect their perception in flavour mixtures. Suppressive and synergistic interactions, as well as blending effects can modify the perceived quality and/or intensity of a volatile in mixture (Laing et al., 2002, Marshall et al., 2006, Weiss et al., 2012).

As a result, volatiles with lower OAVs can become essential in the flavour, while volatiles with higher OAVs can become superfluous (Grosch, 2001).

This study suggested the presence of interactions between volatiles in the flavour mixtures. Despite OAVs above 1, the removal of individual volatiles was not always detected in the strawberry and savoury flavours. Suppressive interactions or flavour blending could have caused volatiles in the strawberry and savoury flavours to lose their individual aroma. As a result, assessors could not detect the removal of individual volatiles in the flavour mixtures.

Olfaction is a synthetic sensory system, and humans do not detect individual volatiles, but odours as a whole. Due to this perceptual blending, humans are very poor at detecting or identifying volatiles in complex flavour mixtures (Cain et al., 1998, Laing et al., 2002, Marshall et al., 2006, Weiss et al., 2012, Laing and Jinks, 2001, Laing and Francis, 1989, Livermore and Laing, 1998, Jinks and Laing, 1999). One hypothesised mechanism for perceptual blending is that interactions between volatiles result in the formation of new spatial patterns (Shepherd, 2006, Giraudet et al., 2002). Each volatile is associated with a characteristic pattern of activated and inhibited receptors (see section 1.2.2.2). Presenting volatiles in mixture can generate new spatial patterns, by modifying the number and type of activated receptors. For example, competition interactions between volatiles at receptor level can reduce the spatial pattern produced by a single volatile and lead to a loss of information about this volatile.

Synergistic interactions could have caused volatiles with lower OAVs to become key volatiles in the flavour mixtures. For example, 3-mercapto-2-butanone played a key role in the savoury flavour, despite a relatively low OAV compared to other volatiles. At a cognitive level, odour processing is modulated based on memory, experience, emotions, and behavioural states (Chaput et al., 2012, Ishii et al., 2008, Grossman et al., 2008, Wilson et al., 2006). Based on this observation, it can be hypothesised that factors such as congruency could modulate interactions between aromas. 3-Mercapto-2-butanone has a pleasant 'meat' and 'onion' aroma. The presence of the other volatiles from the meaty block with a congruent aroma could enhance the perception of 3-mercapto-2-butanone in the savoury flavour.

6.3.1.2.2 Retronasal perception

In this study, no relationship was observed between rOAVs of individual volatiles and d' values measured retronasally. The high variation and inaccuracy of the rOAVs used in this study could explain this result (this will be discussed in section 6.3.1.2.3). Another hypothesis for this observation is that the retronasal delivery of volatile mixtures induced stronger interactions between volatiles, compared to orthonasal delivery. Two mechanisms can be suggested:

(1) The mixing of volatiles with saliva could modify their physico-chemical properties and induce new interactions between volatiles. Some volatiles such as 2,3-butanedione and ethyl hexanoate can interact with mucins in saliva

(Friel and Taylor, 2001, Buettner and Schieberle, 2000). However, interactions between volatiles and saliva appeared to be too slow to have a significant effect in the time scale studied here (Linthorpe et al., 2002, Buettner and Schieberle, 2000).

(2) The cognitive response to a volatile depends on its delivery route (Small et al., 2005) (discussed in section 5.3.3.3). The presence of stimuli associated with retronasal perception (such as oral stimuli) could induce extra cognitive interactions between olfactory signals, compared to orthonasal delivery, and generate new interactions between volatiles.

6.3.1.2.3. Limits of using the PhD study and further experiments

In the present PhD study, the detection thresholds for the volatiles in the flavour models were directly taken from the literature. As discussed in section 5.1.2, detection thresholds are often very broad and very dependent upon the methodology used. Due to the high variability of the OAVs calculated from detection thresholds, it was difficult to establish a relationship between OAV and d' measured in this study. To overcome the large variation and inaccuracy of OAVs, further experiments could focus on measuring the detection thresholds of the assessors that conducted the discrimination tests in this PhD study (see section 5.1.2). These types of experiments would allow calculating more relevant and accurate OAVs. Comparing d' and OAV for each individual volatile in the flavour models could confirm the hypothesis of interactions between volatiles in the strawberry and savoury flavour models.

6.3.2 Part 2: Investigating interactions between volatiles within the savoury flavour

6.3.2.1 Interactions within the fatty block

Table 29 lists the d' values measured in the 'group omission testing'. Results from the previous chapter showed that the removal of each individual volatile from the fatty block (E,E-2,4-decadienal, 12-methyltridecanal and 1-octen-3-one) was not significantly detected orthonasally ($p > 0.05$). This raised the question of the role of the fatty volatiles and whether removal of the whole block would affect the quality of the flavour.

Table 29: d' values measured in 'group omission testing' focusing on the fatty flavour block

Volatiles	d'
Whole fatty block: E,E-2,4-decadienal, 12-methyltridecanal, and 1-octen-3-one	0.95
Pair 1: E,E-2,4-decadienal, 12-methyltridecanal	1.54*
Pair2: E,E-2,4-decadienal, 1-octen-3-one	1.28*
Pair 3: 12-methyltridecanal, 1-octen3-one	- 1.09

Samples were delivered orthonasally. * Indicates a significant difference between the original flavour model and omission sample (Signed square root Pearson statistic, $p < 0.05$)

The removal of the whole fatty block was not significantly detected orthonasally ($p = 0.128$). This was surprising as aldehydes from the fatty flavour block are thought to give the characteristic fatty aromas to cooked meat (Mottram, 1998). This finding may be explained by considering the cognitive strategy used by assessors. Previous results showed that a majority of assessors might have used a beta-strategy to answer the same-different

tests conducted orthonasally in this study (section 4.3.1). When using the beta-strategy, assessors draw an imaginary line between two categories: 'reference sample' (here the original flavour model) and 'different sample'. In this study, it was possible that the sample omitting the whole fatty block was still perceived to be a balanced savoury flavour, and was thus still categorised as a 'savory flavour'. As the savoury flavour was described as a 'savory flavour' to the assessors, assessors would answer 'same' when presented with the pair original flavour/original flavour - fatty block.

It was shown in the previous section that the removal of E,E-2,4-decadienal individually was not significantly detected ($p = 0.183$). However, assessors could detect the removal of the pairs E,E-2,4-decadienal + 12-methyltridecanal and E-2,4-decadienal + 1-octen-3-one ($p = 0.003$ and 0.020 , respectively). This suggests the presence of synergistic interactions between E,E-2,4-decadienal and the other volatiles from the fatty block, which are responsible for the key role of E,E-2,4-decadienal in the savoury flavour.

E,E-2,4-decadienal ('deep-fried', 'fatty' aroma) plays an important role in the aroma of beef and vegetable gravy (Christlbauer and Schieberle, 2009), French fries (Wagner and Grosch, 1998) and meat flavour (Calkins and Hodgen, 2007). It can be hypothesised that although the sample omitting the whole fatty block was still perceived as balanced, the removal of the pairs involving E,E-2,4-decadienal resulted in an unbalanced flavour that could be discriminated from the complete savoury flavour model.

6.3.2.2 Interactions between 4-hydroxy-2,5-dimethyl-3-furanone and other volatiles in the savoury flavour

Table 30 shows the d' measured in omission testing, in presence or absence of 4-hydroxy-2,5-dimethyl-3-furanone in the savoury flavour. The d' values measured were higher in the presence of 4-hydroxy-2,5-dimethyl-3-furanone in the mixture. This suggests that the presence of 4-hydroxy-2,5-dimethyl-3-furanone increases the assessors sensitivity to the removal of all individual volatiles from the meaty flavour block. Student t-test did not show a significant effect of 4-hydroxy-2,5-dimethyl-3-furanone on the overall d' ($p = 0.069$).

Table 30: Values of d' measured in omission testing, in presence or absence of 4-hydroxy-2,5-dimethyl-3-furanone in the savoury flavour.

Omitted volatile	d'	
	Absence of 4-hydroxy-2,5-dimethyl-3-furanone	Presence of 4-hydroxy-2,5-dimethyl-3-furanone
2-Methylpropanal	1.11*	1.27*
2-Furfurylthiol	1.17*	1.23*
3-Mercapto-2-butanone	0.86	1.63*†
2-Methyl-3-furanthiol	1.1	1.32*
3-Methylthiopropional	0	0.81
E,E-2,4-Decadienal	- 0.94	- 1.04
12-Methyltridecanal	0.5	0.86
1-Octen-3-one	- 0.57	- 0.59
Indole	- 1.07	1.08 †

Samples were delivered orthonasally. * Indicates a significant difference between the original flavour model and omission sample (Signed square root Pearson statistic, $p < 0.05$). † Indicates a significant difference with the d' measured in absence of 4-hydroxy-2,5-dimethyl-3-furanone (Student t-test, $p < 0.05$)

4-Hydroxy-2,5-dimethyl-3-furanone was trademarked as “Furaneol™” by Firmenich, in 1975. Furaneol™ is known for its flavour enhancing properties, and was protected by patents for 20 years, for its enhancing effect on fruit flavour (by Firmenich) and on savoury flavour (by Unilever). Furaneol™ is used as a flavour enhancer in food, beverages and perfume, for the preparation of sweet flavours, such as strawberry, pineapple, caramel, and savoury flavours, such as cooked and roasted flavours (Rowe, 2005). In particular, furaneol™ gives special character to chicken and beef flavour

(Taylor and Hort, 2007). It was shown that furaneolTM can also enhance fruity and creamy odour impressions (Ziegler, 1997), sweetness (Green et al., 2012, Labbe et al., 2007, Reineccius, 2005) and umami taste (Tsutsumi and Kawasaki, 2010). The enhancing effect of furaneolTM on sweet flavours could be due to the congruency between “sweet” odours and the “caramel-like” odour of furaneolTM.

The results from this PhD thesis suggest synergistic interactions between furaneolTM and other volatiles from the meaty flavour block. It was hypothesised that furaneolTM has ‘odour enhancing properties’, boosting the contribution of 3-mercapto-2-butanone and 2-methyl-3-furanthiol to the savoury flavour. This finding agrees with previous work on the flavour enhancing properties of furaneolTM.

In this PhD study, the synergistic interactions involving furaneolTM could be due to congruency between furaneolTM and other volatiles from the meaty flavour block. 4-Hydroxy-2,5-dimethyl-3-furanone, 2-methyl-3-furanthiol, and 3-mercapto-2-butanone are all products of Maillard reaction and key volatiles in cooked meat (Christlbauer and Schieberle, 2009, Kerscher and Grosch, 1997, Mottram, 1998, Gasser and Grosch, 1988). The intense, savoury, roasted meat aroma of 2-methyl-3-furanthiol (Mottram, 1998, Gasser and Grosch, 1988) and the sulfury, cooked meat and fried onion aroma of 3-mercapto-2-butanone (Madruga, 1994) are both congruent with the sweet

caramel-like aromas of 4-hydroxy-2,5-dimethyl-3-furanone (Christlbauer and Schieberle, 2009).

6.4 Conclusions

OAVs are very useful and still used extensively to select key volatiles for the recombination of flavours (Guth and Grosch, 1999, Schieberle and Hofmann, 1997, Pino and Fajardo, 2011, Chetschik et al., 2010, Grosch, 2001). However, the current study shows that OAVs do not always predict the relative importance of the volatiles in flavour mixtures. This chapter suggested different types of interactions between volatiles. Suppressive and blending interactions could have caused a volatile with higher OAVs to become of minor importance in the flavour mixtures. On the contrary, synergistic interactions could have caused volatiles with lower OAVs to become of major importance in the flavour mixtures. This highlights the importance and ecological validity of using sensory omission testing to identify the key volatiles in flavours. However, due to the high variability of the OAVs used in this PhD study, it was difficult to establish a relationship between OAV and d' measured in this study. Further experiments would be required to measure more precise and accurate OAVs for the individual volatiles in the strawberry and savoury flavour models.

In this chapter, the same-different approach was used for the first time to investigate interactions between volatiles in mixtures. The same-different approach associated with the Thurstonian d' offers an innovative approach

and provides new insights that could contribute to the understanding of flavour. Omission studies showed that 4-hydroxy-2,5-dimethyl-3-furanone (furaneolTM) was involved in synergistic interactions with other volatiles from the meaty block in the savoury flavour. This finding supports previous work on the flavour enhancing properties of furaneolTM on savoury flavours and is of major interest for the food industry which can use furaneolTM as a flavour enhancer.

This chapter also brought useful information regarding the optimisation of the flavour models. Here, the savoury flavour model could be optimised by removing both 12-methyltridecanal and 1-octen-3-one, as the removal of this pair was not significantly detected by assessors. In the strawberry flavour diluted in water, gamma-decalactone could be removed as it was not significantly perceived ortho- or retronasally. However, any further simplification of the model would involve further discrimination tests to verify that assessors cannot detect the difference.

Chapter 7. Investigating interactions between volatiles and tastants in flavour perception.

7.1 Introduction

7.1.1 Interactions at different levels

When tastants are added to a volatile mixture, interactions can occur between volatiles and tastants and impact on flavour perception (Buettner and Beauchamp, 2010). These interactions are not only the physico-chemical interactions that impact on flavour release, but cross-modal interactions may also occur at a cognitive level (Auvray and Spence, 2008) (see section 1.2.3). Cross-modal interactions are a well-known phenomenon and have been discussed previously in section 1.2.3.

It is now considered that the congruency between taste and smell is a major factor contributing to taste-aroma interactions (Petit et al., 2007, Delwiche, 2004). In particular, congruency plays a major role in taste-induced enhancement of aroma (Frank and Byram, 1988), which is also true for the interaction of fruitiness with sweetness or sourness. It is vital for the food industry to have a good understanding of these interactions, from a formulation (and therefore cost) perspective as they can have a strong effect on consumer perception.

7.1.2 Investigating interactions between volatiles and tastants

Poinot *et al.* (2013) reviewed methods used for the analysis of cross-modal interactions within food flavours. Descriptive sensory analysis and dynamic

sensory analysis are the main methods used to highlight cross-modal interactions. In descriptive sensory analysis, a trained panel is used to evaluate the intensity of several attributes in order to establish a sensory profile. In dynamic sensory analysis, the intensity of an attribute is evaluated during food consumption. The main limitations of these methods, as Poinot *et al.* (2013) points out, are their complexity (various sensations assessed at one time), their inability to explain the origin of the interactions, as well as not completely suppressing the possibility of taste–aroma confusion and attribute dumping. Furthermore, these methods can fail at detecting subtle differences in perception.

Understanding the impact of tastants on the perception of individual volatiles in a flavour and its subsequent effect on overall perception is likely to require methods which can detect subtle differences in perception, and consequently discrimination testing presents a suitable approach. To the author's knowledge, sensory omission experiments have not been used to investigate taste-aroma interactions within a flavour. This approach could constitute a relevant method to better investigate taste-aroma interactions in a panel of consumers, as it suppresses the response bias due to taste-aroma confusion and attribute dumping, and allows subtle changes to be evaluated. Furthermore, the use of a panel of naïve consumers constitutes a more ecologically valid approach to measure consumer perception.

7.1.3 Objectives of this chapter

This chapter investigated the occurrence of taste-aroma interactions in both the strawberry and savoury flavours by assessing the impact of congruent tastants (individually or in mixture) on the perceived flavours.

In the first part of this chapter, physico-chemical interactions between volatiles and tastants were assessed in the strawberry and savoury flavour mixtures, as physico-chemical interactions between volatiles and tastants can lead to changes in volatile release (Friel et al., 2000, Hollowood et al., 2002, Da Porto et al., 2006). Same-different tests were carried out to determine if the addition of tastants, alone or in a mixture, impacted on the orthonasal perception of the flavours.

In the second part, cross-modal interactions between volatiles and tastants were evaluated using omission experiments. A new series of omission experiments were carried out to determine if the addition of congruent tastants, alone (strawberry flavour) or in a mixture (savory flavour), impacted on the retronasal sensitivity to the removal of individual volatiles. Sucrose and citric acid were used as congruent tastants for the strawberry flavour. A mixture of salt, MSG, IMP and proline was used as a congruent taste for the savoury flavour.

7.2 Materials and methods

This study was divided into two parts. In part 1, same-different tests were conducted orthonasally to determine if assessors could perceive significant differences between the flavour in water and the flavour in water + tastants. Tastants were added to the flavours either alone or in a mixture, as their combined effects could affect the volatile release in the headspace.

In part 2, a new series of omission tests (Straw6, Straw7 and Sav6) were carried out in the presence of congruent tastants. Results were compared with previous omission tests in absence of tastants (Straw4 and Sav2) to determine whether omission of volatiles was more noticeable in the presence of tastants.

7.2.1 Preparation of the flavours

Strawberry and savoury flavours and their corresponding omission samples were prepared as described in section 2.2.1.1. The strawberry flavour in PG was kept at 4° C and used up to 8 days after preparation. The savoury flavour and corresponding omission samples were prepared freshly on the day preceding the sensory sessions.

7.2.2 Dilution in mineral water and addition of tastants

The strawberry and savoury flavours (and corresponding omission samples) were diluted in mineral water at 0.75 % and 0.1 % w/w, respectively, as described in section 2.2.1.2.

Sucrose was added to the strawberry flavour and corresponding omission samples at 2 % w/w (as described in section 2.2.1.3). Citric acid was added to the strawberry flavour and corresponding omission samples at 0.05 % w/w (as described in section 2.2.1.3).

Salt, IMP, MSG and proline were added to the savoury flavour and corresponding omission samples, alone or in combination (as described in section 2.2.1.3). The concentrations used were 3.6 %, 0.0526 %, 0.8 %, and 2.5 % w/w, respectively.

Flavour samples diluted in water or water + tastants were kept at 4° C and used within 24 hours. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure flavour samples were at room temperature (20° C ±2° C).

7.2.3 Part 1: Physico-chemical interactions between volatiles and tastants

7.2.3.1 PH measurement

It is known that the pH of a solution can affect volatile release into the headspace (Guyot et al., 1996, Baldwin et al., 1973, Leksrisompong, 2008). To determine if the addition of tastants had an effect on the pH, the pH of the

original flavour models in water was measured (as described in section 2.2.2) in the presence and absence of tastants.

7.2.3.2 Sensory sessions: Evaluating the impact of physico-chemical interactions on volatile release

As the addition of tastants can impact on volatile release, and hence affect flavour perception (Guyot et al., 1996, Da Porto et al., 2006, Ventanas et al., 2010a), sensory experiments were carried out to determine if the addition of tastants, alone or in a mixture, impacted the orthonasal perception of the flavours.

7.2.3.2.1 Strawberry flavour

The sensory sessions were carried out as described in section 2.2.4. One hundred assessors performed a series of same-different tests to compare, orthonasally, the flavour in water with (1) the flavour in water + sucrose, and (2) the flavour in water + citric acid. As the results showed no effect of sucrose and citric acid added individually, another series of same-different tests were conducted to investigate the effect of the presence of the tastant mixture (sucrose + citric acid) on the volatile release. The flavour in water was compared, orthonasally, against the flavour in water + citric acid + sucrose. A 5 minute break was allocated after every 2 same-different tests to limit sensory fatigue and carry-over effects.

7.2.3.2.2 Savoury flavour

The sensory sessions were carried out as described in section 2.2.4. One hundred assessors performed series of same-different tests on the savoury flavour delivered orthonasally. Same-different tests were conducted to investigate the effect of salt, IMP + MSG, and proline added individually. The savoury flavour in water was assessed against (1) the flavour in water + salt, (2) the flavour in water + IMP + MSG, and (3) the flavour in water + proline. Another series of discrimination tests were also conducted to investigate the effect of the presence of the tastant mixture (salt + IMP + MSG + proline) on volatile release. The savoury flavour in water was assessed, orthonasally, against the savoury flavour in water + salt + IMP + MSG + proline. A 5 minute break was allocated after every 2 same-different tests to limit sensory fatigue and carry-over effects.

7.2.3.3 Data analysis

Signed square roots of the Pearson statistic were used on the data obtained from the same-different tests described above, to determine if the presence of tastants (individually or in mixture) had a significant effect on the perception of the orthonasal flavours delivered orthonasally (at $\alpha = 0.05$) (see section 2.2.7.3).

7.2.4 Part 2: Cross-modal interactions between volatiles and tastants

7.2.4.1 Sensory sessions: impact of tastants on the detection of volatile removal

Sensory experiments were carried out to determine if the addition of tastants, alone (strawberry flavour) or in a mixture (savory flavour), impacted on the assessor sensitivity to the removal of individual volatiles in flavour mixtures.

7.2.4.1.1 Strawberry flavour

Sessions Straw6 and Straw7 were carried out to determine the independent effects of sucrose and citric acid on assessor sensitivity to the removal of individual volatiles from the strawberry flavour. For each session, 100 assessors each carried out 9 same-different tests to compare each one of the 9 omission samples (n-1) with the original strawberry flavour (n). Samples were delivered retronasally, as described in session 2.2.4. Within a session, after three and six tests, assessors were allocated a 5 minute break to limit sensory fatigue and carry-over effects.

Session Straw6 was compared with Straw4 (conducted retronasally in absence of tastants) to determine the effect of sucrose on the assessor sensitivity to the removal of individual volatiles. Session Straw7 was compared with Straw4 (conducted retronasally in absence of tastants) to determine the effect of citric acid on the assessor sensitivity to the removal of individual volatiles.

7.2.4.1.2 Savoury flavour

Session Sav6 was carried out to determine the effect of the congruent tastant mixture on the assessor sensitivity to removal of individual volatile from the savoury flavour and hence highlight any interactions. One hundred assessors each carried out 10 same-different tests to compare the original savoury flavour (n) with each one of the 10 omission samples (n - 1). Samples were assessed retronasally, as described in session 2.2.4.

Data from this session Sav6 were then compared with session Sav2 (conducted retronasally in absence of tastants) to determine if the presence of the tastant mixture affected assessor sensitivity to the removal of individual volatiles.

7.2.4.2 Data analysis

Thurstonian d' values were compared to investigate the effect of tastants on the assessor sensitivity to the removal of individual volatiles. d' values were estimated using the differencing model, as described in section 2.2.7.1.

Student t-tests ($\alpha = 0.05$) (Excel 2010, Microsoft, USA) on d' were used to determine if the addition of tastants had a significant effect on (1) the overall d' values obtained from omission testing and (2) individual d' obtained for each individual volatile.

Signed square roots of the Pearson statistic were used on the data collected from sessions Straw6, Straw7 and Sav6 to determine if there was a significant

difference between the original flavour model and each omission sample (at $\alpha = 0.05$) (see section 2.2.7.3).

7.3 Results and discussion

7.3.1 Part 1: Physico-chemical interactions between volatiles and tastants

7.3.1.1 Strawberry flavour

The presence of sucrose or citric acid added individually had no effect on the orthonasal perception of the strawberry flavour ($d' = -1.2$ and 0.51 , and $p = 0.5$ and 0.34 , respectively). These results are in accordance with previous studies showing that the release of the most important volatiles in the strawberry flavour (ethyl butyrate, ethyl acetate, and ethyl hexanoate) was not affected by the presence of sucrose (10 %) or acid (0.3 %) (Pfeiffer et al., 2006).

However, although individually the presence of sucrose and citric acid did not significantly affect the flavour, the combination of both sucrose and citric acid did ($d' = 1.12$, $p = 0.047$). Different mechanisms can be suggested: (i) Physico-chemical interactions could occur between tastants, and between tastants and volatiles (ii) More likely, both sucrose and citric acid have a small effect on volatile release, and the combination of both effects becomes noticeable by assessors. Small molecules such as sugars and acids can chemically interact with volatiles and decrease their concentration in the headspace or, in contrary, increase their release into the headspace via 'salting out' effects (Hewson et al., 2008, Nahon et al., 1998) (see section 1.2.3.1.1).

Sucrose was shown to affect the release of some volatiles (such as cis-3-hexen-1-ol and 2,3-butandione) into the headspace, at concentration as low as 5 % w/w (Rabe et al., 2003, Hansson et al., 2001). The effect of sucrose on volatile release depends on the physicochemical properties of the volatile (Friel et al., 2000). In this study, the addition of citric acid (0.05 % w/w) decreased the pH of the strawberry flavour in water (from 7.7 to 4.9). This change in pH could affect the release of volatiles into the headspace. It was shown previously that the perceived intensity of butyric acid increased when the pH decreases (Guyot et al., 1996, Baldwin et al., 1973). Furthermore, Leksrisompong *et al.* (2008) showed that the pH of a solution can affect the partition coefficient of certain volatiles, such as 4-hydroxy-2,5-dimethyl-3-furanone and 2,3-butandione, and thereby influence their detection threshold.

One way to test the hypothesis of a combined effect of sucrose and citric acid on the volatile release into the headspace would be to measure instrumentally (using gas chromatography) the release of each individual volatile into the headspace in the presence of i) sucrose at 2% (w/w), ii) citric acid at 0.05% (w/w), and iii) both sucrose at 2% (w/w) and citric acid at 0.05% (w/w) in combination.

7.3.1.2 Savoury flavour

When they were present individually, salt, MSG + IMP and proline did not affect the orthonasal perception of the savoury flavour (d' =0.58, 0.70, and -

0.1, and $p = 0.31, 0.26$ and 0.5 , respectively). However, a significant difference was perceived orthonasally between the savoury flavour in water and the savoury flavour in water + tastants ($d'=1.47, p < 0.001$), showing that the tastant mixture significantly affected the release of volatiles. As observed previously for the strawberry flavour, the combination of the individual effects of tastants on the volatile release could be responsible for the changes in the perceived flavour.

Salt (Saint-Eve et al., 2009, Ventanas et al., 2010a, Ventanas et al., 2010b) and MSG (Maga and Lorenz, 1972, Maga, 1983) have a potential salting out effect, as their presence can increase the release of volatiles in the gas phase. The effect of salt on volatile release depends on the chemical properties of the volatile (Ebeler et al., 1988, Yang and Peppard, 1994). Ventanas (2010a) showed that the presence of salt increased the perceived intensity of certain odours (nutty, cocoa, broth-like odours) at a concentration as low as 0.5 %. This concentration was lower than the salt concentration used in this study 0.36%. This suggests a possible effect of salt on the release of the savoury volatiles into the headspace, which was not detected by assessors in this PhD study.

The results in the literature are controversial concerning the effect of MSG at different concentrations on the volatile release into the headspace. MSG and IMP were shown to individually increase the concentration of beef stock volatiles into the headspace (Maga and Lorenz, 1972, Maga, 1983). The effect

was even stronger when both MSG and IMP were added to the beef stock. The MSG and IMP concentrations used by Maga and Lorenz (1972) and Maga et al. (1983) were lower compared to the concentrations used in this PhD study (0.0526 % and 0.8 %, for IMP and MSG, respectively). Other studies suggested no effect of MSG at low concentrations. Using dynamic headspace analysis, Pionnier *et al.* (2002) showed that MSG at 0.023 % (w/w) had no effect on the release of selected volatiles. Furthermore, sensory analysis reported no effect of MSG (up to 1 %) on the odour intensity of flavour solutions (Kemp and Beauchamp, 1994).

Proline was able to decrease the volatility of compounds (Guichard, 2002). However, Pionnier *et al.* (2002) showed that proline (0.044 %) had no effect on the release of selected volatiles. The concentration of proline used in this study was 2.5 % w/w and therefore was unlikely to affect the release of the savoury volatiles into the headspace.

7.3.1.3 Discussion

For both the strawberry and savoury flavours, tastants added individually had no effect on the perceived flavour. However, a significant change was perceived orthonasally when all the tastants were added simultaneously to the flavours. As the effect of non-volatile compounds on the volatile release depends on the physico-chemical properties of the volatile (Da Porto et al., 2006, Ebeler et al., 1988, Friel et al., 2000), the presence of tastants could change the volatile concentration ratio in the flavour. The concentration ratio

of the volatiles is crucial in flavours (Le Berre et al., 2008a, Lytra et al., 2013, Pineau et al., 2009). In particular, results on the stability of the savoury flavour (see section 3.3.2) and fractional omission experiments (see section 5.3.2) both showed that assessors can be very sensitive to a change in the volatile concentration ratio in flavours. Therefore, assessors might have been able to detect, orthonasally, the difference in the volatile concentration ratios between the flavour in water and the flavour in water + tastant mixture.

Individually the presence of sucrose and citric acid did not significantly affect the volatile release in the strawberry flavour. As this result was able to rule out any possibility of physicochemical interactions between sucrose or citric acid and the volatiles in the strawberry flavour, any interaction observed in the strawberry flavour between volatiles and tastants was most likely to have occurred at a cognitive level. However, this study showed that the combined effect of sucrose and citric acid on the volatile release into the headspace could affect the orthonasal perception of the strawberry flavour. This result was not pertinent for the omission experiments conducted in this research as the effect of sucrose and citric were only assessed individually. However, this should be considered in any future work as it might impact sensory perception in real food systems

Looking at the savoury flavour, there was a significant effect of Salt, MSG, IMP and proline, added in combination, on the volatile release in the savoury flavour. This result has to be taken into account in the next session on the

effect of the congruent tastant mixture on the assessors sensitivity to the removal of individual volatiles in the savoury flavour.

7.3.2 Part 2: Cross-modal interactions between volatiles and tastants

7.3.2.1 Strawberry flavour

Table 31 shows the d' values obtained for each of the 3 sets of samples (water, water + sucrose, and water + citric acid). The presence of sucrose increased all the d' values, except for 2,3-butandione and methyl dihydrojasmonate, but there was no significant effect of sucrose on the overall d' values (Student t-test, $p = 0.35$).

Table 31: Estimates of d' for each omission test under the three experimental conditions (water, water + sucrose, and water + citric acid) for strawberry flavour. Samples were delivered retronasally.

Volatile	d' water	d' water + sucrose	d' water + citric acid
Ethyl butanoate	- 0.71	0.7†	1.48*†
Ethyl hexanoate	0	0.74	0.91
Methyl dihydrojasmonate	0.69	- 1†	1.19*
2,3-Butandione	0.82	- 0.69†	0.67
Butanoic acid	- 0.86	0.43†	1.51*†
Gamma-decalactone	- 0.57	- 0.23	0.62†
4-Hydroxy-2,5-dimethyl-3-furanone	- 0.52	1.02†	0.33
Methyl(E)-3-phenylprop-2-enoate	- 1.11	0.67†	1.48*†
Cis-3-hexen-1-ol	0.92	1.03	- 0.46†

* Indicates a significant difference between the original flavour model and the omission sample (Pearson signed square root statistic, $p < 0.05$). Each discrimination test compared samples in the same condition: water, water + sucrose, or water + citric acid.

† Indicates a significant difference between i) the d' measured in water + sucrose and the d' measured in 'water only' and ii) the d' measured in water + citric acid and the d' measured in 'water only' (Student t-test, $p < 0.05$)

Looking at the individual volatiles, the d' values measured for butanoic acid, ethyl butanoate, 4-hydroxy-2,5-dimethyl-3-furanone and methyl(E)-3-phenylprop-2-enoate increased significantly in presence of sucrose (Student t -tests, $p < 0.001$). Although the removals of 4-hydroxy-2,5-dimethyl-3-furanone and cis-3-hexen-1-ol were not detected significantly in presence of sucrose, the associated p -values were close to significance ($p = 0.077$ and 0.077 , respectively).

Sucrose can enhance retronasal odour perception (Green et al., 2012), and in particular the presence of sucrose in a strawberry flavour enhanced perceived flavour intensity (Pfeiffer et al., 2006). This present study enables its effect on individual volatiles of the overall flavour to be established. The importance of ethyl butanoate and 4-hydroxy-2,5-dimethyl-3-furanone within a strawberry aroma has been discussed previously (Larsen et al., 1992). Ethyl butanoate has a 'pineapple-like' aroma (Fenaroli et al., 1975) and is part of the fruity/floral flavour block, which is congruent with a sweet taste (Prescott, 1999). 4-Hydroxy-2,5-dimethyl-3-furanone and sucrose are a congruent aroma-tastant combination (Green et al., 2012). In addition, 4-hydroxy-2,5-dimethyl-3-furanone is added to the strawberry flavour blend as part of the caramel block. Caramel is sweet smelling and is a product of heating sucrose, and is therefore congruent with sucrose (Schifferstein and Verlegh, 1996). Sucrose has been previously shown to enhance the perceived aroma of 4-hydroxy-2,5-dimethyl-3-furanone (Green et al., 2012). It is therefore likely

that the perception of ethyl butanoate and 4-hydroxy-2,5-dimethyl-3-furanone was enhanced in the sucrose-containing original sample to such an extent that their absence was more evident in the respective omission samples.

The Thurstonian d' for butanoic acid was significantly higher in the presence of sucrose ($p < 0.001$). This suggests that the number of assessors that could detect the removal of butanoic acid increased when sucrose was added to the strawberry flavour mixture. Butanoic acid is characterised as having an unpleasant smell, acrid taste and sweet aftertaste. There are several ways to interpret the significant effect of butanoic acid on the strawberry flavour in the presence of sucrose. First, the presence of a sweet aftertaste is congruent with the sucrose tastants. Its absence in the omission sample may have therefore enabled discrimination against the original sample. A second hypothesis is that the incongruence between acrid taste and pleasant sucrose was evident in the sample containing butanoic acid enabling discrimination between the two.

Table 31 shows that citric acid also increased the assessors sensitivity to the removal of individual volatiles. The d' values measured in omission tests increased significantly in the presence of citric acid (Student t-test, $p = 0.046$). Furthermore, the removal of 4 individual volatiles, ethyl butanoate, butanoic acid, methyl(E)-3-phenylprop-2-enoate and methyl dihydrojasmonate, was significantly detected in the presence of citric acid ($p = 0.004, 0.005, 0.006$

and 0.032, respectively). Looking at the individual d' values, the presence of citric acid increased all the d' values, except for 2,3-butanedione and cis-3-hexen-1-ol. Student t -tests on d' values showed that this increase was significant for butanoic acid ($p < 0.001$), gamma-decalactone ($p = 0.002$), ethyl butanoate ($p < 0.001$), and methyl(E)-3-phenylprop-2-enoate ($p < 0.001$).

Ethyl butanoate, ethyl hexanoate, and methyl dihydrojasmonate constitute the fruity/floral block of the strawberry flavour, and therefore are congruent with the taste of citric acid experienced in fruits. Although methyl(E)-3-phenylprop-2-enoate is from the caramel flavour block, it has been described as having a strawberry, sweet, cinnamon odour (Burdock, 2010). Butanoic acid would most likely be congruent with citric acid as they are both acids and hence may enhance perception of each other. This congruency would explain the significant effect of omitting butanoic acid from the flavour.

Sour and sweet tastes are the most prevalent tastes in fruits, which make them congruent with the strawberry flavour. The present study shows that sucrose and citric acid play a critical role in the perception of the quality of a strawberry flavour and enable the removal of particular volatiles to be detected. Pfeiffer *et al.* (2006) showed that perceived strawberry flavour intensity increased with an increase in sucrose or acid content.

7.3.2.2 Savoury flavour

Table 32 shows the effect of the presence of the tastant mixture on retronasal sensitivity to the removal of volatiles from the savoury flavour. There was no

significant effect of the tastant mixture on the overall d' values ($p = 0.628$). Student t-tests on individual d' did not show any significant effect of the addition of tastants ($p > 0.05$).

Table 32: Estimates of d' for each omission test under the two experimental conditions (water and water + tastant mixture) in savoury flavour.

Volatile	d' water	d' water + tastant mixture
2-Methylpropanal	0.9	0.82
2-Furfurylthiol	1.43*	1.5*
4-Hydroxy-2,5-dimethyl-3-furanone	1.33*	1.15*
3-Mercapto-2-butanone	1.36*	1.03
2-Methyl-3-furanthiol	0.45	0.83
3-Methylthiopropional	- 0.2	0.61
E,E-2,4-Decadienal	- 0.26	- 0.55
12-Methyltridecanal	- 0.67	- 0.72
1-Octen3-one	0.76	0.75
Indole	0.63	- 0.49

* Indicates a significant difference between the original flavour model and omission sample (Signed square root Pearson statistic, $\alpha = 0.05$). Each discrimination test compared samples in the same condition: water only or water + tastant mixture.

7.3.2.3 Discussion

Interestingly, no cross-modal interaction between taste and aroma was observed for the savoury flavour. This observation is comparable to the results from Godinot *et al.* (2009), that showed a significant odour-induced taste enhancement for sweetness, but no significant enhancement of saltiness by congruent tasteless aromas was observed. Godinot *et al.* (2009)

concluded that it was more difficult to enhance saltiness with aroma compared to sweetness.

A first hypothesis for this observation is the influence of affective factors on taste-aroma interactions at a cognitive level. There is strong evidence showing that the physiological significance of tastants, such as their related nutritional and caloric properties play a major role in taste-aroma interactions (Mesulam, 1998, Green et al., 2012, Lim and Johnson, 2011, Rudenga et al., 2010). Umami, salt and sweet tastes are all nutritive tastes (Scott and Plata-Salaman, 1991), but their associated physiological factors are different. Sweet taste indicates the presence of fast-acting carbohydrates that can trigger reward mechanisms, such as dopamine release (Lenoir et al., 2007, Hajnal et al., 2009), whereas umami and salt can signal other macronutrients like proteins and complex carbohydrates. These different physiological functions and psychological constructs might result in stronger taste-aroma interactions in sweet flavour, as they indicate the presence of high caloric food and fast-acting carbohydrates.

Another hypothesis relies on the fact that drinking the savoury flavour solution was less pleasant for the assessors compared to drinking the strawberry flavour solution. Some assessors commented that they found the savoury flavour rather unpleasant. One reason for this unpalatability might be that the presentation of the flavour in water at room temperature was confusing for the assessors, as this type of savoury flavour is usually

experienced in warm and more viscous food products, like soups and broth. It has been shown that the degree of liking can modify the eating or sniffing patterns of a food product (Delconte et al., 1992, Bensafi et al., 2003). Furthermore, De Araujo et al. (2003) showed that the orbitofrontal cortex activation was correlated not only to the congruency of the olfactory and taste stimuli, but also to the pleasantness of their combination. As the orbitofrontal cortex is thought to be involved in the integration of the different stimuli in the perception of flavour (Abdi, 2002, de Araujo et al., 2003), the pleasantness of the stimuli could influence cross-modal interactions between taste and aroma. In this study, the fact that the savoury flavour was perceived as rather unpleasant might have limited the cross-modal interactions between taste and aromas.

A third hypothesis is based on the modulation effect of the tastant concentration on taste-aroma interactions. Nasri *et al.* (2011) showed that the enhancement of saltiness by sardine aroma decreased with increasing saltiness and that the perceived intensity of sardine aroma increased with the salt concentration in water. It must be noticed that the volatile and tastant concentrations used in this PhD study were not equi-intense. According to Green *et al.* (1996), the sucrose concentration used for the strawberry flavour model was higher compared to the equivalent volatile concentration (equivalent PEA) (Green et al., 1996) (see section 2.2.1.2). More research is clearly needed to investigate the effect of the tastant concentration on the

interactions between volatiles and tastants. Further experiments could measure the effect of tastants at equi-intense concentrations on the assessors sensitivity to the removal of individual volatiles.

7.4 Conclusions

This study has emphasised the need to consider the flavour in the relevant matrix, as it has demonstrated taste-aroma interactions at both physico-chemical and cognitive levels. Physico-chemical interactions between volatiles and other components in the food matrix have been widely reported and can affect the volatile release (Friel et al., 2000, Hollowood et al., 2002, Da Porto et al., 2006). However, only a few studies have measured the effect of interactions between volatiles and tastants using sensory analysis (Ventanas et al., 2010a). Sensory studies can detect subtle difference in flavours, as the human nose is very sensitive to changes in the concentration of volatiles.

The omission approach used in this study highlighted cross-modal interactions within the strawberry flavour, particularly where congruency between tastes and aromas could be identified. Notably, the presence of sucrose enhanced the perception of the removal of butanoic acid, ethyl butanoate, 4-hydroxy-2,5-dimethyl-3-furanone and methyl(E)-3-phenylprop-2-enoate. The major changes observed in d' occurred in the presence of citric acid, which allows the significant detection of the removal of ethyl butanoate, butanoic acid, methyl(E)-3-phenylprop-2-enoate and methyl dihydrojasmonate in the

strawberry flavour. This emphasises the importance of citric acid in strawberry flavour.

This study has shown how omission testing can provide a simple approach that allows an advanced understanding of taste-aroma interactions. This approach has proved to be efficient and promising, especially at the level of subtle changes which may not be able to be registered on rating scales. First, by removing any possibilities of halo dumping, this study brings a strong argument for cognitive interactions between taste and aroma. Secondly, omission experiments allow the exploration of taste-aroma interactions in more detail, enabling the assessment of interactions between individual volatiles and tastes within the whole flavour. Finally, the use of a large panel of untrained assessors gives a more ecologically valid view of multimodal perception within a flavour.

Chapter 8. General discussion

The research project presented here fully defines and evaluates a new approach in omission testing, using same-different testing associated with the Thurstonian measure d' . This chapter presents and discusses the main and novel findings of the research project, as well as the implications for the understanding and analysis of flavours, and the formulation of food flavour models. Some directions for further work are also suggested.

8.1 Main findings

8.1.1 Stability of the strawberry and the savoury flavours

Assessing the stability of the flavours used within this research project was crucial, as changes caused by aging could confound results of the discrimination testing. The data highlighted major differences between the two flavours and provided crucial information which helped in designing future sensory sessions. The strawberry flavour was stable over time and could be used for up to a week for the sensory experiments, whereas the savoury flavour was very unstable and changed significantly after only 4 days. Consequently, the experimental design was adapted for investigation with the savoury flavour and it was prepared the day before the sensory sessions.

The differential results obtained with the strawberry and savoury flavour clearly highlight the need to conduct stability tests on each particular flavour before proceeding with sensory experiments of this nature. Furthermore, sensory studies coupled with instrumental analysis provided a new insight

into the perception of flavour, as this facilitates the determination of when changes in the flavour are actually detectable by a consumer panel and hence are commercially relevant. The results also highlight the importance of assessing the stability of a flavour sensorially as well as instrumentally, as some changes that are not detectable instrumentally could be perceived by a sensory panel. On the contrary, some changes observed instrumentally were not detected by the sensory panel.

This research project proposes an innovative approach to assess the stability of flavour over time using sensory discrimination testing coupled with instrumental measurements. This approach proved to be relevant and sensitive to small changes in flavour mixture, and could be used by the food industry as an advanced approach to measure the shelf life of food products.

8.1.2 Evaluation of the same-different approach

Determination of the cognitive strategy used by assessors to answer the same-different tests in this study was essential, as an assumption on the cognitive strategy is necessary to obtain a sensitive estimate of the Thurstonian d' . Although the tau-strategy is conventionally assumed (O'Mahony and Rousseau, 2002), the beta strategy can also be used to answer the same-different test (Rousseau, 2001). In this study, Thurstonian modelling showed that some assessors could have used a beta-strategy when the flavour samples were delivered orthonasally. However, the results on

cognitive strategy were inconclusive and both the tau- and beta-strategies could be appropriate.

The Thurstonian d' was then used to compare the new approach using the same-different test with the traditional approach using the triangle test. The triangle and same-different tests have been compared previously, but this was the first time that both the triangle and same-different tests were compared in omission experiments. It was evident that the same-different approach was more sensitive compared to the triangle approach, supporting results from previous studies on discrimination testing. Furthermore, the superiority of the same-different test over the triangle test could be quantified using the d' values: the same-different test was 1.2 to 3.5 times more sensitive than the triangle test. This was attributed to lower memory, carry-over and fatigue effects in the same-different tests. This study is the first to demonstrate the use of the same-different test for omission testing and to show its advantages over the triangle test. The triangle test is widely used as a standard method for discrimination testing within the food industry. Here, we propose the same-different test associated with the Thurstonian d' as an alternative approach, as it constitutes a more relevant and more sensitive approach for discrimination testing. When coupled with the Thurstonian d' , the same-different test allows measuring the magnitude of the difference between the evaluated samples.

8.1.3 Determination of the key volatiles in flavours, and comparison between ortho- and retronasal sensitivities

A successful demonstration of the novel application of the same-different approach to determine the relative importance of individual volatiles in the strawberry and the savoury flavours has been presented. In accordance with the literature, the most important volatiles in a strawberry flavour model were cis-3-hexen-1-ol, ethyl hexanoate, 4-hydroxy-2,5-dimethyl-3-furanone and ethyl butanoate. In particular, cis-3-hexen-1-ol played a key role in the strawberry flavour model, as its removal could be detected by a significant number of assessors (it exhibited the highest d' values), both ortho- and retronasally. This result suggests that cis-3-hexen-1-ol is a major compound of strawberry flavours. Regarding the savoury flavour, 2-methylpropanal (from the top note) and the sulfur compounds from the meaty flavour block played a major role in the perception of the flavour. This is the first work to show the value of the same-different approach and the Thurstonian modelling in identifying the relative importance of individual volatiles in a sweet and a savoury flavour model.

The study also successfully demonstrated the application of the same-different approach to 'fractional omission testing'. Results confirmed the key role of 2-furfurylthiol in the savoury flavour. It was the first time that the approach using the same-different test was used for 'fractional omission testing'. 'Fractional omission testing' is particularly important to assess the effect of a decrease in a volatile concentration on the perceived flavour, as

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the human nose can be very sensitive to a change in concentration of a single volatile in flavour mixtures.

Omission testing was also used for the first time to compare ortho- and retronasal sensitivities to the removal of individual volatiles in mixtures. For both the strawberry and the savoury flavours, orthonasal perception was more sensitive to the removal of volatiles and this was attributed to a different efficiency in delivery to the olfactory receptors. This finding has implications for the analysis of flavour mixtures used in food and beverage products which are consumed, rather than simply sniffed. In this case, retro- as well as orthonasal analysis should be conducted, as the perception of the flavour can vary significantly between the two delivery routes. The application of the same-different approach in the above scenario provides a significant change in the approach to understand the relative contribution of volatile to a flavour mixture.

8.1.4 Interactions between volatiles in flavour mixtures

This work is the first to use the Thurstonian measure d' in omission testing and to determine if OAVs of the aroma compounds reflect the relative importance of individual volatiles in the strawberry and the savoury flavour models. Results showed that, due to interactions between volatiles in mixture, OAVs did not reflect the relative importance of the volatiles in the flavours, as measured by d' . Although OAVs are still used extensively to select the key volatiles for the recombination of flavours (Grosch, 2001), this

highlights the relevance of using sensory omission testing to identify the key volatiles in flavours.

The same-different approach was used as a novel approach to highlight interactions between volatiles within the savoury flavour. 'Group omission testing' was used, where two or more volatiles are removed from the flavour model, before comparing the new sample to the original flavour. Investigation of interactions between the volatiles within the fatty flavour block showed that E,E-2,4-decadienal played a key role in the savoury flavour, due to its involvement in synergistic interactions with other volatiles in the fatty flavour block. Further omission testing showed that the presence of 4-hydroxy-2,5-dimethyl-3-furanone (furaneolTM) in the savoury flavour increased the assessors sensitivity to the removal of other individual volatiles. This is of major interest for the flavour industry to use this ingredient as a flavour enhancer.

8.1.5 Interactions between volatiles and congruent tastants

The final part of this study investigated interactions between volatiles and tastants. Preliminary discrimination testing showed that the presence of a tastant mixture could affect volatile release through physico-chemical interactions within the mixture, and thereby modify the perceived flavour. These results confirm that the assessors can be very sensitive to the volatile concentration ratio in flavour mixtures. Sensory studies can detect subtle

difference in flavours, as the human nose is very sensitive to changes in the volatile concentration.

The same-different approach demonstrated the ability to evaluate the impact of a tastant on specific volatiles in the overall flavour. It was the first work to use this approach to look at taste-aroma interactions and describe the interactions between a tastant mixture and specific volatiles. Cross-modal interactions between taste and aroma were highlighted within the strawberry flavour, particularly where congruency between taste and aroma could be identified.

This was the first time that omission experiments were used to investigate taste-aroma interactions. The omission approach opens up opportunities for improved understanding of cross-modal interactions between aromas and tastes. First, by removing any possibilities of halo dumping, this study brings a strong argument for cognitive interactions between taste and aroma. Secondly, omission experiments enabled the assessment of interactions between individual volatiles and tastants within the whole flavour. Finally, the use of a large panel of untrained assessors gives a more ecologically valid view of multimodal perception within a flavour.

8.2 Implications

8.2.1 Advantages of the new approach in omission testing

Accurate and precise methods are needed to determine the contribution of volatiles in food flavour models. The new approach for omission testing

evaluated in this study is simple and efficient and allows an advanced understanding of flavour perception. The approach used in this study presents two major innovations.

- This was the first study to use the same-different test for omission testing. The same-different approach was proven to be more sensitive than the triangle approach. The same-different approach constitutes an effective robust approach for sensory omission testing and presents a major advantage in omission experiments, due to its lower carry-over, memory and fatigue effects.
- This study pioneers the use of the Thurstonian d' for omission experiments. This is of high interest to determine the relative importance of individual volatiles within a flavour.

The same-different approach opens up opportunities for improved understanding of interaction between aromas and tastes, but also interactions across other sensory modalities. This approach could be beneficially employed in the formulation and application of flavours in food and beverages.

8.2.2 Optimisation of food flavour models

The new approach in omission testing can be used to optimise food flavour models used in the flavour industry. This study has direct applications, as results show that the flavour models can be simplified. Results on the strawberry flavour diluted in water imply that the number of volatiles could be reduced further without affecting the quality of the flavour. For example, the removal of gamma-decalactone was not significantly perceived ortho- or retronasally (see section 5.3.3.1) even in the presence of congruent tastants (see section 7.3.2.1).

Omission testing on the savoury flavour indicated that the removal of 3-methylthiopropional was not significantly detected by assessors, ortho- or retronasally (see section 5.3.3.2), even in the presence of congruent tastants (see section 7.3.2.2). Furthermore, 12-methyltridecanal and 1-octen-3-one could be removed as a pair without affecting the savoury flavour (see section 6.3.2.1).

However, it is important to check the consequences of removing several volatiles at a time before simplifying the models further. In the absence of other particular volatiles, the removal of what appeared to be an insignificant volatile may have a significant effect. For example, the removal of E,E-2,4-decadienal or 12-methyltridecanal was not detected individually in the savoury flavour, but the removal of both volatiles was significantly detected orthonasally. It is also possible that in presence of other components of the

food matrix, such as tastants, the removal of what appeared to be insignificant volatiles may have an effect on the perceived flavour.

8.2.3 Assessment of food flavour models

Clearly results indicated that the manner of testing (orthonasal vs retronasal, in presence of tastants and in the relevant food matrix) should be relevant to the end use of the flavour under investigation. First, this study demonstrates the importance of assessing flavours retronasally. The perception of the flavour can vary significantly between the two delivery routes (ortho- and retronasally). For example, although cis-3-hexen-1-ol and ethyl hexanoate were essential in the orthonasal perception of the strawberry flavour, retronasal assessment showed that these volatiles could be removed without impacting the perception of the flavour. This shows the importance of assessing flavours retronasally for products that are designed to be consumed. Orthonasal perception should also be considered as it plays a role in the consumer perception of food and beverage products. This study showed interactions between volatiles and between volatiles and tastants at different levels, from physico-chemical level before consumption, to peripheral and central interactions during consumption.

Interactions between volatiles and other components of the food matrix can also affect the perception of flavour. For example, texture (Hollowood et al., 2002) and carbonation (Hewson et al., 2009) can affect the perception of flavours. The new approach in omission testing opens up opportunities for

improved understanding of interaction between volatiles and other sensory modalities, such as texture and chemesthetic effects. This approach could be beneficially employed in the formulation and application of flavours in food and beverages.

8.2.4 Potential impact of this research study

This research project could have a broad impact on the understanding and formulation of flavours, as well as on the use of discrimination testing within the food industry. The triangle test is widely used as a standard method for discrimination testing within the food industry. This PhD study has shown that the same-different test with a sureness rating was more sensitive, particularly for products with strong carryover and memory effects, such as flavour perceived orthonasally. Furthermore, when coupled with the Thurstonian d' , the same-different test allows measuring the magnitude of the difference between the evaluated samples. Therefore, we propose the same-different test associated with the Thurstonian d' as an alternative to the commonly used triangle test, as it constitutes a more relevant and more sensitive approach for discrimination testing.

A number of areas were identified in omission research in which improvements could be made, both in terms of the sensory methodology adopted and manipulation of omission samples. This study pioneers the use of the Thurstonian measure d' for omission testing as an action standard. The approach proposed in this thesis represents an improved opportunity for the

evaluation of the key components of flavour and could be used in the flavour industry to optimise food flavour models. This is not only of high interest to determine the key volatiles in flavour mixtures, but most notably, the Thurstonian d' allows the determining of the relative importance of the different volatiles within a flavour mixture.

This PhD study has also shown how omission testing can provide a simple approach that allows an advanced understanding of food flavours. Omission studies could not only be used to identify the key compounds of flavour, but also to compare ortho- and retronasal perceptions, and to investigate intra- and intermodal interactions with other key flavour components such as tastants and trigeminal stimuli. The new approach of using omission experiments to investigate cross-modal interactions has proved to be efficient and promising, especially at the level of subtle changes which may not register on rating scales. This approach could be beneficially employed in the formulation and application of flavours in food and beverages.

Finally, this research study highlights the importance of testing food flavour retronasally and in the relevant matrix, as intra- and cross-modal interactions can have a strong effect on the perception of a flavour. Results showed that orthonasal omission tests may not best represent how flavours perform during consumption. This finding has implications for the analysis of flavour mixtures used in food and beverage products which are consumed, rather than simply sniffed. In this case, we recommend retronasal as well as

orthonasal analysis, as the perception of the flavour can vary significantly between the two delivery routes. This study also emphasises the need to consider the impact of other matrix components to understand consumer perception of flavour. It is possible that, in the presence of other components of the food matrix, the removal of what appeared to be insignificant volatiles may have an effect on the perceived flavour.

8.3 Further work

8.3.1 Characterisation of the 'mature' savoury flavour

In this research project, omission studies were conducted on the fresh savoury flavour: the flavour was prepared freshly every day preceding the sensory sessions. Instrumental and sensory results showed that the savoury flavour used in this study stabilised after 2 weeks. Furthermore, the mature flavour seemed to be more pleasant as it was described as more 'rich' and 'rounded' by assessors, compared to the fresh flavour. As the 2 weeks aged savoury flavour appeared to be stable and more pleasant, one alternative would have been to age the flavour prior to sensory testing.

Further omission testing could be conducted to characterise the mature savoury flavour. Furthermore, comparing results from omission testing on the fresh and mature savoury flavour could help gain a better understanding of the maturation effects on the perceived flavour. This type of study would provide further information on the chemistry of the maturation of flavours,

and would allow measuring the impact of the changes on the sensory perception of the flavour.

8.3.2 Determining the cognitive strategy used by individual assessors

Results on cognitive strategy were inconclusive in the current study. It was hypothesised that some assessors spontaneously adopted a beta-strategy to answer the same-different tests, while others adopted the usual tau-strategy. Further experimental work is required to determine the cognitive strategy used by assessors to answer the same-different tests in ortho- and retronasal omission testing. One way of investigating the cognitive strategy would be to use a small number of assessors to carry out a large number of same-different tests, and use ROC fitting to determine the cognitive strategy used by individual assessors. This type of study is ambitious as it requires assessors to carry out a large number of discrimination tests (up to 2,000 in Santosa *et al.* (2011)).

The long version of the same-different test, where each assessor would test two pairs of stimuli, one pair the same and one pair different, could be used instead. The effects of the experimental design (repeated exposure), the delivery method (ortho or retronasally), and the complexity of the samples (simple vs. complex flavour mixture) on the cognitive strategy could also be investigated.

8.3.3 Comparing the triangle and the same-different approach

More experimental work could be carried out on the strawberry flavour to compare the same-different and the triangle approaches and to overcome the limitations highlighted in this study (see section 4.3.2.2.2). Following the method used by Rousseau *et al.* (1997, 1998), the same assessors should perform both discrimination tests in order to avoid variability related to assessor sensitivity. Adapting this method to omission testing would require all assessors to conduct two complete sets of omission tests: one using the triangle test, and the other using the same-different test. The triangle and same-different tests should be presented in a balanced order to avoid any learning effects. One hundred assessors were used in this study for all omission experiments and this appears to be a reasonable number to compare the triangle and same-different approaches. Using the same number of assessors to carry out both triangle and same-different tests implies that the statistical power has to be taken into account when comparing the tests sensitivities (Rousseau *et al.*, 1998, Lau *et al.*, 2004, Rousseau *et al.*, 1999).

8.3.4 Using the tetrad test in omission experiments

Recently, more interest has been shown regarding the tetrad test (ASTM WK32980) for discrimination testing, as this test was more powerful compared to the triangle test, and more precise in estimating d' (Ennis and Jesionka, 2011, Ennis, 2012, Ennis *et al.*, 2014). Therefore, the tetrad test could be a suitable alternative in omission studies. Tables to estimate d' for

the tetrad test are available (Ennis et al., 1998, Bi and O'Mahony, 2013). However, the tetrad test requires the evaluation of four samples, which could generate additional perceptual noise related to fatigue and memory effects. If the increase in noise is large enough, the tetrad test could lose its theoretical power advantage over the triangle and same-different tests. This study has shown that carry-over, memory effects and fatigue were particularly important for flavour samples delivered orthonasally. In this case, the approach using same-different testing might be more appropriate. An experimental comparison would be necessary to understand the tetrad test in practice.

8.3.5 Further investigation of taste-aroma interactions

8.3.5.1 Combined effects of sucrose and citric acid

This study highlighted cross-modal interactions between sucrose and citric acid and the volatiles in the strawberry flavour. Sucrose and citric acid presented individually enabled the removal of particular volatiles to be detected. Pfeiffer *et al.* (2006) showed that perceived strawberry flavour intensity increased with an increase in sucrose or acid content, and that this effect was even stronger when the two tastants were present in combination. Here, it would be interesting to increase the complexity of the sample and investigate the effect of the combination of both sucrose and citric acid on the detection of volatile removal. It can be postulated that the presence of both tastes would result in further increases in d' , compared to the tastant

presented alone. Of further interest are methyl dihydrojasmonate and cis-3-hexenol, where opposing effects of sucrose and citric acid were observed. Complex interactions between tastants and volatiles could lead to suppressive effects of the tastants. For example, if the presence of sucrose reduces the perception of acidity, would the removal of methyl dihydrojasmonate still be noticeable?

8.3.5.2 Effects of incongruent tastants

Congruency plays a major role in the enhancement of aromas by taste (Petit et al., 2007, Delwiche, 2004, Frank and Byram, 1988). However, sensitivity enhancement has also been associated with incongruent odour-taste pairs, such as pineapple - brothy (Delwiche and Heffelfinger, 2005). The presence of incongruent tastes could also increase the assessor sensitivity to the removal of individual volatile, by making the incongruence between taste and aroma more evident in the flavour sample. For example, this study showed a significant effect of sucrose on the assessor sensitivity to the removal of citric acid. It would be interesting to investigate the effect of incongruent tastes (such as salty or bitter tastes) on the assessors sensitivity to the removal of individual volatiles in the strawberry flavour. Further omission tests could be conducted on the strawberry flavour delivered retronasally, in the presence of sodium chloride, or caffeine, for example.

8.3.5.3 Effect of tastant concentration

The concentration of tastants can have an effect on taste-aroma interactions (Nasri et al., 2011). This study highlighted cross-modal interactions between sucrose and citric acid and the volatiles in the strawberry flavour. It would be interesting to assess the effect of different sucrose and/or citric acid concentrations on the assessors sensitivity to the detection of the removal of volatiles within the strawberry flavour.

Looking at the savoury flavour, omission testing did not show interactions between the savoury tastant mixture and individual volatiles. Increasing the concentration of the savoury tastant mixture might enhance taste-aroma interactions within the flavour, as Nasri *et al.* (2011) showed that the perceived intensity of sardine aroma increased with the salt concentration in water.

8.3.6 Assessing the effects of more complex food matrices on the perception of flavours

Simple models like the strawberry and savoury flavour models used in this study are useful to develop our understanding of food flavour. However, real food matrices are much more complex and it is a challenge to predict the sensory properties of complex foods and beverages. Interactions between volatiles and other components of the food matrix can modify the perception of the flavour. Therefore, the strawberry and savoury flavour models used in

this study may require balancing before being added to a more complex food matrix, in order to conserve the desirable flavour.

The strawberry flavour used in this study could be incorporated into a sweet beverage or a smoothie. In this case, the flavour profile would be influenced by the viscosity of the beverage. Hollowood *et al.* (2002) showed that increasing the viscosity of the matrix decreased the perceived intensity of volatiles in strawberry flavour. Non-volatile compounds such as phenolic compounds in fruit juices could also interact with the volatiles and modify their release (Plotto *et al.*, 2004, Jung *et al.*, 2000, Dufour and Bayonove, 1999). For example, the detection threshold of some volatiles measured in an orange juice matrix was much higher than the threshold measured in water (Plotto *et al.*, 2004). If carbonation was added to the beverage, the effect of carbonation on the perceived strawberry flavour should also be considered (Hewson *et al.*, 2009).

The savoury flavour could be incorporated in a food gel for cats or dogs, or a savoury broth. The proteins, lipids and carbohydrates contained in the food matrix may interact with volatiles. It was shown that proteins, starch and other hydrocolloids can bind volatiles and decrease their release (Taylor and Linforth, 2010, Jones *et al.*, 2008, Guth and Fritzler, 2004, Heng *et al.*, 2004, Seuvre *et al.*, 2004, Frost *et al.*, 2005). Pionnier *et al.* (2002) showed that the mineral fraction of the food matrix affected the volatile release in camembert cheese. While the emulsion structure of the matrix could also affect the

flavour release (Frost et al., 2005, Bakker and Mela, 1996), a gel-like food would increase the time of mastication, which can modify the release of volatiles and result in a different flavour profile (Buettner and Schieberle, 2000).

8.4 Conclusions

To conclude, the same-different approach was shown to be an effective, robust and sensitive approach in sensory omission testing. The same-different approach was successfully applied to (1) determine the relative contribution of individual volatiles in flavours delivered ortho- or retronasally, (2) investigate interactions between specific volatiles in mixtures, and (3) assess interactions between tastants and specific volatiles within flavour mixtures. The same-different approach brings a novel contribution to sensory science as it opens up opportunities for a deeper understanding of flavour and could be beneficially employed in the formulation and application of flavours in food and beverages.

Appendix

1. Different types of interactions between compounds in binary mixtures (Breslin, 1996)

Box 1. Definitions of types of potential interactions between two mixed compounds of different tastes

Linear

Suppression: (for suprathreshold concentrations)

Suppression is a linear (or subtractive) process that bears direct analogy with subthreshold hypoadditive mixing as well as with subtractive mixing (see below), except that it is applied here to suprathreshold compounds. As shown in Fig. 1, it is most clearly depicted by a rightward shift of the concentration-intensity function along the concentration axis in the presence of a second compound, so that every concentration point along the curve is perceived as less intense. One should note that the function itself is not linear and will not result in the same magnitude decrease at different positions along the concentration-intensity curve (see Fig. 2)^{5,6}.

Enhancement: (for suprathreshold concentrations)

Enhancement is a linear (or additive) process that bears direct analogy with subthreshold hyperadditive mixing (see below), except that it is applied here to suprathreshold compounds. As shown in Fig. 1, it is most clearly depicted by a leftward shift of the concentration-intensity function along the concentration axis in the presence of a second compound, so that every concentration point along the curve is perceived as more intense. Again, one should note that the function itself is not linear and will not result in the same magnitude increase at different positions along the concentration-intensity curve (see Fig. 2)^{5,6}.

Additive mixing: (for subthreshold concentrations)

Additive mixing may be the same process that governs enhancement and suppression. Additive mixing involves the determination of the threshold concentration for compound A in the presence of a subthreshold concentration of compound B. Simple (complete) additivity means that, when the concentrations of A and B are reduced below their individual threshold concentrations and mixed together, the detection threshold for the mixture will occur when each compound is at 50% of its concentration threshold (or 30:70; 70:30; 40:60; 60:40, etc.), so that the sum of the percentages of the individual threshold concentrations for A and B equals 100%. This usually results in nearly additive combinations of compounds A and B (Ref. 7).

Hyperadditive mixing: (for subthreshold concentrations)

Hyperadditive mixing is defined in the same way as additive mixing except that the threshold for the mixture is obtained when the compounds are at concentrations corresponding to percentages of

their individual thresholds that, when totalled, sum to less than 100% (e.g. 45% for A and 50% for B, or 45% for A and 45% for B, etc.)⁷. Hyperadditive mixing bears a direct parallel to the suprathreshold phenomenon of enhancement.

Hypoadditive mixing: (for subthreshold concentrations)

Hypoadditive mixing is defined in the same way as additive mixing except that the threshold for the mixture is obtained when the compounds are at concentrations corresponding to percentages of their individual thresholds that, when totalled, sum to more than 100% (e.g. 55% for A and 50% for B, or 55% for A and 55% for B, etc.)⁷. Hypoadditive mixing bears a direct parallel to the suprathreshold phenomenon of suppression.

Subtractive mixing: (for subthreshold and suprathreshold mixtures)

Subtractive mixing involves the determination of a threshold for compound A in the presence of a suprathreshold background concentration of compound B (Ref. 7). This usually results in compound B elevating the threshold for compound A.

Nonlinear

Masking: (for suprathreshold concentrations)

Masking is a nonlinear process in which the addition of compound A decreases the intensity of compound B in a manner that goes beyond linear reductions in intensity. As shown in Fig. 3, it is most clearly depicted by a decrease in the slope of the function and a rightward shift of the concentration-intensity function along the concentration axis in the presence of compound B, so that every concentration point along the curve is perceived as less intense. One should note that the function itself is not linear and will not result in the same magnitude decrease at different positions along the concentration-intensity curve (see Fig. 2)^{5,8-10}.

Synergism: (for suprathreshold concentrations)

Synergy is a nonlinear process in which the addition of compound A increases the intensity of compound B in a manner that goes beyond linear increments in intensity. As shown in Fig. 3, it is most clearly depicted by an increase in the slope of the function and a leftward shift of the concentration-intensity function along the concentration axis in the presence of compound B, so that every concentration point along the curve is perceived as more intense. One should note that the function itself is not linear and will not result in the same magnitude increase at different positions along the concentration-intensity curve (see Fig. 2)^{5,8-10}.

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