Cross-modal interactions in complex food matrices

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Thesis

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Contents

Chapter 1

General Introduction

Health concerns related to sodium, sugar and fat intake make it necessary to reduce those components in industrial produced food.

Overconsumption of sugar and fat has been related to obesity. In Europe 30-80% of adults are overweight, varying over countries [1]. In particular, soft drinks constitute the leading source of added sugars in the diet, and the total energy intake positively correlates with soft drink consumption [2-5]. Dietary fat has been identified as one of the main contributors to obesity [6-9]. Overweight and obesity are associated with increased risk of cardiovascular disease, diabetes mellitus, musculoskeletal disorders and several types of cancers [10]. The total dietary salt intake has been estimated to range between 9-12 g/day (3.5-5 g sodium) in western society, which is more than double the amount of the recommended 5 grams a day [11, 12]. 75 to 80% of the salt intake can be sourced to processed foods [13, 14]. Excessive sodium intake can lead to increased blood pressure, coronary heart diseases, diabetes and stroke [15, 16].

Because of these concerns food manufacturers are trying to lower salt, sugar and fat content in industrial produced foods. One of the main consequences of decreasing salt, sugar and fat contents is a change of sensory characteristics leading to consumer rejection [17]. Reducing salt, sugar and fat contents, while maintaining consumer acceptability has become an important challenge for the food industry.

Several strategies have been applied in the past to reduce the sodium content in food products, including gradual reduction [18], replacing of sodium-salts with potassium-salts [14], addition of yeast extracts and flavour potentiators [19] [14] or modification of the distribution of salt in the product [20]. However, those concepts are limited to only allow reductions up to 20-30% [21]. Furthermore, the use of sodium replacers often leads to off-tastes, such as bitter notes and therefore consumer rejection. Artificial sweeteners are widely used in the reduction of the sugar content. However, metallic off-taste and the lingering of taste impressions after consumption of the product also led to rejection. Reducing fat, as well as the use of fat replacers changes the flavour and texture dramatically, again leading to product rejection by consumers [22-24].

The fact that no substantial reductions of salt, sugar and fat in foods can be achieved without reducing consumer appreciation calls for alternative strategies to reduce salt, sugar and fat. One of those alternative strategies might be enhancement of taste and modification of texture by the use of aromas and aroma components [25-27]. Knowledge

about the optimal use of these odour compounds, as described in this thesis, is crucial for proper application in food products.

Taste Perception

Taste is one of the five senses. It can be differentiated between sweet, sour, bitter, salty and umami taste. Taste is a chemical sense that detects non-volatile components by taste receptor cells in the taste buds. The taste buds, which are located on the surface of the tongue, along the soft palate, and in the epithelium of the pharynx and epiglottis, detect taste stimuli dissolved in water, oil or saliva. Sour and salty taste are detected via ion-channels, whereas bitter, sweet and umami taste are G-protein coupled receptors [28, 29].

Interaction of taste components with taste receptor cells result in a decrease of the tension difference between the outside and inside of the receptor cells, the depolarisation (as shown in Figure 1-1). When depolarised, a Ca^{+} influx through ion channels produces an action potential and transmits an impulse to the taste nerve fibers that are located alongside the receptor cells.

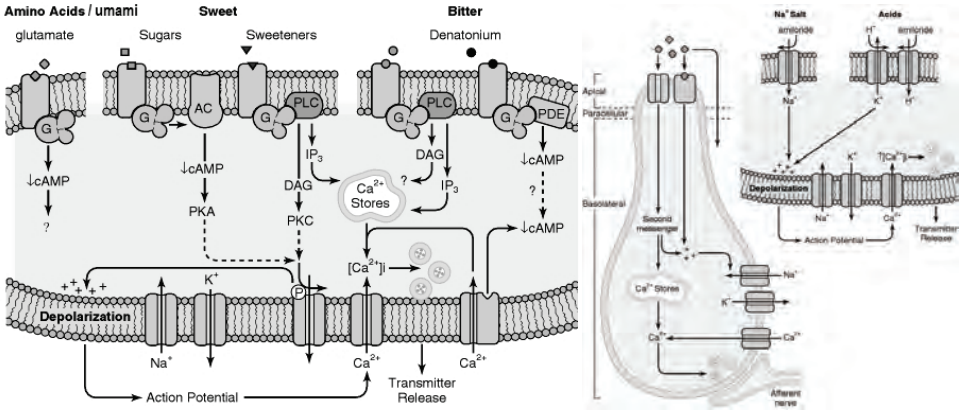

Figure 1-1: The mechanism of taste perception[30]

Saltiness is perceived when Na^{+}-ions enter the receptor cells via Na^{+}-channels. Ion channels in the wall of a taste cells allow Na^{+} ions to enter, the cell depolarises and opens voltage-regulated Ca^{2+} gates, flooding the cell with ions. Consequently neurotransmitters are released, signalling to the brain [30].

In humans, sweetness is detected by the receptors T1R2 + T1R3 that bind to glucose. These receptors then activate a G-protein, which then directly or indirectly activate the TRPM5 channel through several intercellular messengers (e.g. PLC-ß2 and DAG). When TRPM5 is activated, the cell depolarises and Ca^{2+} enters the cell through depolarization-activated Ca^{2+} channels. Subsequently this leads to neurotransmitter release and the sweet taste signal to the brain [31].

Aroma Perception

Like taste, smell is a chemical sense. Smell (olfaction) is the detection of volatile odour compounds by the olfactory sensory neurons in the olfactory epithelium which is located on top of the nasal cavity (Figure 1-2). The olfactory sensory neurons lead into the olfactory bulb and proceed to the forebrain (prosencephalon).

Three different cell types are located in the olfactory epithelium, the sensory olfactory neurons, basal cells and supporting cells. While the basal cells generate the sensory olfactory neurons, the support cells are the structure cells of the olfactory epithelium.

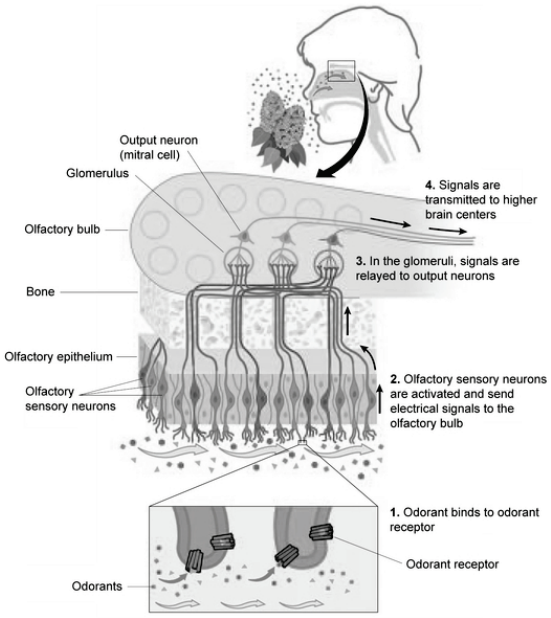

Figure 1-2: The human olfactory system[32]

After sniffing, an odour passes through the superior nasal concha of the nasal passage and dissolves in the nasal mucosa. They are then detected by the olfactory receptors on the dendrites of the olfactory sensory neurons.

Hydrophilic compounds are solubilised in the mucus layer; hydrophobic compounds bind to an olfactory binding protein (OBP's) and then are transported through the mucus layer to the receptor cells [33]. Every receptor cell contains one of the approximately 1000 different transport proteins. Binding of the volatile component to the olfactory receptor leads to an action potential in the olfactory receptor neuron. G-proteins are activated and two mechanisms of signal transmission can be followed to depolarise the receptor cells. The first way involves the stimulation of odour specific adenylate cyclase that synthesizes cAMP (cyclic adenosine-mono-phosphate). The second way is the stimulation of phospholipase C that converts lipid phosphadityl inositol bi-phosphate in the cell membrane into inositol triphosphate (IP_3). Both cAMP and IP_3 trigger signal transduction opening cyclic nucleotide-gated ion channels (CNGs). The resulting membrane depolarisation causes the signal to the brain [32, 34, 35].

Different receptor proteins react differently to certain odourous components. As a result, a different activation pattern of the olfactory epithelium exists for each odorous component and mixtures of different compounds. Different combinations of stimuli with different stimulus intensities result in perception of thousands of different odours[36]. Not necessarily resembles a combination of odours the mere sum of perceptional qualities of the single components[37-40]. The patterns behind the combinatory olfactory receptor neuron activation in odour mixtures are not entirely known, yet.

An odour is perceived when the concentration of volatiles in the headspace phase reaches the odour threshold level. The odour threshold is defined as the level at which the human nose can detect a volatile compound. The odour threshold differentiates between individuals, but is determined by averaging the level at which subjects detect a certain odour or a mixture of odours [41]. Odours can lead to activation at sub threshold level as shown by Walla et al. [42], who observed subconscious olfactory processing at odour stimulation between 200 and 500 ms after stimulus onset. Between 600 and 900 ms after stimulus presentation conscious odour processing was observed.

Texture Perception

The texture of food can be described as the mechanical, structural and surface properties of a product. Texture is a multi-parameter attribute that covers a multitude of properties like hardness, thickness, smoothness, slipperyness or viscosity of a product. Senses involved in texture perception are mainly touch and audition, although also vision can influence texture perception [43-45]. Three types of texture sensations can be distinguished. Kinesthetics describe texture perception during mastication, while somesthetics are perceived sensations by other tactile stimulation that does not require the oral manipulation of the food. The third sensation describing food texture is the trigeminal sensation, which detects temperature, pungency (e.g. CO_2 stimulation) and astringency. Although trigeminal somatosensory receptor neurons transmit trigeminal sensations, no single receptor type can be ascribed to texture perception.

Cross-modal interactions

Whenever we consume food all our senses are involved in the perception of the food. This entails the simultaneous perception of different sensations such as taste, aroma, colour, flavour, texture, pain and heat. The interactions of the senses are referred to as cross-modal interactions. As depicted in Figure 1-3, all sensory modalities interact in a complex way, with the brain interpreting the signals from different receptors and converting them into an overall taste sensation [46, 47].

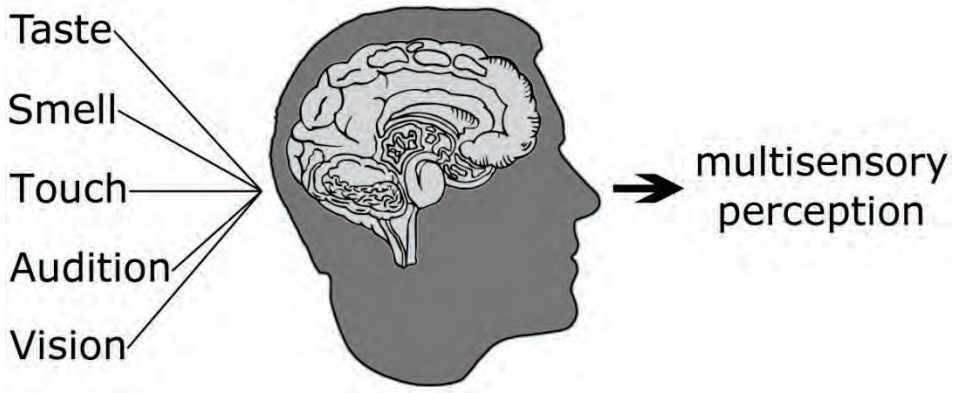

Figure 1-3: All senses are involved in food perception

The most commonly described interaction between sensory modalities in food perception are the integrations of taste and smell. It has been widely described that taste perception is influenced by aromas and vice versa[25, 27, 48-52]. Other cross modal interactions involves the integration of vision, taste and/or aroma [53, 54]. For example observed Petit et al. a change in the description of beverages with colour [55]. It is known that aroma perception strongly depends on the texture of a product [56-58]. However, relatively little is known whether this effect can be reversed, with aroma influencing the perceived texture of food. As reported by Zampini and Spence [59] the perception and pleasantness of food is not only determined by its appearance, smell, and taste, but also by its oral texture and by the sound that it makes during in-mouth break down.

Congruency between stimuli in multi-modal food perception

Congruency between impressions from different sensory modalities is needed in order to enhance taste with odours [60, 61]. Congruency is defined by Schifferstein and Verlegh [62] as the extent to which two stimuli are appropriate for combination in a food product. They showed that strawberry odour enhanced sweet taste in whipped creamwhile the aromas peanut butter, bacon or wintergreen did not enhance sweet taste [62-64]. Djordjevic and co-workers [65] achieved saltiness enhancement with congruent soy sauce odour. Lawrence *et al*. [66] observed a significant saltiness enhancement in model cheeses when a Comté cheese or sardine odour was presented. Carrot odour, not associated with saltiness, did not enhance saltiness.

Stimulus congruency strongly depends on the familiarity with the stimulus pair and therefore with the cultural background of the consumer. In Western culture, vanilla odour and saltiness, or chicken flavour and sweetness are unlikely combinations, and therefore incongruent. This is supported by a study presented by Nguyen et al.[67] who found variations between French and Vietnamese consumers in the extent to which odour/taste pairs were judged as congruent.

Not only taste, but also pleasantness can be influenced by the degree of congruency of the taste/aroma pair, positivly as well as negatively. Schifferstein and Verlegh demonstrated an increase of pleasantness for strawberry odour and sweet taste with increasing strawberry aroma concentration, while they observed the opposite effect

when sweet taste was paired with ham odour, thus incongruent taste/aroma conditions [68].

Aroma induced taste enhancement

As previously described food perception involves all senses. Aroma induced taste enhancement was described in several studies [25, 27, 49-51, 69, 70]. Even below threshold level odours were found to be able to influence taste perception [71, 72]. Aroma induced taste enhancement was reported for several tastes such as sweetness [54, 62, 65, 73, 74], bitterness [75], sourness [76, 77] and saltiness [65, 77-79]. Aromas cannot just enhance taste perception, but also decrease it, usually due to a lack in taste-smell congruency. Stevenson et al. showed a decrease in of sourness with increasing caramel notes.

At present, most of the presented studies on taste-smell interactions focused on simple tastant/water solutions and one odour or odour component, lacking in complexity. Little is known on whether odour induced taste enhancement can be transferred to real life food products increased in structural as well as perceptual complexity and whether it can be an additional tool to reduce sugar, salt and fat contents in industrial produced food.

Interactions between texture and aroma

Physicochemical, as well as cognitive factors can be the origins of interactions between texture and aroma. The physical or chemical binding of volatiles to the matrix of the food can influence the aroma release. The congruency between stimuli, as well as expectation and attention given to the stimulus can be considered as cognitive factors in texture and aroma interactions[80].

Interactions of texture and aroma in food are mainly described as the effect that texture has on aroma release and aroma perception [56, 58, 81-84]. Generally a decrease of aroma release and aroma perception with increasing viscosity, hardness or firmness was observed. Relatively little is known regarding the reversed case where aromas influence the texture of food, an whether aromas might be a tool to overcome texture changes in sugar, salt and fat reduced products.

The complexity of food

In most cases, multi component mixtures of odorants that do not co-occur in daily life will produce a higher perceived aroma complexity than the singular components. As described by Livermore et al. human beings are just able to discriminate between up to four odorants in chemical complex odorant mixtures [85]. If more components were presented the task became more complex and discrimination became more difficult [39, 40, 85, 86]. However Kroeze pointed out, that the complexity of a sensation cannot always be described as the number of stimulus components [87]. A chemical complex mixture might be easily perceived, while single components might be perceptually complex. Furthermore the interaction between different components in multicomponent mixtures might lead to enhancement, suppression or masking of certain flavours [88]. Therefore, when referring to the complexity of the sensory impression of a food it is needed to differentiate between chemical and perceptual complexity. How complex food is, can be defined either by the amount of components it contains (chemical complexity), or the number of attributes that is needed to fully describe the product (perceptual complexity) or the conditions under which it is consumed (contextual complexity).

Temporal aspects in multimodal food perception

Whether multiple sensory input is perceived as one event or more, or as separate events is important in food perception. It has been shown, that if the odour is presented much earlier or later than the taste this affects the products taste [71]. Von Bekesy [89] underlined the importance of temporal factors by showing that the perceived location of an odour (mouth versus nose) and the extent to which an odour and taste were perceived as one sensation or two could be manipulated by varying the time delay between the presentation of the odour and taste. Studies on aroma release from liquids and semisolids have shown that swallowing determines, to a large extent, the retronasal aroma release [90-92]. This was found to be particularly important for (viscous) liquids, which undergo limited oral processing [93]. However, in the past it was difficult to fully control temporal aspects of aroma and taste delivery and therefore most studies lack in temporal validity.

Rationale and outline of this thesis

In this thesis it is investigated whether odour induced taste enhancement can be applied to complex food matrices. It is hypothesised, that an application to chemical and perceptual complex food is possible, however it is further hypothesized that stimulus modification will be necessary to overcome obstacles in the application of odour induced taste enhancement related to stimulus complexity. This thesis further aims on the better understanding of the mechanisms behind cross-modal integration processes in food perception and consequently the optimization of conditions under which odour induced taste enhancement is most effective. The effect of aroma on perceived food texture is investigated, as well as the influence of sensory contrast in taste-taste interactions in order to reduce salt and sugar contents.

First the identification of single aroma components, naturally appearing in apples, that have the capacity to enhance apple juice sweetness was addressed (chapter 2). It was investigated what effect an increase in the concentration of sweetness enhancing aroma component ethylhexanoate has on the overall perception of the apple juice. In chapter 3 it was investigated whether combinations of different aroma components can mask for undesired side effects of ethylhexanoate addition. Chapter 4 focused on gaining knowledge to better understand the mechanisms underlying aroma induced taste enhancement by investigating the impact odour presentation time has with respect to swallowing on the magnitude of odour induced taste enhancement. The aim of the study described in chapter 4 was furthermore to optimize the conditions under which odour induced taste enhancement is applied in order to achieve maximum taste enhancement. This was further addressed in chapter 5, which describes the effect of consumption temperature on the magnitude of odour induced taste enhancement.

As a second objective to this thesis the effect of temporal sensory contrast on taste perception was investigated. This is described in chapters 6 and 7. Chapter 6 shows the effect pulsed stimulus presentation with alternating taste intensities has on saltiness perception. The combination of pulsed tastant presentation with pulsed aroma presentation to further enhance taste perception was investigated in the study described in chapter 7. In this study aroma and taste were either presented pulsed or continuous, as well as in-phase or out-of phase.

The combination of different cross modal integration strategies to enhance sweet taste perception is further described in chapter 8. It was hypothesized that combinatory and even cumulative effects will be found when an increase in the serum release of semi-solid apple juice containing gels is combined with ethylhexanoate induced sweetness enhancement.

Chapter 9 describes two studies. In the first study the impact of the aroma phase on taste, aroma and texture perception in cheese is explored. As a result of the outcome it was investigated whether butter aroma can influence the perceived texture in dairy model gels and whether congruency between stimuli is necessary in order to achieve aroma induced texture modification.

The implications of this research are discussed in chapter 10. This chapter provides furthermore suggestions for applications in industrial food production.

Chapter 2

Gustatory and Olfactory interactions enhance taste perception in a complex beverage

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(submitted)

Abstract

Taste-smell interactions have been described in literature. Studies showing sweet taste enhancement by congruent, i.e. 'sweet', odorants mostly use simple sucrose solutions. It is unclear whether similar effects may be expected for complex food systems where matrix interactions and stimulus familiarity may influence perception.

In many complex food systems sweetness is not only influenced by the level of sugar and sweeteners, but also suppressed by other stimuli, e.g. acids. To study taste enhancement by odorants in a complex system, we investigated the effect of four aroma-relevant esters from apples on the perceived sweetness of apple juice. The used esters, i.e. ethylhexanoate, ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate, are key components in apple aromas. Their observed concentrations depend on the ripening stage of apples. In GC-O analysis studies they are characterized as sweet and/or fruity. To investigate their effects on Apple Juice sweetness, these esters were added to a diluted apple juice concentrate containing a trimmed apple aroma, not including the esters.

Clear differences in sweetness could be perceived among the samples. The ethylhexanoate containing sample was significantly sweeter than the reference sample without ethylhexanoate. The magnitude of sweetness enhancement rose with increasing aroma concentration. Ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate did not affect the perceived sweetness of the samples. Sourness was influenced opposite to sweetness. Not just taste qualities were influenced by the added aroma, but also aroma dependent qualities such as flowery and synthetic notes were enhanced significantly by the presence of ethylhexanoate. It is concluded that the potential of odourants to enhance a product's taste is related to the natural co-occurrence of these odorants with tastants.

Introduction

Sugar reduction is a major challenge for the production of food products by the industry to contribute to lowering the rate of obesity. As a consequence of lowering the sugar content of a product, not only the intensity of sweetness is reduced but also the mouthfeel can change in an unfavourable manner. So far, different strategies have been applied to reduce sugar while maintaining taste and mouthfeel. Long term gradual reduction of sucrose has been applied successfully to several products such as beverages or bakery products without a loss of product acceptability. Sugar could be reduced significantly, while product acceptance was maintained. Sugar replacement by artificial and high potency sweeteners allowed further reductions. This approach affects not only the quality of sweetness, but can induce bitter aftertaste and metallic off-taste [94] and/or change the mouthfeel of the product.

An alternative strategy to reduce sugar in foods is the use of multimodal integration of taste with other sensory modalities. During eating or drinking, different sensory modalities such as taste, smell, sound, vision and touch contribute to the overall perception of the food. Impressions from different modalities but originating from a common source tend to evoke unified sensations from which the contributing modalities cannot fully be reconstructed. Such synthetic multimodal integration was shown for combinations of sound and vision [95, 96], sound and oral texture perception [97] and taste and smell [25, 74, 98-100]. It was shown that multimodal sensory integration can lead to taste enhancement by odorants [53, 101], provided that odour and taste qualities match in a natural way. Such perceptually similar stimulus combinations are referred to as "congruent" [102]. These authors showed that the occurrence of odour-induced sweetness enhancement highly depends on the odorant used. The sweetness of sucrose solutions was enhanced by strawberry odour but not by ham odour. The inverse effect, taste induced odour enhancement, has also been reported. Enhancement of odour sensitivity for congruent odour-taste combinations was observed by Dalton et al. [103]. They found an increase in sensitivity for the odour benzaldehyde in combination with saccharin in contrast to combinations of benzaldehyde with water and mono sodium glutamate that showed no effect or suppression of sensitivity respectively. Schifferstein and Verlegh [102] showed that the degree of congruency, defined as the qualitative similiarity and familiarity to the observer of odour-taste combinations, does not correlate with the degree of taste

enhancement. Hence, an odour can have a high sweetness enhancement capacity even though it might not be the most congruent odorant to that specific taste aspect of the food.

In the past the focus of most studies was on aroma – taste interactions in model systems with reduced complexity such as tastant solutions consisting of sucrose and water with one selected odorant. Although a number of studies demonstrated odour-induced taste enhancement for various tastant-odorant combinations, translation of the concept of odour induced taste enhancement from these simplified models to real food applications remains a challenge. The reason for that is that odorants obtain their taste-enhancing qualities from learned associations between their smell and the accompanying taste [100, 104, 105]. Because real food systems already have a specific odour, the mere enhancement of the smell would not necessarily enhance its taste. It may actually counteract taste enhancement by making the flavour less natural. Moreover in complex food systems different taste qualities such as sweetness or sourness interact with each other. Instead of taste enhancement, adding a specific odorous component to a complex system might result in a qualitative change towards an aroma that is more reminiscent of the taste aspect that should be enhanced. Therefore, odorous components that contribute to a desired quality need to be identified. For apples such a relation between odorant concentration and taste intensity was reported. Poll [106] showed that specific volatile esters were synthesised at higher concentration during later stages of apple ripening, when also sweetness increased. Apple juice produced from late picked and/or long stored apples contained significantly higher amounts of butanoate and hexanoate esters compared to apple juice made from unripe picked or ripe picked apples [107]. Rizzolo [108] and co – workers observed an increase in concentration of ethylbutanoate and ethyl-2-methylbutanoate during storage of paclobutrazol treated apples. Kollmannsberger and Berger [109] reported an increase of ethylbutanoate and ethylhexanoate concentrations during storage of red delicious apples.

Many of the esters occurring in apples produce a sweet aroma impression [110, 111].

In this study, three butanoate esters (ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate) and ethylhexanoate have been selected to investigate the sweetness enhancement capacity of the single esters in apple juice. All of the esters have been

reported to be of importance in apple aroma of different apple varieties and cultivars and have been described as sweet smelling [109, 112-114]. We hypothesize that changing the concentration of these four odour components will result in odour-induced sweetness enhancement and that the magnitude of the sweetness enhancement depends on the odour concentration of these odours. We assume that aromas are congruent with apple juice flavour because of their natural appearance in apples.

To test the hypothesis, two sensory studies were performed. In the first study the four esthers (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethyl-hexanoate) were added individually to apple juice at constant concentration to evaluate their sweetness-enhancing capacity. In addition to sweetness, twelve sensory attributes were evaluated by naïve panelists describing taste, aroma and mouthfeel properties of the apple juices.

The second study focused on the effect of odour concentration of an individual ester on the magnitude of odour induced sweetness enhancement.

Materials and Methods

Materials

An industrial apple juice concentrate, very low in aroma content, (Friesland Campina, Ede, NL) was diluted with deionized water (MilliQ, Billerica, MA) to 8.4° Brix (130 g/L) and 10.4°Brix (150 g/L), respectively.

An apple aroma (IFF, Hilversum, NL) was used as basic apple aroma, which did not include any of the four aroma relevant esters used in these studies (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethylhexanoate). The omitted individual aroma components (ethylhexanoate, ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate) (purity: 98%, pro analysis), were also provided by IFF, Hilversum, NL.

Stimuli were presented in 30 mL medicinal cups with lids (DUNI GmbH & Co KG, Bramsche, Germany). The lids were customized in such a way that a hole of the same diameter as the straw (0.8 mm) was cut into the lid. The straws (PE, 10 cm, SOLO®, Illinois, USA 722X PART# 9084922) were inserted into the holes. The cups were closed with the lids. Odours could not evaporate from the cup before and during the

tasting by the panelist due to the closed lid with inserted straw. This way, the odours were always presented retronasally.

Subjects

Seventeen paid subjects (age 26-61) took part in study 1. The subjects were tested for normal sense of smell and taste, prior to the study. The subjects were naïve with respect to the experimental conditions. However, some took part in earlier food sensory evaluations.

Eighteen paid subjects (age 27-61) took part in study 2. Thirteen of the subjects participating in study 2 also took part in study 1. Like in study 1, subjects were selected accordingly to the same criteria and they were naïve with respect to the context of the experimental conditions.

All subjects that took part in either study 1 or study 2 gave informed written consent prior to the sensory evaluations.

Table 2-1: Composition of all apple juice samples: HEX = ethylhexanoate; ETH = ethylbutanoate; MET = methylbutanoate; E2MB = ethyl-2-methylbutanoate

Experiment	Sample name	Apple juice concentrate [g/L]	Added aroma [ppm]					° Brix
			basic	HEX	ETH	MET	E2MB	
1	1	130	10	6	-	-	-	8.4
	2	130	10	-	6	-	-	8.4
	3	130	10	-	-	6	-	8.4
	4	130	10	-	-	-	6	8.4
	reference	130	10	-	-	-	-	8.4
2	1	150	10	0.04				10.4
	2	150	10	0.2				10.4
	3	150	10	1				10.4
	4	150	10	2.5				10.4
	5	150	10	5				10.4
	6	150	10	7.5				10.4
	reference	150	10					10.4

Methods

Study 1: Odour induced sweetness enhancement by single esters naturally occurring in apples

Five apple juices were prepared using an apple juice stock solution (130g/L apple juice concentrate, diluted to 8.4ºBrix). Individual odourants were added to the stock solution (Table 2-1). All apple juices contained 10 ppm basic apple aroma. Four of these contained one of the four esters (ethylhexanoate, ethylbutanoate, ethyl-2-methylbutanoate and methylbutanoate) at concentrations of 6 ppm, the fifth was the reference without added esthers. All stimuli were presented in duplicates.

The samples were evaluated in three sessions on the perceived intensity of 13 attributes (apple-like, citric, cooked, flowery, fresh, fruity, green, malty, rancid, sour, sweet, synthetic and watery). These attributes were generated by a descriptive panel prior to the study. During the first session the panel rated attribute intensities on a 0 – 12 line scale for 7 randomly selected apple juices, three of which consisted of the reference apple juice. Individual reference scores were calculated for each subject after the first session and were given to each subject and used as reference scores in sessions 2 and 3. Each sample was evaluated with respect to the reference score. Stimulus orders were fully balanced and randomized over subjects.

Study 2: Effect of ethylhexanoate concentration on odour induced sweetness enhancement in apple juice

To achieve a product as close as possible to real apple juice, the concentrate was 10.4º Brix (150g/L; study 2) All apple juices contained 10 ppm of the basic apple aroma. Ethylhexanoate (HEX) was added to the two juices at concentrations of 0.04 ppm, 0.2 ppm, 1 ppm, 2.5 ppm, 5 ppm and 7.5 ppm (Table 2-1). The concentration of 0.04 ppm ethylhexanoate was below detection threshold in apple juice, as was verified in a preliminary triangle test with 18 consumers (data not shown). This stimulus was identified as different with respect to the reference stimuli in 35% of the cases, which is equal to the chance of probability. Therefore it is assumed that 0.04 ppm of ethylhexanoate is below threshold level in apple juice.

The method of sample presentation was the same as in study 1. Eight attributes were selected from the list of twelve, based on relevance to describe the apple juices of study 1 (apple-like, citrus, flowery, fruity, sour, sweet, synthetic, watery).

Data Collection and Analysis

Intensity ratings were collected using an automated data collection system (FIZZ 2.30C, Biosystems, Couternon, France). Subjects evaluated samples on 0 -12 linescales anchored at 0 'not at all' at the left end and 12 'very' at the right end.

PCA analysis was performed using SENPAQ 4.7 (QiStatistics, UK, 2008). In study 1 Multi-Factor-ANOVA testing of intensity ratings for main effects of samples and ester profile (5) as a fixed factor and subjects as a random factor (17) was performed with XLSTAT (Version 2009.1.02, Addinsoft™). Post-Hoc analysis was performed using Tuckey-HSD. In study 2 multi-factor ANOVA tested for main effects of ethylhexanoate concentration (7) as a fixed factor and subjects as a random factor (18). Post-Hoc analysis was performed using Tuckey-HSD.

Average intensity ratings were considered as significantly different, when $p < 0.05$.

Results

Study 1: Odour induced sweetness enhancement by single esters naturally occurring in apples

Four esters (ethylbutanoate, methylbutanoate, ethyl-2methylbutanoate and ethylhexanoate) were tested for their enhancement capacity on sweetness of apple juice. Figure 2-1 shows the sweetness intensity ratings for apple juices varying in aroma composition. ANOVA results showed significant influence of the esters on sweetness ratings [$F(4, 85) = 3.71$; $p < 0.05$] and sourness ratings [$F(4, 85) = 3.08$; $p < 0.05$]. Post-hoc analysis revealed that ethylhexanoate significantly enhanced sweetness ($p < 0.01$), whereas sweetness ratings for ethylbutanoate, ethyl-2-methylbutanoate and methylbutanoate remained at reference level. Sourness ratings were significantly reduced by ethylhexanoate ($p < 0.05$), whereas no effect of ethylbutanoate, methylbutanoate andethyl-2-methylbutanoate on sourness intensity was observed (Figure 2-1).

In addition to the attributes “sweetness” and “sourness” eleven attributes were scored by the subjects. Each of the esters influenced the flavour of the juice differently. Figure 2-2 shows the changes of the flavour profile caused by the esters. The apple juices were found to be significantly different from the reference with respect to their freshness [$F(4, 85) = 2.85$; $p < 0.05$] Post-hoc results show, that freshness of the juices was suppressed by ethylhexanoate ($p < 0.01$), ethylbutanoate ($p < 0.05$) and methylbutanoate ($p < 0.005$), while ethyl-2-methylbutanoate had no significant effect.

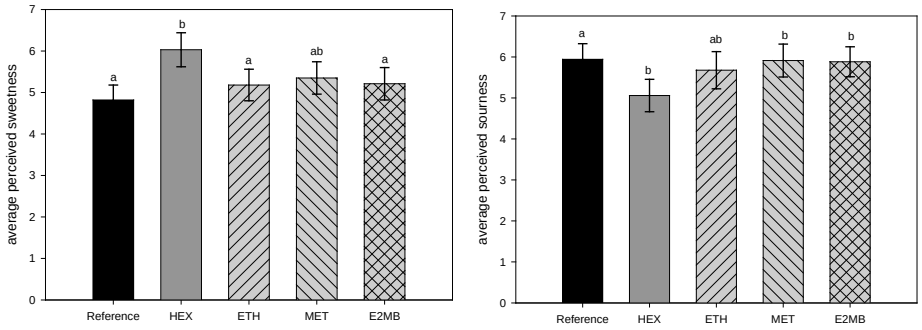

Figure 2-1: Sweetness and sourness intensity of apple juices containing different esters in comparision to reference: HEX = ethylhexanoate; ETH = ethylbutanoate; MET = methylbutanoate; E2MB = ethyl-2-methylbutanoate

Fruitiness was influenced significantly [$F(4, 85) = 4.460$; $p < 0.01$] by the addition of these esters. Highest fruitiness enhancement was caused by addition of ethylhexanoate ($p < 0.01$), while addition of the other esters had no influence on fruity notes. Citric, cooked, green, malty, rancid and watery notes as well as apple-likeness were not influenced significantly by ester addition.

Significant effects of ester addition were found for the attributes synthetic [$F(4, 85) = 6.06$; $p < 0.001$] and flowery [$F(4, 85) = 4.46$; $p < 0.005$]. Figure 2-3 shows the effect of the four esters on synthetic and flowery. Ethylhexanoate enhances intensity ratings on the attributes “synthetic “and “flowery” significantly with respect to the reference juice (“synthetic”: $p < 0.05$; “flowery”: $p < 0.05$). Ethylhexanoate addition therefore resulted in a significant change in the overall product quality. Ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate showed no significant effect on the attributes “synthetic” and “flowery”.

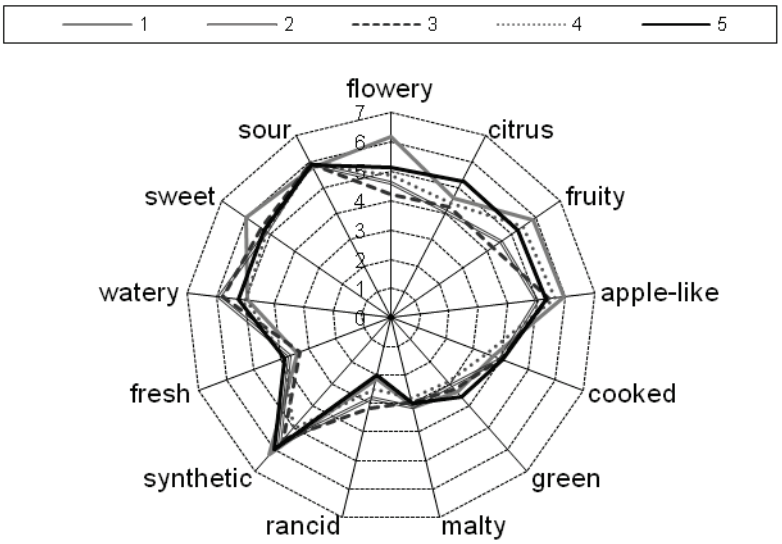

Figure 2-2: Flavour profiles as of apple juies containing different esters in comparision to reference: HEX = ethylhexanoate; ETH = ethylbutanoate; MET = methylbutanoate; E2MB = ethyl-2-methylbutanoate

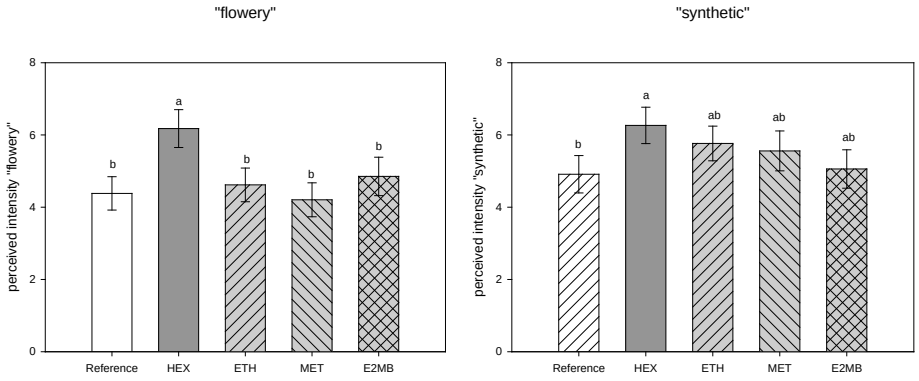

Figure 2-3: Intensity of the aroma attributes flowery & synthetic of apple juices containing different esters in comparision to reference: HEX = ethylhexanoate; ETH = ethylbutanoate; MET = methylbutanoate; E2MB = ethyl-2-methylbutanoate

Study 2: Effect of ethylhexanoate concentration on odour induced sweetness enhancement in apple juice

Study 1 showed a sweetness enhancing effect of ethylhexanoate at an odour concentration of 6 ppm additional ester. The effect of variation of the ethylhexanoate concentration on six different attributes (sweet, sour, flowery, synthetic, fruity and apple-like) is described in Figure 2-4 (0.04 ppm, 0.2 ppm, 1 ppm, 2.5 ppm, 5 ppm and 7.5 ppm ethylhexanoate (Table 2-1).

Overall a significant effect of increasing ethylhexanoate concentration was observed for the attributes sweet [$F(6, 117) = 3.09$; $p < 0.01$], synthetic [$F(6, 117) = 6.29$; $p < 0.001$] and fruity [$F(6, 117) = 5.88$; $p < 0.001$]. Sour, flowery and apple-like ratings were not influenced significantly. However, trends of sourness enhancement and floweriness enhancement with increasing ethylhexanoate concentration are displayed in Figure 2-4.

At 0.04 ppm ethylhexanoate did not affect any of the attribute ratings significantly. At increasing ethylhexanoate concentrations sweetness intensity ratings showed initially a slight decrease followed by an increase at higher odour concentrations. The sweetness enhancement through ethylhexanoate was found highest at 5 ppm ($p < 0.01$) and finally decreased again at 7.5 ppm ($p < 0.05$) additional ethylhexanoate. Sourness as well as floweriness showed a similar drop in intensity ratings as sweetness at ethylhexanoate concentrations below 1 ppm. Also syntheticness followed this observation finally resulting in a significant increase at ethylhexanoate concentration above 2.5 ppm ($p < 0.001$). As for sweetness ratings a decrease in synthetic notes at 7.5 ppm followed the highest synthetic intensity at 5 ppm. Fruitiness increased significantly at ethylhexanoate concentrations above 2.5 ppm ($p < 0.001$) compared to the reference juice. The sample containing 2.5 ppm ethylhexanoate was rated highest in fruitiness followed by a slight decrease in fruitiness at higher ethylhexanoate concentrations.

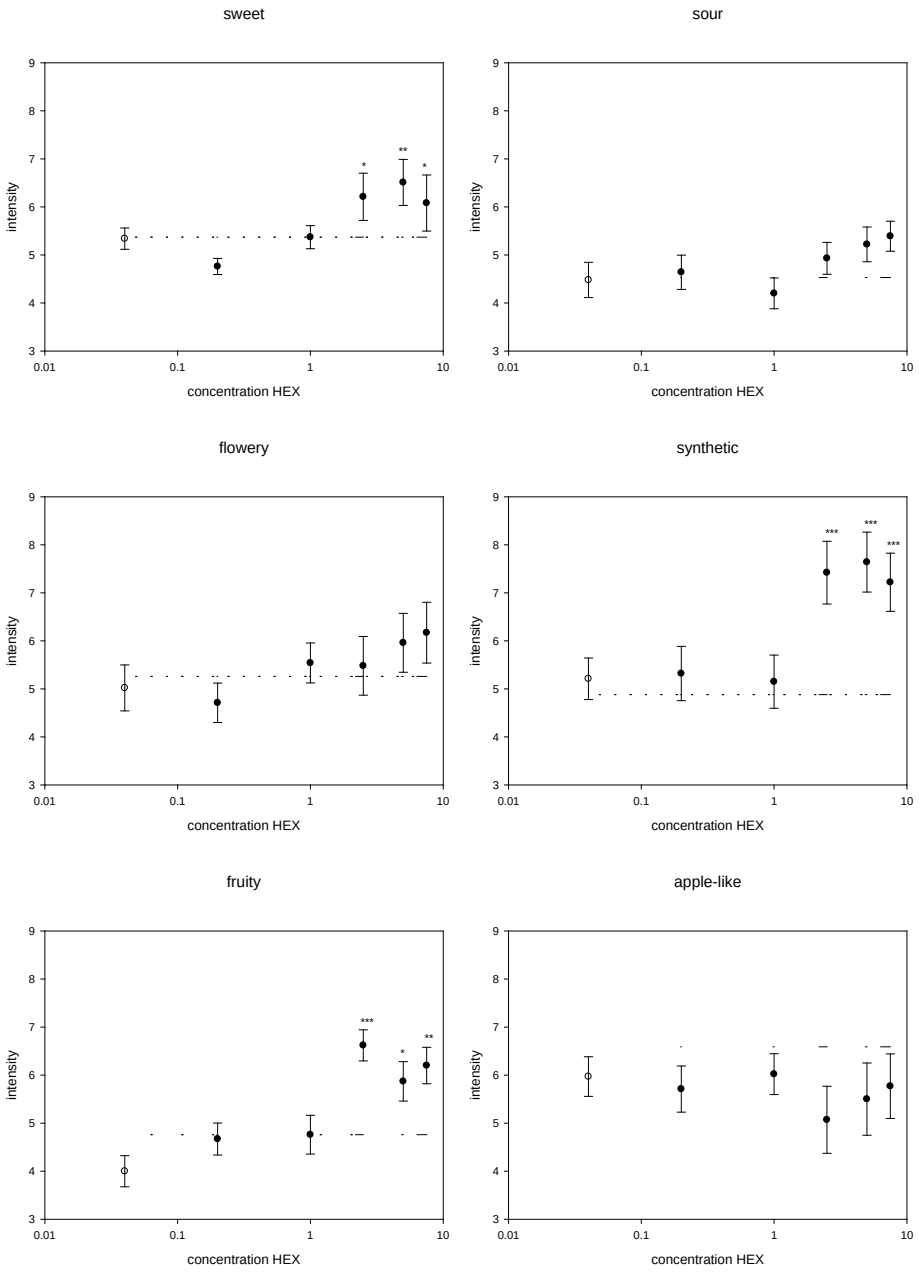

Figure 2-4: Intensity ratings of six taste and aroma related qualities in apple juice containing ethylhexanoate at different concentrations compared to reference; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; ----- = reference ratings

Discussion

It has been reported, that the four esters used as odorants in this study are key components in sweet apple aroma [107, 114-116]. Therefore all four esters have been selected to test sweetness enhancement in apple juice. Although all components were selected on basis of the same criteria, only the addition of ethylhexanoate does result in odour induced taste enhancement. Guichard et al. [117] linked the sensory quality of 8 different Calvados samples to the presence of certain chemicals including ethylbutanoate and ethylhexanoate as well as overall ester content. Samples containing high amounts of ethylhexanoate were described as fruity while samples high in overall ester content did not show fruity characteristics. Therefore, the contribution of ethylhexanoate to the aroma quality of Calvados, a liquor made of apples, was similar to its contribution to apple juice aroma in the present study. Also in Calvados, fruitiness was linked to overall product quality of the samples. Fruitiness therefore is considered an important factor in the quality of apple-based drinks. Although not significant, fruitiness scores in this study were higher for the ethylhexanoate containing samples compared to the reference. That could mean that the panel considered samples containing ethylhexanpoate of higher quality compared to the other samples and and as a consequence other attributes considered as positive in apple juice, such as sweetness, might have scored higher as well.

The fruity smell of ethylhexanoate in general could be the key to its sweet taste enhancement capacity. Komthong et al. [110] used GC-O to describe the smell of the esters used in the present study. Despite the fact that all four aroma components are described as apple-like, only ethylhexanoate has been described as fruity as well, while ethylbutanoate and ethyl-2-methylbutanoate are described as aged or even sweet. In a study focusing on Cantaloupe melons the authors could correlate the presence of ethylhexanoate with the fruity and sweet taste of the melons [112]. This correlation was not reported for the presence of ethylbutanoate evaluated in the same study. Furthermore, this study shows that the sweetness enhancing potential of ethylhexanoate lies beyond its use in apple juice or apple-related products but can be transferred to other fruit products. As congruency of odour and taste is one of the main driving factors behind odour induced taste enhancement [68] it is assumed that products containing fruits containing ethylhexanoate as an odour-active compound

such as apricots, pineapple or melon are further candidates for application of ethylhexanoate induced sweetness enhancement.

Apple aroma consists of many other sweet smelling components not belonging to the chemical group of esters [107, 114, 115]. The present study focused on the addition of esters as sweetness enhancing components in apple juice due to the correlation of increased sweetness with increased presence in matured apples as reported by Poll [106, 118, 119]. Just one out of four esters showed sweetness enhancement. However, this does not mean that other aroma active components congruent to apple juice would not effectively be able to enhance sweetness in apple juice.

In all studies a strong correlation between sweetness and sourness scores was found. The contribution of sourness scores was found to behave reciprocal to sweetness scores, which is in line with the general observation that sweetness suppresses sourness and vice versa in binary taste mixtures [100, 120, 121], even when these tastes are modulated by smell: 'Sweet-smelling' odours enhance sweetness while suppressing sourness (Djordjevic et al. [122] and Prescott et al. [27]). The suppression of sourness clearly shows that taste perception as a whole is influenced by the odour instead of just one taste quality. The sweet smell does not just add a sweet aroma impression to the juice, but changes sweetness itself and therefore sourness as well.

Our results show not only sweetness enhancement, but also enhancement of undesired notes such as "flowery" and "synthetic". Up to now, model systems have been used to describe odour taste interactions that were generally of limited complexity. Those studies focused on description of mainly just taste related attributes such as "sweet" and "sour". The present study shows that simple changes in aroma composition limited to the presence of only one component needed for sweetness enhancement, might affect the overall apple juice perception by generating off-notes.

No sweetness enhancement was found for addition of 0.04 ppm ethylhexanoate addition. A gradual increase in perceived sweetness was observed from 1 ppm ethylhexanoate onwards. While the magnitude of sweetness enhancement is highest at a concentration of 5 ppm the juice at 5 ppm ethylhexanoate concentration was still described as overall apple-like and fruity by the panel. However, at a concentration of 7.5 ppm the magnitude of sweetness enhancement decreases. It seems that at more

dominant odour intensity, the capacity to enhance sweetness is reduced. A possible explanation for this could be that if the concentration of the aroma component, in this case ethylhexanoate, is too high, the odour becomes too dominant resulting in the product becoming unbalanced. If the product is unbalanced and flavour characteristics change, the congruency of product and odour might not be given anymore. Hence, taste enhancement cannot be achieved anymore by addition of this specific odour.

While sweetness and synthetic notes were rated highest in concentrations of 5 ppm fruitiness was judged to be highest at 2.5 ppm and flowery notes were still enhanced at concentrations of 7.5 ppm. This clearly shows that optimal odour concentrations differ for different attributes. As a result the decision which odour concentration should be used to achieve the optimal product reduced in sugar needs to involve more consideration than just looking at the optimum concentration for sweetness enhancement. Knoop and co-workers showed that the complexity of food products require further adjustments in odour composition in order to achieve maximal sweetness enhancement of apple juice while preserving other appreciated sensory properties [123].

Conclusions

It is concluded that ethylhexanoate enhances the sweetness of apple juice, a complex real food product. Along with the odour induced sweetness enhancement sourness is reduced, showing that taste-taste suppression also occurs if taste intensities change due to odour addition. Undesired notes such as flowery and synthetic are enhanced as well by ethylhexanoate. Ethylhexanoate induced sweetness enhancement can be achieved with concentrations above 1 ppm and is most effective at concentrations around 5 ppm. Subthreshold addition of ethylhexanoate did not influence sweetness in apple juice.

Results clearly indicate that sweetness enhancement in real food products is more complex than in model mixtures and might require additional adaptations of the aroma profile in order to keep a balanced and/or desired flavour profile.

Acknowledgements

We thank Ilse Polet and Celine Brattinga for assisting with the sensory studies. We thank Friesland-Campina and IFF for generously providing the ingredients for this study.

Chapter 3

Masking off-flavours in apple juice induced by sweetness enhancing component ethylhexanoate

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(submitted)

Abstract

Esters naturally appear in apple juice contributing to the aroma of the juice. During apple maturation, *some* esters are produced at increasing concentration. The amount of these esters correlates with the perceived sweetness of apples and apple juice. As such, ethylhexanoate is a potent odor which enhances sweetness intensity in apple juice. Ethylhexanoate does not only enhance sweetness but also undesired aroma attributes such as “flowery” and “synthetic”.

This study aims at maintaining the ethylhexanoate induced sweetness enhancement and suppressing off-flavors introduced by ethylhexanoate addition by altering the relative concentrations of other naturally appearing aroma components.

Twelve combinations of four naturally in apple aroma occurring esters (ethylhexanoate, ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate) were tested in combination with each other. Odour induced sweetness enhancement by ethylhexanoate was observed only when methylbutanoate was not present in the ester combination. The undesired enhancement of the attributes “flowery” and “synthetic” caused by ethylhexanoate addition were successfully masked in the apple juice by changing the relative concentrations of ethylbutanoate,ethyl-2-methyl-butanoate and ethylhexanoate. By those means sweetness enhancement and scores for desired attributes such as “apple-like” remained at the level produced by ethylhexanoate alone. Compared to the reference, attributes such as “fresh” or “fruity” were neither affected by ethylhexanoate nor by the combination of the ethylbutanoate, ethyl-2-methyl-butanoate and ethylhexanoate.

We conclude that manipulation of the aroma composition of a familiar, real food can enhance taste intensity while suppressing undesired off-flavors resulting in a balanced product. However, we furthermore conclude that the prediction of changes in a products flavour after aroma alteration remains challenging.

Introduction

Aroma components can enhance sweetness in complex food products such as beverages [26, 74, 99]. Earlier work [124] demonstrated that the sweetness of apple juice increased, when the concentration of one naturally in apples occuring aroma component (ethylhexanoate) was increased. However, also undesired product properties such as flowery and synthetic notes were perceived stronger upon addition of ethylhexanoate. Hence, manipulating an aroma in order to reduce sugar concentrations in food production can result in off-flavours and leading to less consumer acceptance.

Dislike of a flavour is one of the most common reasons for consumers to reject a food [125]. The principal driver for consumers to reject food products are off-flavours, which can be described as undesired changes of a products flavour [126, 127]. The creation of off-flavours in foods can have many reasons, such as micro organisms [128, 129], contamination by chemicals [130, 131], enzymatic reactions, non-enzymatic browning, light induced changes in flavour and appearance or oxidation during product ripening and storage. In apple juice, Siegmund and Poellinger-Zierler [132] showed a correlation between microbiological spoilage of the juice and the occurance of off-flavours. In addition, changed or unbalanced aroma compositions can also result in off-flavours. Therefore, to maintain consumer acceptance, it is important to monitor the aroma balance whenever changing a product's flavour profile.

One possibility to reduce undesired flavours in foods is flavour masking. Flavour masking has been applied successfully to a large range of products by addition of new flavour components to foods [133]. However, the introduction of new flavour components can leave the product unbalanced. Instead, off-flavours could be masked by rebalancing the concentrations of individual aroma components native to the food, rather than by introducing new components. However, it is currently not possible to predict the effect individual aroma components have on the perceived aroma of the mixture. For example, not one key component is responsible for the perceived typical aroma of an apple, but a complex mixture of several components. The aroma of an odour mixture can differ strongly from the aromas of its constituting components [37-40]. Olfactory coding of odour mixtures is based on combinatorial receptor activation [36, 134]. In theory a complex odour mixture is deconstructed into molecular features by non-specific olfactory receptors and reassembled as a multiplicity of odour images in

the olfactory cortex [134, 135]. As a consequence, adding an odour to an odour mixture can result in a change of qualities different from those associated with the single component.

In a previous study [124], the addition of ethylhexanoate to apple juice caused a significant change of its aroma. This study investigated the impact of manipulating the concentration of four esters naturally occurring in apples on the sweetness and other sensory aspects of apple juice. Four esters (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethylhexanoate) were added individually to the apple juice. An untrained panel rated the juices on sweetness and 12 other taste and aroma related attributes. It was concluded that the sweetness enhancement induced by increased ethylhexanoate concentrations was accompanied by a deterioration of the aroma quality. Ideally, the restoration of the aroma quality would allow the creation of an apple juice reduced in sugar, while maintaining the aroma quality of the end product.

In a natural, non-manipulated, apple juice all four esters are present (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethylhexanoate) and no off-notes occur. Therefore, at least one combination of the four esters exists for which the apple juice is a balanced product without off-notes. However, this combination of esters does not result in sweetness enhancement of the product. We hypothesize that if one ester is able to induce strong off-notes in such a complex odour mixture, combinations with other esters exist that mask the off-notes while maintaining sweetness enhancement. Therefore, in this study, all possible combinations of the four esters with each other in binary, ternary and quaternary mixtures will be investigated for their ability to enhance sweetness without inducing off-notes.

The main objectives of this study are to determine whether a combination of esters can mask off-notes induced by ethylhexanoate while maintaining ethylhexanoate induced sweetness enhancement and to investigate combinatory effects between different esters.

Materials and Methods

Materials

An industrial apple juice concentrate, very low in aroma content, (Friesland Campina, Ede, NL) was diluted with deionized water (MilliQ, Billerica, MA) to 8.4 °Brix (130 g/L).

Table 3-1: Composition of all apple juice samples: HEX = ethylhexanoate; ETH = ethylbutanoate; MET = methylbutanoate; E2MB = ethyl-2-methylbutanoate

Sample	*Apple juice concentrate [g/L]*	*Added aroma [ppm]*					
		basic	HEX	ETH	MET	E2MB	° Brix
1	130	10	6	-	-	-	8.4
2	130	10	3	3	-	-	8.4
3	130	10	3	-	3	-	8.4
4	130	10	3	-	-	3	8.4
5	130	10	-	3	3	-	8.4
6	130	10	-	3	-	3	8.4
7	130	10	-	-	3	3	8.4
8	130	10	2	2	2	-	8.4
9	130	10	2	2	-	2	8.4
10	130	10	2	-	2	2	8.4
11	130	10	-	2	2	2	8.4
12	130	10	1.5	1.5	1.5	1.5	8.4
13 reference	130	10	-	-	-	-	8.4

An apple aroma (IFF, Hilversum, NL) was used as basic apple aroma, which did not include any of the four aroma relevant esters used in these studies (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethylhexanoate). The omitted individual aroma components (ethylhexanoate, ethylbutanoate, methylbutanoate and ethyl-2-methylbutanoate) (purity: 98%, pro analysis), were also provided by IFF, Hilversum, NL.

Stimuli were presented in 30-mL medicinal cups tightly closed with lids (DUNI GmbH & Co KG, Bramsche, Germany). The lids had custom-made holes of the same diameter as the straws used (SOLO®, Illinois, USA 722X PART# 9084922, PE, o.d. = 8 mm,

l = 10 cm). Straws were inserted into the holes so that odours could not emanate from the cup before and during the tasting by the panelist. This way, the odours were always presented retronasally.

Subjects

Seventeen paid subjects (age 26-61; 13♀, 4♂) participated in the study. The subjects were tested for normal sense of smell and taste, prior to the study. The subjects were naïve with respect to the experimental conditions. However, some took part in earlier food sensory evaluations. All subjects gave informed written consent prior to the sensory evaluations.

Method

Closed cups with an inserted straw where used to allow retronasal odour stimulation.

Thirteen apple juices were prepared using an apple juice stock solution (130g/L apple juice concentrate, diluted to 8.4º Brix). All stimuli were presented in duplicates.

Odorants were added to the base juice, according to the different compositions described in Table 3-1. All apple juices contained 10 ppm basic apple aroma. In addition, the samples contained combinations of the four esters (ethylhexanoate, ethylbutanoate, ethyl-2-methylbutanoate and methylbutanoate) in an overall concentration of 6 ppm. One sample did not contain the additional esters to be used as a reference sample.

The samples were evaluated in three sessions on the perceived intensity of 13 attributes (apple-like, citric, cooked, flowery, fresh, fruity, green, malty, rancid, sour, sweet, synthetic and watery). These attributes were generated by a descriptive panel prior to the experiment.

During the first session the panel rated attribute intensities on a 0 – 12 line scale for 7 apple juices three of which consisted of the reference apple juice. Individual reference scores were used by each subject and each sample was evaluated with respect to the reference score. Stimulus orders were fully balanced and randomized over subjects.

Individual reference scores were calculated for each subject after the first session and were given to each subject and used as reference scores in sessions 2 and 3. In session 2 and 3 thirteen apple juices were evaluated by the panel. Each sample was evaluated with respect to the reference score. Stimulus orders were fully balanced and randomized over subjects.

Data Collection and Analysis

Intensity ratings were collected using an automated data collection system (FIZZ 2.30C, Biosystems, Couternon, France). Subjects evaluated samples on 0 -12 linescales anchored at 0 'not at all' at the left end and 12 'very' at the right end.

PCA analysis was performed using SENPAQ 4.7 (QiStatistics, UK, 2008). Multi-Factor-ANOVA testing of intensity ratings for main effects of samples and ester profile (13) as a fixed factor and subjects as a random factor (17) was performed with XLSTAT (Version 2009.1.02, Addinsoft™). Post-Hoc analysis was performed using Tuckey-HSD. Average intensity ratings were considered as significantly different, when $p < 0.05$.

Results and Discussion

Multi-Factor ANOVA analysis showed that the sweetness of the samples varied significantly amongst samples [$F(12, 221) = 2.34$; $p < 0.01$]. Additionally, the malty aroma [$F(12, 221) = 2.10$; $p < 0.05$] and freshness [$F(12, 221) = 2.63$; $p < 0.01$] of the apple juices were influenced significantly by ester addition. The authors previously observed a significant influence of ethylhexanoate addition on the attributes flowery and synthetic (Knoop et al.; submitted). Those results could be confirmed in this study, as both attributes were significantly influenced by addition of different ester combinations (flowery: [$F(12, 221) = 2.21$; $p < 0.05$]; synthetic: [$F(12, 221) = 3.20$; $p < 0.001$]). Post-Hoc analysis later revealed, that ethylhexanoate containing samples indeed contributed most to those effects. The attribute ratings for citrus, apple-like, cooked, green, watery and sour were not influenced significantly by the addition of different ester combinations.

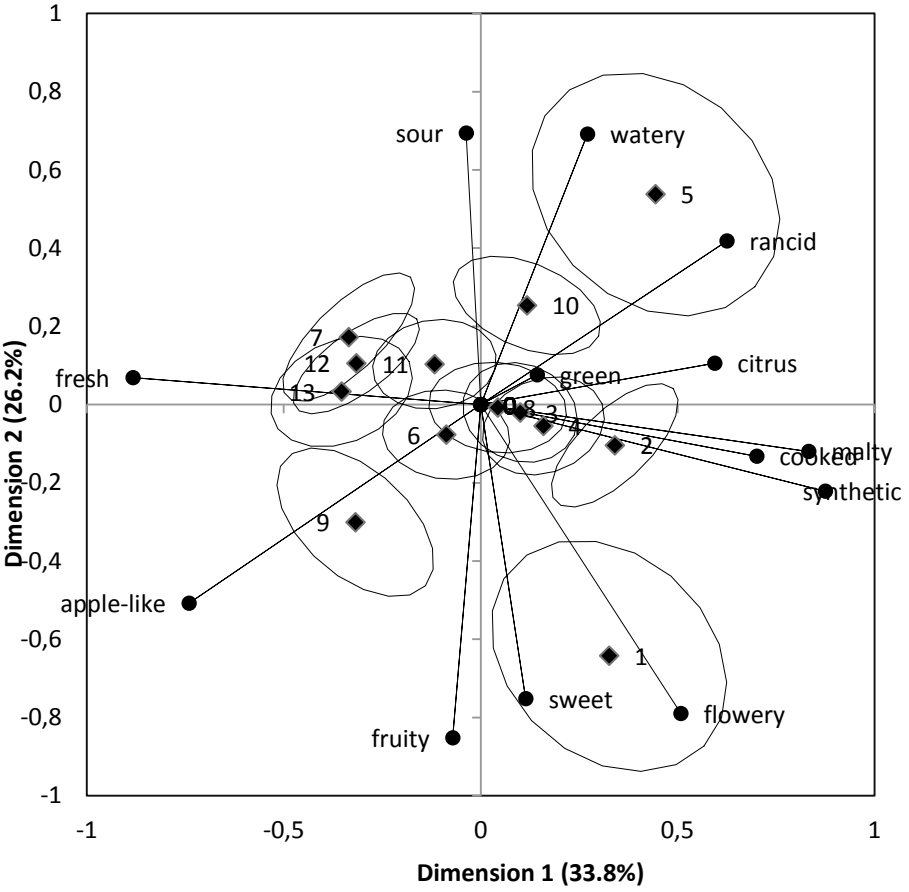

Figure 3-1: PCA-Bi-plot showing sample mean scores in relation to the attribute loadings on the first two principal components. Ellipses indicate sample confidence intervals.

Figure 3-1 shows the factor loadings on the first two principal components and the corresponding sample scores combined in a bi-plot. These give an impression of the overall changes in the flavour profile caused by addition of different ester combinations (dimension 1 vs dimension 2). Samples 1, 5, 9 and 10 (composition see table 1) show clear changes in the flavour profile with respect to the reference sample. While addition of ethylhexanoate (sample 1) leaves the apple juice flavour sweet yet flowery, the addition of ethylbutanoate and methylbutanoate (sample 5) makes the

flavour quality of the apple juice more watery and rancid. More desirable changes in the overall flavour profile are caused by the addition of a combination of ethylhexanoate, ethylbutanoate and ethyl-2-methylbutanoate (sample 9), shifting the flavour towards apple-like and fruity notes while maintaining its sweetness. When ethylbutanoate is replaced by methylbutanoate (sample 10) the flavour profile becomes more watery and rancid and less sweet yet more sour. Sample 2, containing ethylhexanoate and ethylbutanoate, is dominated by a synthetic off-flavour. The flavour profiles resembling most the original product (sample 13) were those of the sample containing all four esters (sample 12) and the sample containing methylbutanoate and ethyl-2-methylbutanoate (sample 7) (Figure 3-1). These results therefore suggest that a combination of methylbutanoate and ethyl-2-methylbutanoate produces the most 'natural' apple juice, qualifying the combination of these esters as the best candidate to mask ethylhexanoate off notes. However, this is not the case, as this combination (sample 10) resulted in malty off-notes and a significant reduction in freshness ($p < 0.05$). Those are different characteristics that cannot be ascribed to ethylhexanoate addition, but to the addition of the whole mixture.

Literature supports these observations: addition of odour components to an odour mixture can lead to changes in the odour quality that show no qualitative resemblance to any of the odour components added [136-140]. This aspect of odour mixtures is referred to as synthetic mixture perception, as opposed to analytical mixture perception [27, 141]. An explanation for non-retraceable shifts of aroma quality after addition of certain odour components could be the way olfactory receptors are activated by odour mixtures. Olfactory receptors (OR) are sensitive to a wide range of odorant molecules. Different odour components in a mixture can bind to the same receptor. Duchamp-Viret et al. [142] as well as Rospars et al. [143] showed that in binary odour mixtures the activation patterns of olfactory receptor neurons (ORN) differed strongly from the activation caused by the single odours. Both studies showed that ORN activation by mixtures of limonene and menthol are not a mere addition of the activation patterns caused by the single components but resulted in a non-additive change in the temporal response patterns. Rospars et al. concluded that especially for natural odours that are very complex, like in this study apple juice aroma, it is not possible to predict changes in activation as too many interactions between the odours

Sample	*Attribute*												
	sweet	*sour*	*apple*	*citric*	*cooked*	*flowery*	*fresh*	*fruity*	*green*	*malty*	*rancid*	*synthetic*	*watery*
1	++	--	-	-	+	++	--	++	/	++	+	++	-
2	+	/	--	+	++	+	--	/	/	++	/	++	/
3	+	/	--	-	/	+	--	+	+	++	+	+	+
4	+	/	-	/	+	+	--	+	/	++	+	+	+
5	/	/	--	+	+	-	--	--	/	++	++	+	+
6	/	/	/	-	+	-	--	-	--	++	+	+	-
7	+	+	--	--	-	/	/	+	+	+	/	-	+
8	+	+	--	-	+	+	-	+	/	++	/	-	+
9	++	-	/	-	-	/	-	/	/	+	/	/	-
10	/	+	-	/	+	+	--	-	+	++	+	/	+
11	/	+	--	--	+	+	--	/	-	+	+	+	+
12	/	+	-	/	/	/	-	/	-	/	-	-	-
13 (ref)	*4.82*	*5.94*	*6.44*	*4.79*	*3.35*	*4.38*	*4.82*	*5.11*	*3.47*	*2.20*	*1.91*	*4.91*	*5.20*

Table 3-2: Effects of ester addition on all sensory properties of all apple juice. Numbers of sample 13 represent the mean intensity of the reference ++ = significant enhancement ($p < 0.05$); + = enhancement (difference > ±0.2 compared to reference and $p > 0.05$); / = no change compared to reference score (± 0.2); - = suppression (difference > ±0.2 compared to reference and $p > 0.05$); -- = significant suppression ($p < 0.05$)

take place. On a perceptual base this can be seen in our study as well. The addition of ester combinations resulted in unpredictable changes in the aroma profile. A more systematic approach therefore is needed in order to better understand which possible interactions may occur between aromas involved in sweetness perception.

Table 3-2 shows the contributions to attribute ratings of esters added in comparison with the reference apple aroma (for composition see Table 3-1). Figure 3-2 summarizes the mean scores for sweetness of all juices which showed odour-induced sweetness enhancement (as a trend or significant). Sweetness enhancement could be observed for the single ester ethylhexanoate (sample 1), all binary ester mixtures including ethylhexanoate (samples 2, 3, 4) a binary mixture including methylbutanoate and ethyl-2-methylbutanoate (sample 7) and ternary mixtures of ethylhexanoate with ethylbutanoate and methylbutanoate (sample 8) or ethyl-2-methylbutanoate (sample 9). Only ethylhexanoate alone (sample 1) and sample 9 enhanced sweetness significantly ($p < 0.05$) compared to the reference. The other combinations (sample 2, 3, 4, 7) showed trends of sweetness enhancement, however not significant ($p > 0.05$).

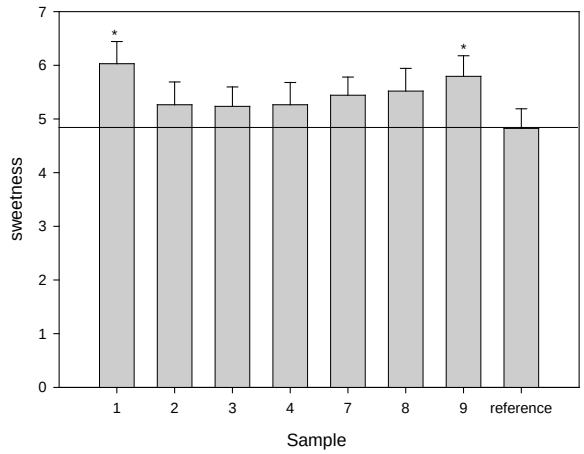

Figure 3-2: Sweetness ratings of all samples that showed sweetness enhancement. * indicates significant enhancement ($p < 0.05$).

As reported previously, the addition of ethylhexanoate to apple juice enhances flowery and synthetic off-notes significantly (Knoop et al., submitted). In the present study, trends of flowery enhancement were found for samples 2, 3 and 4. Sample 2 enhanced synthetic notes significantly ($p < 0.01$) (Figure 3-3). This is in agreement with previous studies. As post-hoc comparisons between the sweetness ratings for the reference sample and samples 2, 3, 4 and 8, respectively, did not indicate significant sweetness enhancement they were not further evaluated on their off-flavour masking capacities. Sample 9 (containing a ternary combination of the esters ethylhexanoate, ethylbutanoate and ethyl-2-methylbutanoate) showed significant sweetness enhancement. In addition, this sample did not produce flowery and synthetic ratings different from the reference sample. Moreover, besides its enhanced sweetness, no further changes in the flavour profile of sample 9 were observed in comparison with the reference. It can be concluded, that a combination of ethylhexanoate, ethylbutanoate and ethyl-2-methylbutanoate increases sweetness significantly while maintaining the overall flavour profile of the juice.

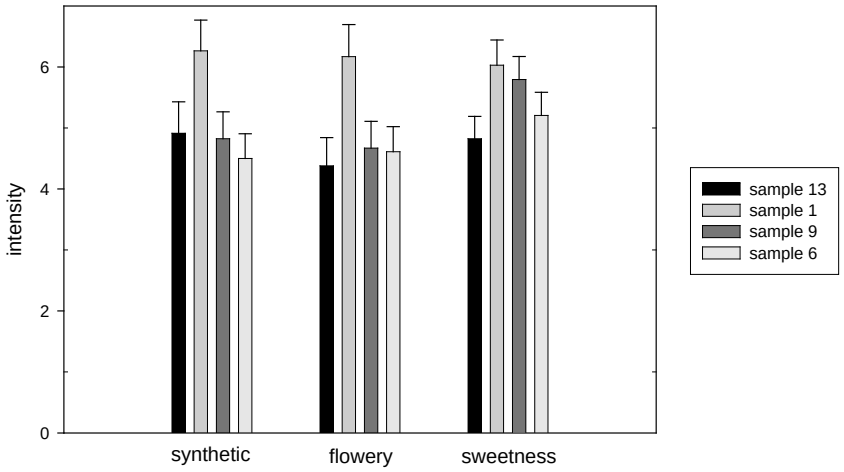

Figure 3-3: Mean scores for flowery note, synthetic notes and sweetness for samples 1, 6 and 9 in comparison to reference sample 13. Error bars indicate the standard error.

In this experiment concentrations of the single components vary between the samples to keep the overall concentration of added odour constant at 6 ppm. One might conclude that the ethylhexanoate concentration (2 ppm of sample 9) was too low to induce off-notes. Although previous studies showed that the magnitude of

ethylhexanoate induced floweriness and syntheticness depended on the ester concentration, previous work (Knoop et al., submitted) showed a significant increase of such notes from concentrations of 1 ppm and higher. Therefore, the addition of 2 ppm ethylhexanoate to sample 9 can be considered as sufficient to induce off-notes. Thus, the absence of off-notes can be ascribed to masking effects of ethylbutanoate and ethyl-2-methylbutanoate addition.

Sample 9 was described as more 'round' by the sensory panel, with which they meant that all aspects of apple aroma are produced by this sample. This is in contrast to sample 2 which revealed very dominant ethylhexanoate induced 'flowery' notes. A possible reason for the masking effect of ethylbutanoate and ethyl-2-methylbutanoate on flowery and synthetic notes produced by ethylhexanoate in apple juice could be combinatory effects of individual esters in odour mixtures. As can be seen in Figure 3-3, the combination of ethylbutanoate and ethyl-2-methylbutanoate (sample 6) reduces synthetic notes. When these components are combined with ethylhexanoate in apple juice (sample 9), the ethylhexanoate-induced synthetic note is masked. However, combinatory effects cannot explain the masking of floweriness, as sample 6 shows a slight increase in floweriness, but in combination with ethylhexanoate (sample 9), that itself increases synthetic notes significantly, no effect on syntheticness was observed.

This study demonstrates that, at the present, interactions between single odour components in complex food matrices cannot be predicted nor understood from elementary odorant contributions to the food aroma. In order to exploit odour induced taste enhancement to compensate for tastant reduction on an industrial scale it will be necessary to test potentially taste enhancing components systematically and individually in complex, realistic systems.

Conclusions

Addition of esters and ester combination to apple juice changes the overall product flavour. Sweetness enhancement can be induced by single aroma components but might induce off-flavours. A natural, non-manipulated apple juice containing the complete set of esters does not show those off-notes. In this study the addition of a combination of all four esters did not cause off-notes either. It can be concluded that addition of ester mixtures can counterbalance the ethylhexanoate addition.

Linking of certain changes of product qualities to specific esters is difficult as the esters interact with each other and therefore different properties are influenced by ester combinations than by certain esters alone.

Masking off-flavours in apple juice induced by ethylhexanoate addition is possible by addition of ethylbutanoate as well as ethyl-2-methylbutanoate. In this combination sweetness enhancement could be maintained without inducing off-notes.

Acknowledgements

Ilse Polet and Celine Brattinga are thanked for helping to carry out the experiments. We also thank Friesland Foods and IFF for generously providing ingredients for this study.

Chapter 4

Synchronicity of retronasal aroma release with swallowing effects taste intensity

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Abstract

Synchrony of auditory and visual stimuli defines whether these will affect each others perception. Sound sensitivity was found to be enhanced when synchronously presented with visual stimulation. Similar cross modal interactions occur during food consumption. It was shown that aroma influences taste and vice versa. Little is known as to what extent synchrony of chemosensory stimuli affects their cross-modal integration.

This study aims at investigating effects on taste perception as a function of the degree of synchrony between retro-nasal presentation of a sweetness enhancing aroma component and the moment of swallowing.

Aliquots of 5 mL apple juice were served to 18 subjects by gustometer. Olfactometric presentation of the sweetness enhancing aroma ethylhexanoate was at predefined 1-s intervals before, during and after swallowing. The exact moment of swallowing was recorded via two VMG-sensors placed on the left masseter and the thyroid of the subjects, which allowed an exact assessment of the synchrony of aroma presentation with swallowing. Retro-nasal aroma presentation resulted in significant sweet taste nhancement. Time-intensity ratings during and line-scale ratings after stimulus presentation were evaluated as a function of aroma-swallowing synchrony. Significant differences were found for the magnitude of sweetness enhancement depending on synchronicity of aroma presentation with swallowing. No sweetness enhancement occurred, if the aroma was presented synchronous to swallowing. Highest sweetness enhancement was found if the aroma was presented either 2.5 – 1.7 s before or 2.6 – 3.6 s after swallowing. An asynchronous presentation of odour stimulation with respect to swallowing was found to be most effective to induce maximum taste enhancement.

Introduction

During food consumption all senses are involved in its perception. For instance, the colour of a drink has been identified to strongly influence the flavour [55, 144-146]. Sound was found to influence texture perception profoundly [147, 148]. Zampini and Spence [97] showed that potato chips were perceived more crispy when simultaneously presented with more "crisp" sounds, i.e. by amplifying the sound frequencies produced from 2 to 20kHz. The reason for this phenomenon is that humans naturally integrate synchronous stimuli from multiple modalities into unified impressions. The McGurk effect shows the influence of vision on auditory perception [95] while the 'bounce illusion' demonstrates an influence on the visual perception of motion induced by sound [149]. Violentyev et al. [150] demonstrated the creation of visual illusions induced by tactile stimulation. Visual illusions such as multiple flashes have previously been described by Shams et al. [96, 151]. In their experiments participants were presented with single flashes and various numbers of beeps. Depending on the number of beeps, participants perceived multiple flashes although just one flash was presented. Furthermore they reported that sound-induced flash illusions (SIFIs) depended on the synchronous presentation of both stimuli. They demonstrated that if stimuli were presented asynchronously, visual-acoustic interactions disappeared. In fact, the magnitude of SIFI decreased with increasing temporal asynchrony between stimuli.

The most common multimodal integration process in food perception is that of taste–smell integration. Taste and smell interactions have been subject to various studies [26, 74, 98, 124, 152-155]. It was shown that aroma perception can be influenced by various tastants and vice versa [25, 100, 156]. Not much is known, whether synchronicity as reported for visual-acoustic interactions also apply to interactions of taste and smell. One study by Pfeiffer et al. [71] studied the effects of stimulus synchrony on odour-induced taste enhancement. They evaluated the effect of presenting the aroma component benzaldehyde on the taste threshold of a saccharin solution. If the odour was presented simultaneously with the taste, it lowered the perception threshold of saccharin. However, this effect disappeared when they presented odour and taste asynchronously. In their study, the temporal delay between gustatory and olfactory stimulation was created by spitting out the tastant solution followed by sniffing the odour. In the synchronous condition, combined odour/tastant solutions were swallowed. Swallowing is a crucial part in the typical consumption of food. It should

be noted that, under normal eating conditions, the act of swallowing provokes odour delivery at the olfactory epithelium through the oropharyngeal-nasopharyngeal pathway [90, 157-159]. Therefore, odour stimulation is typically delayed with respect to (oral) taste stimulation. Nonetheless, because this delay should be considered natural, we hypothesize that odour presentation at the moment of swallowing is the optimal condition for odour-induced taste enhancement whereas advancing or delaying the odour delivery with respect to the moment of swallowing would imply a perceptual desynchronization of odour and taste resulting in reduced odour-induced taste enhancement. Previously, we proved that ethylhexanoate enhances sweetness perception in apple juice [124, 155]. In the present study, we investigate whether the magnitude of ethylhexanoate-induced sweetness enhancement in apple juice is influenced by the temporal synchrony between odour presentation and the moment of swallow. We tested this by delivering the stimuli via fully synchronized gustometry and olfactometry allowing a complete control over stimulus timing. Vibrio-myographic (VMG) measurements of swallow actions at high temporal resolution allowed the exact assessment of odour-swallow desynchrony. This approach should provide further insight into the temporal dynamics of the mechanisms underlying multimodal odour and taste integration.

Materials and Methods

In this study the authors differentiate between the indicated moment of swallow (IMS) and the real moment of swallow (RMS) assuming that reaction times to actually perform the task after indication via screen event will differ not only inter-individually but also individually for each stimulus presentation. Therefore all IMS values are corrected with respect to the real moment of swallow as measured by VMG.

Stimuli

An industrial apple juice concentrate, very low in aroma content, (Friesland Campina, Ede, NL) was diluted with deionized water (MilliQ, Billerica, MA) to 10.4° Brix (150 g/L).

An apple aroma (IFF, Hilversum, NL), which did not include ethylhexanoate, was used as basic apple aroma. The basic apple aroma was added to the juice in a concentration of 10ppm, to restore the apple aroma that was removed during the industrial

processing of the concentrate. Ethylhexanoate (purity: 98%, pro analysis), was also provided by IFF, Hilversum, NL.

Table 4-1: Olfactory odour presentation times for 8 stimuli. Odour was presented retronasally for a duration of 1 s per stimulus.

Stimulus	$odour_{start}$ [s]	$odour_{end}$ [s]
1	-	-
2	12	13
3	14	15
4	16	17
5	17	18
6	18	19
7	19	20
8	21	22

In this study, oral stimuli were presented to the subjects under eight different odour timing conditions via Gustometer and Olfactometer (Table 4-1)

Odour presentation times varied with respect to the IMS that occurred 15 s after the start of each stimulus sequence (Figure 4-1). In all conditions apple juice was presented as liquid stimuli in amounts of 5-mL per stimuli, directly into the subjects mouth (see Gustometry). The eight stimulus conditions included one presentation of plain apple juice only, not accompanied by retronasal odour and seven combinations of apple juice combined with the ethyl-hexanoate odour presented retronasally at seven different moments around the IMS (Figure 4-1, Table 4-1). Stimuli were chosen to represent a wide range of odour presentation times with respect to the RMS.

Mineral water (SPA, reine, Spadel Nederland BV., Made, NL) was delivered via Gustometer in amounts of 5 mL to rinse the mouth after each stimulus.

Olfactometer delivery conditions

Odorous stimuli were presented retro-nasally by an Olfactometer (OM2s; Burghart Instruments, Wedel, Germany) which allows application of odours without causing concomitant mechanical or thermal sensations (Kobal and Hummel, 1988). The air saturation chamber of the olfactometer was filled with 10 mL diluted odour (ethylhexanoate: 4000.ppm in propylenglycol). The odorant concentration was 1000

ppm, as this concentration was found to be sufficient to provide a clearly perceivable odour peak without overpowering the overall apple juice aroma intensity and without producing further odour lingering during test trials. The total flow-rate was 4.1 L/min, resulting from mixing 1.14 L/min of odorized air with 3.98 L/min humidified air. The temperature and humidity of the dilution air stream was kept constant (36,5 ºC; >80% relative humidity). Odour pulses of 1-s duration were presented to the subjects in a constant air stream that was presented by a tube positioned under endoscopic control by a licensed physician (Heilmann and Hummel, 2004). The opening of the tube was placed in the epipharynx, approximately 8 cm from the naris. The tubes were fixated to the nose by adhesive tape.

Gustometer delivery conditions

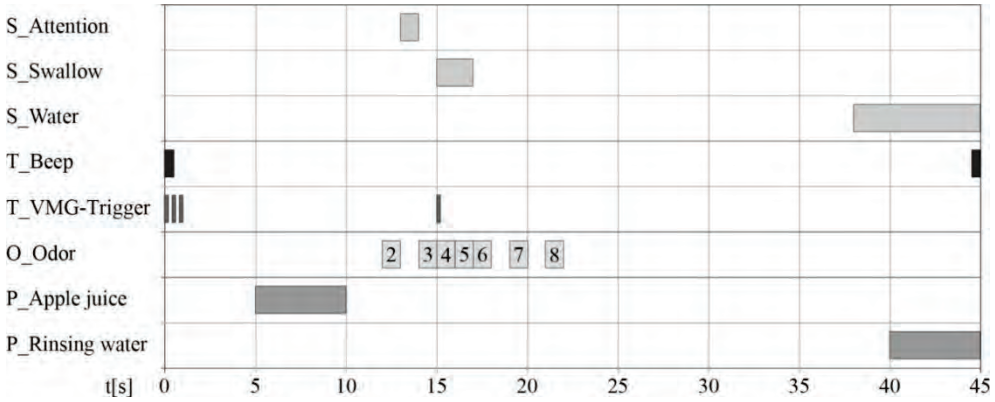

Figure 4-1: Schematic overview of experimental setup over time as programmed for the Gustometer software. S = screen event; T = trigger event; O = olfactory stimulation; P = pump event

The apple juice was presented to the subjects using a gustometer. The gustometer is a computer-controlled, multi-channel delivery system for liquids, which allows precise control of stimulus timing, quantity and composition [160]. These stimulus parameters are assured by mixing predefined liquid flows produced by eight pumps (KNF Stepos FEM03.18RC, KNF, Verder, Vleuten, NL). Teflon tubing (1.6mm inner diameter) connects the pumps to a 8-channel-manifold in which different liquids are mixed. From here, oral stimuli and rinsing water are delivered at the distal portion of the subject's anterodorsal tongue through a 5-cm long Teflon tube (1.6 mm inner diameter).

Four pumps were used in parallel to deliver apple juice (5 mL at a cumulative pump speed of 120 mL/min during 2.5 s) and four pumps to deliver rinsing water to the subjects' mouths (10 mL at a cumulative pump speed of 120 mL/min during 5.0 s; see Figure 4-1.

Vibrio-myography-measurements (VMG)

Vibrations produced by contracting muscles can be detected by piezo-electrical sensors when placed in contact with the skin overlying the muscle. The vibro-myographical (VMG) signal that these sensors produce is proportional to the muscle activity, similar to electromyography, with the advantage that the signal is not sensitive to electrical noise and requires no preparation of the skin. In this study, VMG sensors were placed on the skin, 1 cm lateral to the thyroid and on the left masseter, held in place with double-side tape. Unfiltered VMG-potentials were analogue-to-digital converted (ADC11, Picotechnology Ltd., UK) and recorded on hard disk.

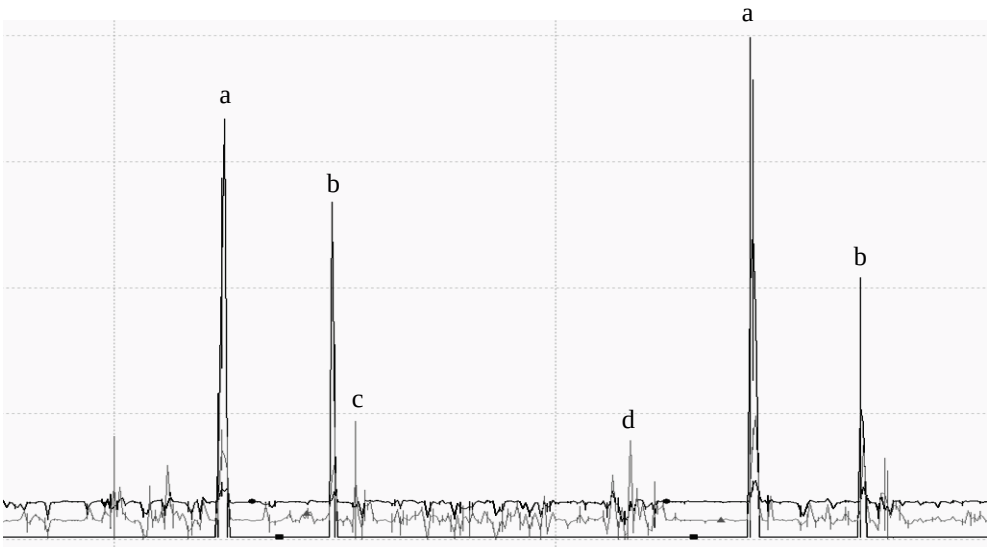

Figure 4-2: VMG- and gustometer trigger signal readings for one subject and one stimuli as generated by the data collection system (PICO). Readings were used to synchronize real moment of swallow (RMS) and odour presentation time. Letters a, b, c and d indicate events recorded by the system. a = trigger signal (gustometer) at the beginning of each stimuli; b = trigger signal generated parallel to the visual event 'swallow' (compare figure 1); c and d = movement of the thyroid generated by swallowing; c indicates swallowing of the stimuli and d indicates swallowing of the rinsing water

All sensors and electrical equipment were optically isolated from the mains and had CE approval. To facilitate time-locking of swallowing events with the presentation of odour pulses, the VMG recordings also included markers for stimuli generated by the olfactometer/gustometer control software (Figure 4-2).

Subjects

Nineteen paid subjects (age 18-60; 16♀, 3♂) participated in the study. The subjects were tested for normal sense of smell and taste, prior to the study. The subjects were naïve with respect to the experimental conditions. However, some took part in earlier food sensory evaluations. All subjects gave informed written consent prior to the sensory evaluations.

Sensory Study

The study entailed three sessions (one training, two experimental) and all subjects completed the full study. The training session lasted 30 minutes, the experimental sessions lasted 45 minutes.

In the first session, subjects were familiarized with the setup and trained on the experimental procedure. Ten apple juices were presented to the subjects via the gustometer. No accompanying odours were presented in this session in order to minimize discomfort for the subjects while practicing the procedure. In each of the two experimental sessions, subjects evaluated the sweetness intensity of the eight apple juice – timed odour combinations in triplicates and in randomized and balanced order. For this, they used computer-controlled time-intensity ratings during stimulus presentation and line-scale rating after each stimulus. In session 2 and 3, two dummy samples were presented to the subjects prior to the experiment for warm-up purposes.

Figure 4-1 shows the experimental design of this study. Each stimulus presentation sequence lasted 45 seconds followed by a 15 seconds brake. The start of a stimulus sequence was introduced by a beep (0 – 0.5 s). Parallel to the beep, three trigger signals lasting each 200 ms were send to the VMG recording system to facilitate the time-locking of events during data analyses. Over a period of five seconds (5 – 10 s) 5mL of apple juice were pumped into the subjects mouth. The subjects were asked to keep the juice in their mouth until instructed to swallow. From 13 – 14 seconds

subjects received the screen instruction to stay alert. This screen event was followed by the instruction to swallow (15-17 s). Parallel to the instruction to swallow a trigger signal was send to the VMG recording system to indicate the desired moment of swallow (IMG) (Figure 4-1, Figure 4-2). An odour pulse was presented retronasally during 1 second at variable onset times (Table 4-1), with the exception of the blank stimulus (stimulus 1), for which no odour was presented. Thirty-eight seconds after initiation of the stimulus sequence, another screen event alerted the subjects that water was about to be pumped into their mouths. For a duration of 5 seconds rinsing water was pumped in the panellists mouth (40 – 45 s). Subjects were free to swallow the rinsing water whenever they wanted.

Data Collection and Analysis

Time-intensity (TI) ratings were recorded by an automated data collection system (Dynascore 2.01, DNS Multimedia Factory GmbH, Hamburg, Germany). During stimulus evaluation, subjects indicated actual sweetness intensities with a computer mouse by moving a cursor up and down on an vertical scale in the computer screen (anchored on the bottom 'not sweet' and on the top 'very sweet'). Intensity scores (0-100) were collected every 0.2 seconds during 45 seconds for each stimulus. TI measurements produced curves of 225 data points each. For each stimulus evaluation, the Area under the curve (AUC) was calculated as a measure of perceived taste over time [154, 161, 162]. In addition, the time at which TI ratings reached a maximum (T_{max}) was assessed for each stimulus evaluation.

Besides the time-intensity ratings, subjects marked overall sweetness intensities on a 10 cm line-scale (0-100 mm; anchored on the left 'not sweet' and on the right 'very sweet') printed on paper.

Odour pulses were initiated at 7 different moments before, at, and after the intended moment of swallowing (i.e. starting between 5 s before until 4 s after the end of the swallow instruction). Because subjects show a clear variation in the timing of the real moments of swallowing (RMS), the exact moments of odour pulse presentations relative to the real moments of swallowing [$t_{OD/S}$] were calculated by subtracting the time of the RMS [t_{RMS}] from the time of odour pulse presentation [t_{OD}]:

$$t_{OD/S} = t_{OD} - t_{RMS}$$

Analysis of variance (ANOVA) was carried out on sweetness scores consisting of AUC and line scale ratings with subjects as a random factor (19 subjects) and odour pulse timing as a fixed factor defined in two different ways: First, sweetness scores were categorised according to the 8 time categories of the experimental design, i.e. representing the 7 odour presentation times relative to the IMS not corrected for RMS and the no-odour condition. In the second ANOVA, sweetness scores related to stimuli involving odour presentations were assigned to 16 time categories, representing consecutive time windows of odour presentations relative to the RMS (Table 4-2). The limits of each time category were set in such a way that each category contained 50 sweetness scores. As a first category the blank condition in which no odour was presented was included in the analysis with n = 114.

All ANOVA calculations were performed with SENPAQ 4.7 (QIStatistics, UK, 2008. Post-Hoc analysis used Tuckey-Kramer-HSD statistic as compensation for inflation of the significance level due to multiple comparisons. The significance level used was 0.05.

Results

Effects of odour presentation time on odour induced taste enhancement

The main objective of this study was to evaluate the impact of odour presentation time during consumption on the magnitude of ethylhexanoate-induced sweetness enhancement in apple juice. Sweetness intensity ratings for all eight odour timing conditions, but not corrected for the real moment of swallow (RMS) are presented in Figure 4-3. Analysis of variance (ANOVA) for the effects of nominal odour presentation times, i.e. not corrected for the RMS, showed significant effects of odour presentation time with respect to the instructed moment of swallow on time-intensity ratings
[$F(7, 760) = 4.32$; $p < 0.001$] and line-scale ratings [$F(7,760) = 6.96$; $p < 0.0001$]. Post-hoc analysis revealed that, in both cases, this was due to the no-odour stimulus (stimuli 1) that produced significantly lower sweetness ratings than the other stimuli ($p < 0.0001$). No statistically significant differences were found between ratings of samples 2 – 8.

Table 4-2 gives an overview of the category formed for odour presentation times with respect to the RMS and the corresponding TI- and line-scale sweetness ratings. RMS corrections resulted in odour presentation times ranging from -6 sec before to + 6 sec after the moment of swallowing. For both ratings, sweetness intensity differed significantly between odour presentation times (TI [$F(16, 618) = 2.08$; $p < 0.01$]; line-scale [$F(16, 618) = 2.24$; $p < 0.005$]). The variations in sweetness intensity at different odour presentation times before, during and after the RMS are shown in Figure 4-4 and Figure 4-5. Figure 4-4 shows the total variation as observed using time-intensity ratings during stimuli delivery, while Figure 4-5 shows the relative variation for both rating methods in normalized form, equaling average ratings for the blank

Table 4-2: Overview of categories of odour presentation times with respect to the RMS (n=50). The RMS is set to be 0s. The category width is shown in columns 2 and 3. ANOVA results for both rating methods are shown in columns 5 and 7. Mean values associated with the same letter are not significantly different ($p < 0.05$).

Category	*Category width*		*ANOVA results*			
			Time-intensity		*Line-scale*	
	$t_{odour\_start}[s]$	$t_{odour\_end}[s]$	*AUC*	*significance*	*intensity ratings*	*significance*
1	-	-	1107.06	de	54.88	b
2	(-6.0)	(-3.8)	1346.26	abc	63.94	ab
3	(-3.8)	(-3.2)	1279.40	abcd	64.82	ab
4	(-3.2)	(-2.5)	1244.60	bcde	67.66	ab
5	(-2.5)	(-1.7)	1497.40	a	85.54	a
6	(-1.6)	(-1.2)	1227.79	bcde	65.00	ab
7	(-1.2)	(-0.8)	1218.24	cde	62.52	b
8	(-0.8)	(-0.4)	1253.32	bcde	66.12	ab
9	(-0.4)	0.2	1185.26	cde	63.64	ab
10	0.2	0.7	1245.82	bcde	64.56	ab
11	0.7	1.2	1212.70	cde	67.44	ab
12	1.2	1.8	1300.23	abc	65.86	ab
13	1.8	2.6	1282.64	abcd	70.46	ab
14	2.6	3.6	1448.07	ab	63.38	ab
15	3.6	4.1	1199.29	cde	64.84	ab
16	4.1	5.4	1237.61	bcde	63.52	ab
17	5.4	6.9	1042.61	e	68.73	ab

odour presentation condition (stimuli 1) to 1. Odour-induced sweetness enhancement was observed for all categories, independent of the moment of odour presentation. However, the magnitude of sweetness enhancement differed considerably between

odour presentation time categories. Most sweetness enhancement was found for categories 5 ((-2.5s) – (-1.7 s)) and 14 (2.6 s – 3.6 s). Post-hoc analysis showed that stimuli in these categories were perceived significantly higher in sweetness than the reference (category 1) [category 5: ($p < 0.0001$) and category 14: ($p < 0.0001$), respectively]. These samples were also perceived sweeter than stimuli accompanied with odours presented in the period close

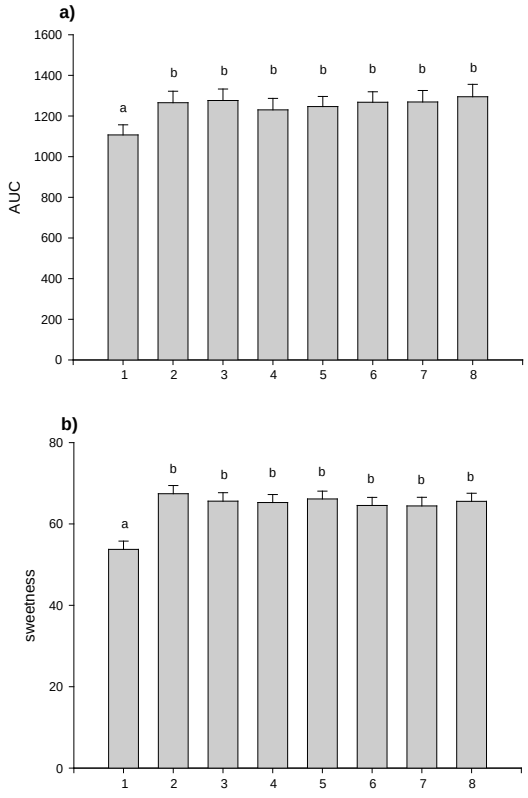

Figure 4-3: AUC converted time-intensity ratings and linescale ratings before RMS correction.

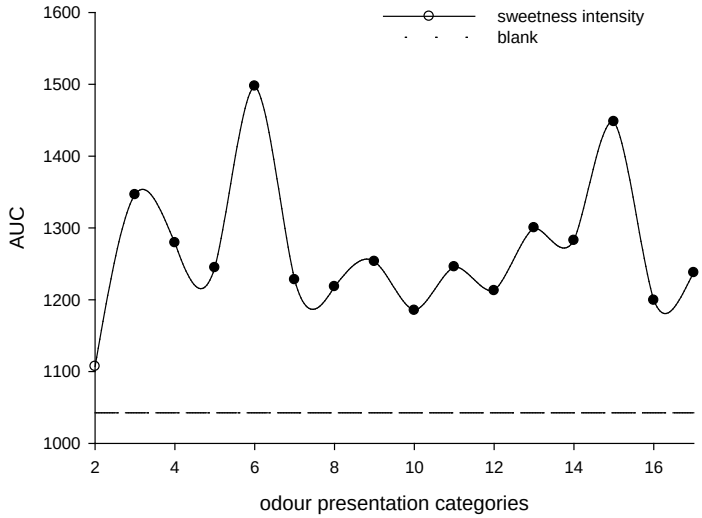

Figure 4-4: Changes in the magnitude of odour induced taste enhancement for all odour presentation time categories. The blank sample represents category 1 (non-odour condition)

to the RMS, represented by category 9 ((0.4 s) - (0.2 s)) [(Category 5: $p < 0.0005$ and ; Category 14: $p < 0.01$, respectively]). Intensity ratings for this time category were found to be lowest of all odour-present stimuli and closest to those generated for the blank odourless condition without additional ethylhexanoate stimulation, in absolute values as well as in post-hoc analysis, making it the one inducing the least sweetness enhancement. Sweetness ratings produced for odours presented between Categories 2 ((-6.0 s and) – (-3.8 s)) and 12 ((1.2 s) – (1.8 s)) were also found to be significantly different from ratings for the blank odourless condition stimuli [(sample 1) (category 2: $p < 0.005$ and ; category 14: $p < 0.05$, respectively]), but these ratings did not differ significantly from ratings for category 9, around the RMS. Intensity ratings for other odour timing categories showed variation in sweetness intensity, however they were neither significantly different from ratings for the blank odourless condition , nor from stimuli with odours presented around the RMS (category 9).

While post-hoc comparisons of line-scale ratings showed overall a significant difference in sweetness over the categories, post-hoc analysis revealed that the only odour time category for which intensity ratings were found to be significantly

different from those produced in category 1was category 5. All other samples were statistically found to be no different from the non-odour condition (Table 4-2).

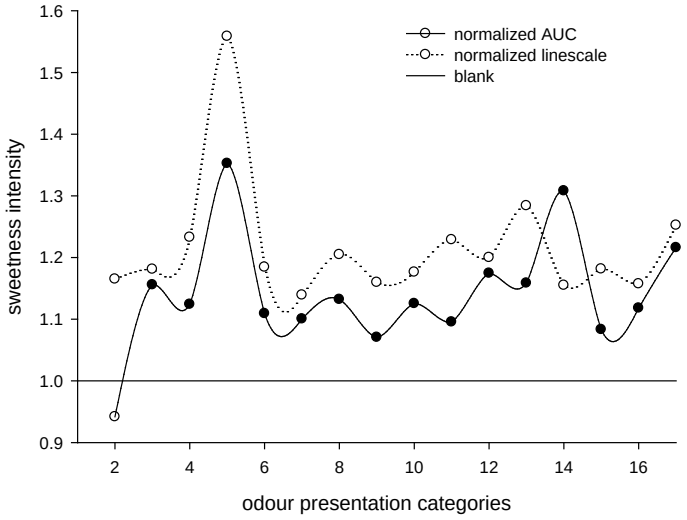

Figure 4-5: Changes in the magnitude of odour induced taste enhancement different odour presentation times. The blank sample represents category 1 (non-odour condition). Results are normalized by dividing these with the observed ratings for odourless stimuli category 1.

TI curve shapes in relation to odour presentation time

TI curves, averaged over subjects per condition, are shown for four different odour timing categories (Figure 4-6). Odour timing conditions shown in the graph are the no-odour condition (category 1), the odour timing conditions showing the highest sweetness enhancement (categories 5 and 14) and the condition in which odour was presented closest to the RMS (category 9). A steep increase in sweetness intensity can be observed for all TI-curves from 5 seconds up to 10 seconds after the start of oral stimulus delivery (apple juice). During this period, TI-curves for conditions with no odour, odour around the RMS and odour after RMS (categories 1, 9 and 14, respectively) are parallel. In contrast, the condition with odour presentation before the RMS (category 5) shows a steeper increase in sweetness intensity, resulting in much higher sweetness intensity ratings after 10 seconds than observed for the other categories. This result can be retrieved in the highest sweetness enhancement at T_{max} that was shown by category 5 at 14.8 seconds with an average rating of 61.8. This was also the highest sweetness intensity rating shown by all of the samples. Categories 1

and 14 reached T_{max} at the same time at 14.8 seconds, although the sweetness at T_{max} of category 14 was rated slightly higher than that of category 1. The maximum sweetness intensity was reached latest at 17.2 seconds for category 9. The average sweetness

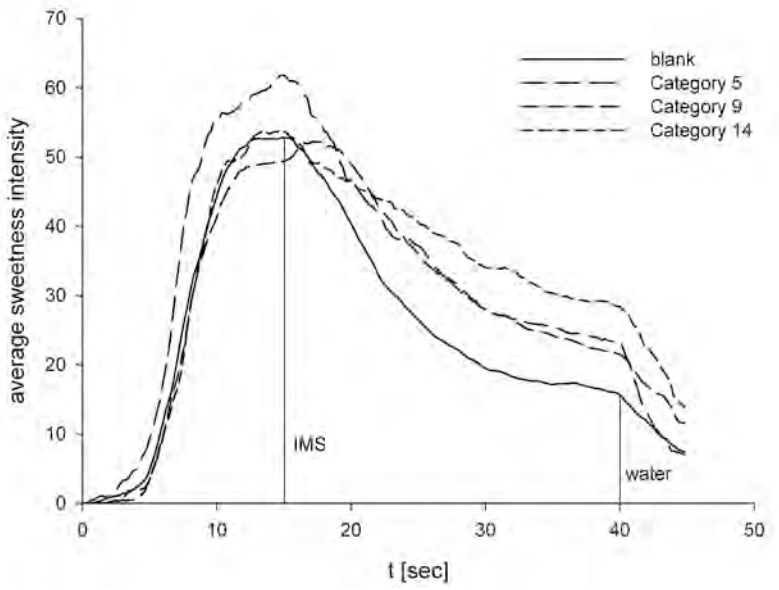

Figure 4-6: Time-intensity curves for categories 1, 5, 9 and 14, curves represent the averaged results of 19 subjects.

intensity rating at Tmax for this category was 52.3. Ratings at the IMS (15 seconds) were lowest for category 9 and highest for category 5. The slowest decrease in sweetness intensity after Tmax was shown by category 14. The decrease in sweetness intensity for categories 5 and 9 was distributing parallel. Category 1 showed the fastest decrease in sweetness intensity, resulting in the lowest average intensity score at 40 seconds, when rinsing water was presented to the subjects. All categories showed a drop in sweetness intensity after 40 seconds.

Discussion

Previously, we showed that an odorant contained in apple juice, i.e. ethylhexanoate, enhances the sweet taste of the juice [124]. During consumption, this odorant can only contribute to the perception of taste after swallowing the juice, which allows the

odorant to stimulate the olfactory epithelium after passing through the nasopharynx (i.e. retronasal odour stimulation). This dependency of odour presentation time on the act of swallowing may imply that the magnitude of odour-induced taste enhancement depends on the synchronisation of the moment of swallowing with the moment of odour presentation. In the present study, we investigated the role of the synchrony between the moment of swallowing an oral stimulus and the moment of presenting an odorant retronasally for its perceived taste. We did so by systematically varying the temporal asynchrony between the odour and the moment of swallowing. The results confirmed that ethylhexanoate enhances the perceived sweetness of apple juice, even under the relatively artificial stimulus presentation conditions employed in the present study, involving both oral and nasal stimulus presentation devices. This sweetness enhancement was observed for all odorant timing conditions, regardless whether the odorant presentation was advanced or delayed with respect to the moment that subjects were instructed to swallow, and did not differ between odour timing conditions.

After correcting the intended odour presentation times for deviations of the actual swallowing times from the instructed swallowing times, the magnitudes of sweetness enhancement showed a clear dependence of odour stimulus timing. We, therefore, conclude that the moment at which subjects swallow after receiving the swallow instruction varies considerably and to the extent that it conceals systematic effects of odour-swallow synchrony. We conclude that a correction for deviations from the instructed swallowing times is required.

In the present study, odour-induced sweetness enhancement was observed regardless of the rating method used (time-intensity ratings and line scale ratings, either corrected for real moment of swallow or not corrected). In contrast with previous studies in which similar results were obtained for sweetness ratings in the context of multiple attribute evaluations, subjects evaluated sweetness only in the present study. The omission of additional attributes in this study may have induced spurious stimulus effects due to “halo dumping”, i.e. the influencing of attribute responses by differences between stimuli in qualities that are not represented by the attributes used [50, 163]. However, the limitation to sweetness as the only product characteristic to be rated was provoked by the use of time-intensity scaling, which demands a continuous focus of the subject to a single attribute. Clark and Lawless [156] described

the effect of halo dumping on time-intensity scoring. They found that the sweetness of a flavoured beverage increased when sweetness was the only attribute offered compared to when flavour and sweetness intensity were both asked for. Hence, a possible contribution of halo dumping to the results of this study cannot be excluded. However, this does not refute the conclusion that the synchronisation between odour presentation and the moment of swallowing affects the perceived sweetness of the oral stimulus. If halo dumping had contributed to the observed differences between sweetness scores, it should have been because odour intensities varied between odour timing conditions. Our previous work showed that this would still have contributed to a sweetness enhancement, had multiple attributes been employed.

Time-intensity ratings were followed by line-scale ratings after stimulus presentation in this study. The differences between the results obtained with both rating methods indicate that, in order to gain insight in the mechanisms governing odour-induced taste enhancement, time-intensity ratings are most discriminative. In addition, time-intensity ratings provide a detailed insight into the temporal dynamics of odour-taste interactions in contrast with line-scale scoring. Both rating methods showed highest sweetness enhancement when the odour was presented prior to the moment of swallow (category 5, (-2.5 s) – (-1.7 s)). However, the time-intensity rating method proved to collect more detailed differences than line-scale scoring.

The main objective of this study was to determine whether the magnitude of aroma induced taste enhancement depends on synchronous odour delivery with the RMS. This study showed that an odour presentation asynchronous with the exact moment of swallowing contributes most to the taste enhancement in food: A delay or advancement of odour presentation with 2.5 s relative to the moment of swallow produced the greatest taste enhancement. Remarkably, the least taste enhancement was observed for odour delivery synchronous with the swallowing action (odour presentations between -0.4 s and 0.2 s from the moment of swallow). The notion that synchronous odour/swallowing events produce the least taste enhancement is further supported by a closer inspection of results for that timing category. Ratings obtained at the exact moment of swallow did on average not show sweetness enhancement at all (results not shown). That asynchronous odour stimulation with respect to the moment of swallowing produces most taste enhancement contradicts the results previously presented by Pfeiffer et al. [71]. In a study investigating the effects of

benzaldehyde on saccharin sweetness, they observed the highest taste enhancement when the odour was presented synchronous to the swallowing of the taste solution. However, the asynchrony between odour and taste stimulation was created by spitting out the stimulus followed by orthonasal sniffing, while in the synchronous condition the taste stimulus containing the odorant was swallowed, followed by retronasal aroma stimulation. For these conditions, not only the odour timing but also the odour presentation paths different, which may explain the higher taste enhancement observed for the synchronous condition. Additionally, the real-time delay between swallowing and odour presentation was not measured in the Pfeiffer study. It may be that the degree of asynchronous presentation was too big. Our results indicate that if the temporal delay between odour stimulation and swallowing exceeds 4 s, the odour-induced taste enhancement is reduced. This is in-line with the results obtained by Shams et al., in a study of sound-induced visual illusions. The probability of perceiving illusionary visual flashes decreased with increasing asynchrony between auditory and visual stimuli [96], although it has to be taken into account, that those results were obtained for two perceptual stimulations alone. The swallow in itself is a mechanical action and therefore an additional dimension. Mechanically, during swallowing the nasopharynx is closed off by elevation of the soft palate [164]. In this phase volatiles cannot enter the nasopharynx and therefore at this particular moment no odourous molecule triggers the involved olfactory receptors. In line with this, Buettner at al. showed that odorous volatiles deriving from food cannot be detected in-nose before or during swallowing, but only just after swallowing [90, 91, 165]. In contrast with realistic eating conditions, our experimental setup allowed odour presentation at synchronous conditions because odours were delivered into the nasopharynx by an olfactometer. As a result, stimulation of the olfactory receptors should have occurred for all odour presentation times. A possible explanation for the absence odour effects at the moment of swallow is that no olfactory information is processed by the brain at that moment. Since olfactory stimulation usually does not occur at the moment of swallow, olfactory processing would be futile then. Electrophysiological or hemodynamic studies of brain responses under similar stimulus synchrony conditions could add to a further understanding of this matter.

The shape of the TI – curves provides further insight in the temporal dependency of odour presentation with respect to the moment of swallow and its effect on taste perception. Figure 4-5 shows the shape of the four curves representing the lowest and

highest AUC values. From its shape it can clearly be seen that the reason for the highest measured sweetness intensity for category 5 is a steep increase of sweetness intensity correlating with an early aroma delivery. This leads to very high sweetness ratings at an early stage resulting in the highest T_{max} value. A lingering effect of the aroma in aftertaste conditions (15 s – 40 s) following swallowing of the taste stimuli prolongs this high sweetness intensity. Category 14 representing a late odour stimulation (table 2) shows the highest sweetness intensity during the aftertaste period, however due to the late aroma delivery sweetness ratings until aroma delivery remained on reference level. The non-odour condition showed lowest sweetness intensity, which can be ascribed to the lowest rated aftertaste period. The condition representing synchronicity between odour presentation and swallowing (category 9) showed the lowest initial sweetness intensity ratings. The curve than increases after swallowing showing impressively that the odour was not perceived before slightly after the moment of swallowing, encouraging the idea that during the moment of swallow afferent olfactory information is either not signalled to the brain or not further processed by the brain. This might also explain the slight drop in intensity just before swallowing for this particular condition, although we would expect this curve to develop parallel to the blank condition at this particular moment in time. That the curve for category 9 further develops almost exactly the way the curve for category 5 develops is another indication that purely binding of odour molecules to the receptors does not lead to activation of related brain areas at the exact moment of swallow and that therefore the effectiveness of odour stimulation is lower, as part of the receptor activation capacity is probably swallowed up.

Acknowledgements

This study was approved by the medical ethical committee of Wageningen University, The Netherlands (Protocol ID NL25364.081.08).

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Chapter 5

Temperature effects on taste and the magnitude of odour induced taste enhancement

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Abstract

Health concerns related to sodium and sugar consumption have led to extensive research on products reduced in sodium and sugar. One of the main consequences of decreasing salt and sugar content is that the sensory characteristics are affected and consumer acceptance is decreased. Odour induced taste enhancement, based on multimodal perception, could contribute to the development of healthy, still tasty products. The aim of this study is to investigate the effect of temperature on taste and the magnitude of odour induced taste enhancement. By combining gustometry, olfactometry and a temperature control device, a fully controlled stimulus delivery was achieved. Subjects received 2.5 ml of either apple flavoured tea or a model broth at 7˚C, 25˚C, 37˚C and 50˚C. Randomly a pulse of ethylhexanoate or sotolon, respectively, was presented to the subjects. Two sensory studies were performed in either fully randomised temperature conditions or random block-wise temperature conditions.

Results showed that in fully randomised conditions no effects of aroma or temperature on either sweet or salty taste were observed. In contrast to previous research the aromas had neither effect on the taste of the products nor on the perceived aroma intensity. The fact that subjects had difficulties determining differences between the two sucrose or NaCl concentrations at randomised temperature conditions, indicated that randomized stimulus presentation makes subjects less sensitive to NaCl or sucrose concentration differences and the task becomes too complex for an untrained panel.

Presentation in block-wise temperature conditions indicated trends of aroma effects, however results have found to be not significant. It is likely that subjects are less distracted by temperature differences presented in a block-wise design rather than in a randomised design.

Introduction

Food is consumed at different serving temperatures. For some foods this temperature is mostly fixed such as salad or ice cream. However, for some products the consumption temperature varies, e.g. tea. Temperature is known to affect the sensory quality of food. It has been shown that the serving temperature of wine changes wine perception. The typical cold serving temperature of white wine will suppress sweetness while enhancing acidity. Serving red wine close to room temperature is thought to enhance its aroma while diminishing perceived bitterness and astringency typically associated with red wine [166].

It has been shown, that an increase of temperature will result in an increase of released volatile components[167-169]. It is therefore expected that aroma compounds become more volatile, thus enter the nasopharynx easier. Not only the temperature, but also food composition, structure, viscosity of the different phases and molecular interactions (with proteins, lipids, carbohydrates) determine whether an aroma will be released into the vapour phase [170]. The distribution of an aroma compound within food depends on its affinity towards these different phases and its ability to release into the vapour phase. At lower temperatures, the concentration of volatiles might be below thresholds levels and thus they will not be perceived. If food is heated, the concentration of volatile compounds in the headspace will increase and released aroma molecules can be perceived. Thus when food is heated the concentration of volatiles in the nasopharynx increases as does the rate of aroma release and the perceived aroma intensity becomes higher [171]. Voirol and Daget [172] observed that the intensity of beef odour increased with increasing serving temperature.

In taste perception taste receptor cells react differently at changing temperatures. Talavera *et al.* [173] showed that sweet taste receptor cells (TRPM5) are highly temperature sensitive, with taste responses strongly enhanced by increasing temperature. It was demonstrated, that TRPM5 currents arise steeply at temperatures between 15°C and 35 °C[173]. This might explain the effect observed by Bartoshuk *et al*. [174], who observed, that sweetness intensity is higher at increased temperatures. Cruz *et al*. [175] observed heat activation of TRPM5 receptor cells without the presence of tastants, a phenomenon called “thermal taste”. Green and George [176], found that not all individuals experience thermal taste. In their study about two out of three subjects did experience thermal taste of at least one taste modality.

It is important to note that the effects of temperature cannot be generalized for all taste modalities because they are restricted to a certain component. In NaCl perception the dependence of temperature on saltiness perception is more complex. The amiloride sensitive epithelial Na^+ channel (ENaC) and a taste variant of the vanilloid receptor TRPV1t are salt receptors on the tongue [177]. The Chorda tympani is a part of the facial nerve system and transmits taste signals to the brain. An increased Chorda tympani response indicates increased sensitivity around 32°C for both Sucrose as NaCl receptors as described by Talavera et al. and shown in Figure 5-1. Work done by Roset *et al.* [178] supports this observation by showing that soups reduced in Na^+ content were perceived more salty at room temperature (22°C) than at serving temperature (65°C).

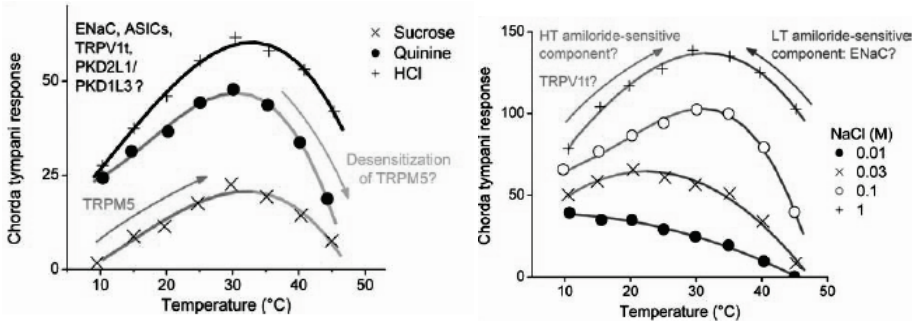

Figure 5-1: Effect of temperature on the role of several ion channels in the gustatory nerve response [177]

Temperature perception can be seen as trigeminal stimulation. At high or low temperatures trigeminal factors may play a role in taste perception. The effects of trigeminal stimulation are very much seized by inter-individual differences; one subject might be stimulated at much lower temperatures than others. To investigate the effect of trigeminal stimulation on taste perception often low concentrations of CO_2 or other pungent compounds such as capsaicin are used. Cowart [179] showed that the addition of CO_2 to sucrose and NaCl solutions decreased sweet and salty taste perception, however, it did not affect overall taste perception. This might indicate that trigeminal stimulation at very low or high temperature will increase trigeminal somatosensory neuron signalling, and thus influence sweet and salt perception. This is also concurrent with observations in previous research [173].

Aside of the chemical and physical effects of temperature on the samples, also psychological factors influence temperature taste perception. In order for the odour induced taste enhancement to have effect, the sample still has to be pleasant [62]. This means that the subjects cannot be presented with samples that are too hot or that have extreme taste. The serving temperature has to be congruent to normal serving temperatures in order to have subject acceptability [180].

We can conclude that literature has shown that odour perception, as well as taste perception are temperature dependent. We therefore hypothesize that odour effects on taste perception will also be influenced by temperature differences. In this study we investigated the influence of four different temperatures on taste perception and the magnitude of odour induced taste enhancement. The odour- taste pairs in this study have previously been proven to be effective with odours enhancing taste perception [181, 182].

Materials and Methods

Materials

Study 1: Effect of temperature on taste and odour induced taste enhancement in apple flavoured tea in fully randomized conditions

The tea extract was provided by Unilever (Dark tea base, Unilever, Vlaardingen, The Netherlands). Commercial available sucrose (Euroshopper, AH, Zaandam, The Netherlands) and citric acid were bought (Merck KGaA, Darmstadt, Germany, lot# 1.00244.0500). The basic apple aroma used to flavour the tea was customized for this study and did not contain ethylhexanoate (IFF, Hilversum, The Netherlands).

Table 5-1: Composition of apple flavoured tea at two different sucrose concentrations

	100% sucrose	**70% sucrose**
Tea extract	0.7g/L	0.7g/L
Sucrose	80g/L	56g/L
Citric acid	1.1g/L	1.1g/L
Apple aroma	35 ppm	35ppm

To test the effect of temperature on odour induced sweet taste enhancement a black tea base with apple aroma was used (Table 5-1). This was chosen because Dutch consumers are familiar with black tea types at different temperature ranges (iced tea to hot tea) and with different flavours. The apple aroma was chosen since previous work showed sweet taste enhancement for this specific odour taste pair [182].

In this studies apple juice was used as corresponding beverage. As apple juice usually is consumed at cold temperatures, this is incongruent with warm temperatures; therefore a tea with apple aroma was used. For odour induced taste enhancement to occur it is important that the apple flavour is perceived by the subject. Like this they can relate product and odour, however the odour should not overpower the tea flavor. A concentration of 35 ppm apple aroma was found to be sufficient to induce a clearly perceivable apple aroma, for both hot and cold serving temperatures. As observed in previous the concentration of sucrose seem to influence the magnitude of odour induced taste enhancement (data not shown in this thesis). Therefore it was chosen to test possible effects for a high (80g/L) and a 30% reduced (56 g/L) sucrose concentration (Table 5-1). The sucrose concentrations used will not significantly increase viscosity of the tea, thus is it not expected that the viscosity will influence taste perception.

All products were prepared one day in advance to the experiment.

Study 2: Effect of temperature on taste and odour induced taste enhancement in a model broth in fully randomized conditions

NaCl (AH, Zaandam, The Netherlands) and sucrose (Euroshopper, Zaandam, The Netherlands) were bought at the local supermarket. Succicnic acid (lot#S3674-250G) and mono sodium glutamate (lot#BCBC5574) were obtained from Sigma-Aldrich GmbH (Steinheim Germany). Ribotide was kindly provided by Unilever (Unilever, Vlaardingen, the Netherlands, lot#3935017, E631/E627, 50:50 ratio).

To test the effect on salt taste enhancement a model broth was designed (Table 5-2). No added fats or viscosifiers were used. It was chosen to not add any fats or viscosifiers at this stage, since this might interfere with the volatility of the odorous compounds. Additionally fat might cause gustometer pump failures as for possible clotting at lower temperatures.

Table 5-2: Composition of a model broth at two different NaCl concentrations

	100% NaCl	70% NaCl
NaCl	8.00 g/L	5.60 g/L
Sucrose	4.00 g/L	4.00 g/L
Succicnic acid	0.14 g/L	0.14 g/L
Mono sodium glutamate (MSG)	0.60 g/L	0.60 g/L
Ribotide	0.20 g/L	0.20 g/L

A broth model was chosen because of its use in both, cold and hot application in Dutch cuisine (hot in bouillons and cold in gazpacho, vichyssoise). Similar to study 1 it was choosen to use two NaCl concentrations (Table 5-2). The NaCl concentrations used are based on commercially used NaCl concentrations in broths of 3.1g sodium/L (so 8g NaCl/ L).

All products were prepared one day in advance to the experiment.

Study 3: Effect of temperature on taste and odour induced taste enhancement in apple flavoured tea in fully block-wise presented temperature conditions

All materials used in this study were the same as in study 1.

Subjects

In study 1 and 2 the same twenty-five subjects (5 ♂ and 20 ♀; age 20- 64) completed the study. All subjects were tested for normal sense of taste and smell prior to the experiment. All subjects have European background and were expected to be familiar with the combination of sweetness and apple aroma and sotolon as part of a broth flavour. In between both studies at least one week time was imposed. Most subjects had experience on gusto- and olfactometer taste-delivery and were used to test large quantities of samples, however, the panel is not considered to be trained on specific modalities tested in these studies.

For study 3 study subjects from the previous panel were recruited. It consisted out of 18 subjects (5♂ and 13 ♀; age 20 – 64). For the study the same subjects were used so

data could be compared. Not all subjects were able to participate, Therefore triplicates instead of duplicates insured that the statistical power was maintained.

All subjects gave written informed consent prior to the experiment.

Methods

Temperature Control

In order to control the temperature of the stimuli three cryostat bath were used to either cool or warm the liquids. They were kept at temperatures of 2.5˚C, 40˚C and 54˚C to achieve serving temperatures of 7˚C, 25˚C, 37˚C and 50˚C. This temperature range was chosen to represent normal serving temperatures. 7˚C is the common serving temperature of cold drinks or foods, like ice tea or cazpacho in this case. 25˚C represents room temperature and at this temperature previous experiments have been performed, making it possible to compare data. 37˚C was selected since, as described, receptor cells are expected to be most sensitive. 50˚C is the common serving temperature of hot foods or drinks, in this case a hot tea or broth.

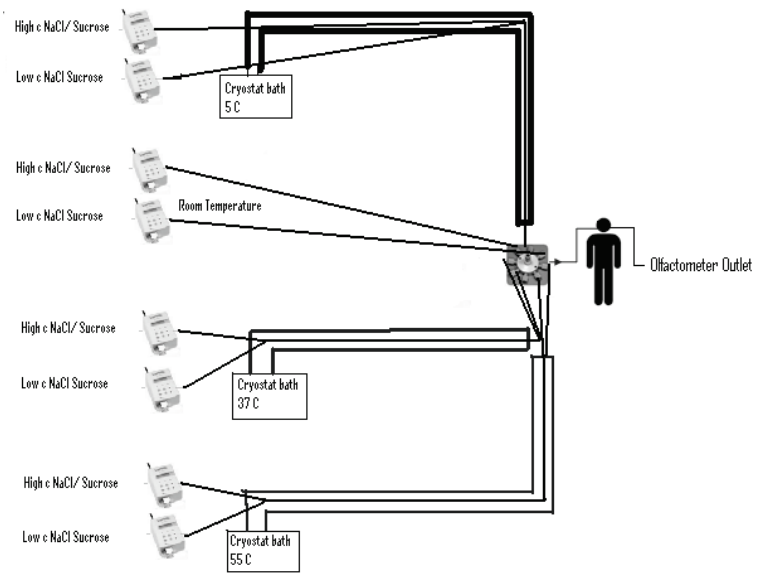

Figure 5-2: Temperature controlling using circulation of cryostat baths.

The water baths have a circulating system which was extended to the manifold. The gustometer tubing was put through the circulation tubing of the water bath Figure 5-2. The length of gustometer tubing that is put trough the circulation was calculated on the basis of the amount of sample that was presented to the subject for each stimulus. In this way the exact amount of sample that is presented was temperature controlled till the manifold. The gustometer tubing was further insulated to ensure optimal temperature use in the circulating system.

The temperature was controlled randomly at the gustometer outlet via a precision fine wire thermocouple probe (Omega engineering K-type, diameter 0.005, length 36", Teflon insulated), attached to an APPA 51 control unit (HTP, Valkenswaard, The Netherlands). The thermometers measure frequency is 2.5 times per second, thus is thought to be sufficient to represent the actual temperature the subjects received.

Figure 5-3 shows the stability with which it was achieved to present the different temperature conditions. The graph shows that the hot samples reach a temperature of 51.2˚C on average, with a maximum temperature difference of 0.5˚C. The body temperature sample is measured at 36.5˚C on average and also has a maximum temperature difference of 0.5˚C. The room temperature sample is on average measured at 25.2˚C, which is quite high for room temperatures; however, this occurs because of the high temperatures in the olfactometer room. The cold sample is on averaged at 7.1˚C with a maximum temperature difference of 0.7˚C. Thus, in randomised temperature conditions the maximum temperature difference over all temperatures is 0.7˚C.

Because room temperature in the olfactometer room increases significantly during the day (from ±20˚C in the morning to ±29˚C at the end of the afternoon), it was tested what the effect of this is on the room temperature sample during the day. The average temperature of the room temperature samples in the morning was 25.7˚C and after the last subject 28.5˚C. This is an average difference of 2.8˚C which stays within acceptable temperature differences for this study.

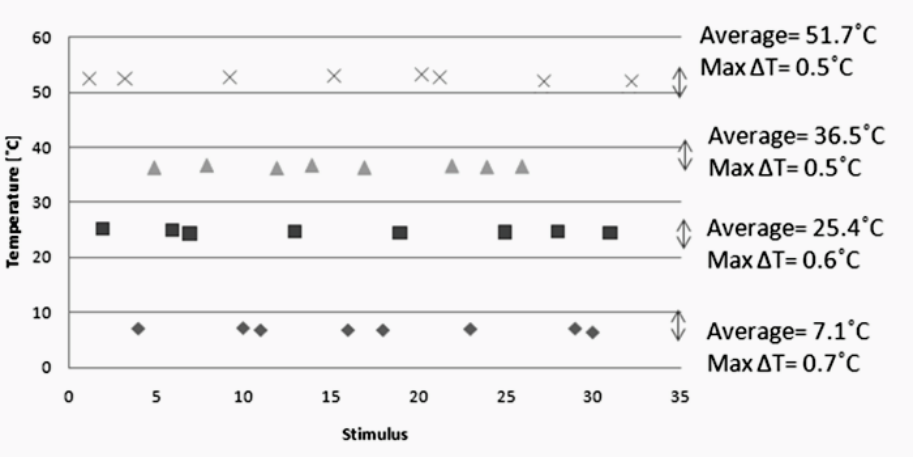

Figure 5-3: Temperature stability using circulation of cryostat baths

Olfactometer settings

Orthonasal aroma stimulation was used in all studies . Stimuli were presented via an olfactometer. In this manner the concentration and timing of the volatile compounds would stay steady and accurately controlled over the different temperatures in which the samples were presented. A commercial oxygen mask used in medicine to deliver oxygen to patients was used for aroma delivery to the subjects. Subjects put the nose piece into the nostrils with opening beyond the nasal valve, pointing toward the olfactory cleft.

Ethylhexanoate or sotolon were presented with the olfactometer in a propylene glycol solution (10% ethylhexanoate or sotolon in propylene glycol). Concentrations of 10% were found to be sufficient in order to deliver clearly perceivable odour pulses but not overpower the product aroma.

Flow rates of 8 L/min (4.1 L/min odour flow and 4.2 L/min air flow in the tea study and 2.03 L/min odour flow and 6.03 L/min airflow in the broth study) were applied in studies 1 and 2 and a flow rate of 4L/min (2.15 L/min odour flow and 2.03 L/min airflow) in the study 3. The air was heated to 42˚C allowing a temperature of 37.5°C when entering the nostrils and was humidified to 80% relative humidity to forestall drying out of the nasal mucosa [93].

According to previous work in our group the aroma should be presented approximately 2.5 seconds before swallowing to achieve maximum taste enhancement induced by the odour. This was achieved by presenting the odour 1.5 seconds before the instruction to swallow was given taking an average delay of 1 second to perform the task into account [183]. The odour was presented before swallowing to assure that the odour lingers in the olfactory cleft while scoring the sample. The stimulus pulse time was 100 ms in study 1 and 500 ms in study 2. 500 ms provided ethylhexanoate induced sweetness enhancement in an apple juice system [183]. A blank sample with no odour presentation was imposed at each temperature condition. The design allowed testing for the effect of temperature on the taste perception and the effect of temperature on the magnitude of odour induced taste enhancement.

Gustometer settings

The gustometer was used to accurately control delivery time and concentration of the liquids. A temperature controlling device for the gustometer was developed to accurately control the temperature of the delivered liquids (Figure 5-2). Subjects kept a mouthpiece between their central incisors and either 2.5 ml apple flavoured tea or broth was presented with the gustometer at a flow-rate of 30ml/min for 5 s. The study design as programmed via the gustometer control software is shown in Figure 5-4.

Sensory study

Studies 1 and 2: Randomized sample temperatures

Each session consisted of two sets of 16 sstimuli (four temperatures, two sucrose/NaCl concentrations, two aroma conditions). The stimuli were presented in fully randomised order. The two sets were separated by a five minute break. In between each sample 15 seconds rest was imposed.

At the beginning of each session subjects received two additional warm-up stimuli to adjust to the product, different temperatures and to remove excess saliva from the mouth. As shown in Figure 5-4, the subjects first were made conscious that the test would start by a beep signal and attention sign on the computer screen. Then this was followed by 2.5 ml tea of broth delivered by the gustometer (5 second pulse, flow 30ml/min) at 7˚C, 20˚C, 37˚C or 50˚C. 1.5 sec before the subject would see the swallow

sign the odour was presented by the olfactometer into the nasal cavity. After swallowing the subjects would rate intensity of the samples. After scoring the subjects received a medicine cup with (tap) water to rinse their palate. In the tea study the subjects received 4 ml and in the broth study 10 ml since the broth is considerably more lingering. The temperature of the rinsing water was at room temperature so subjects were able to neutralize the temperature differences in their mouth in between each sample.

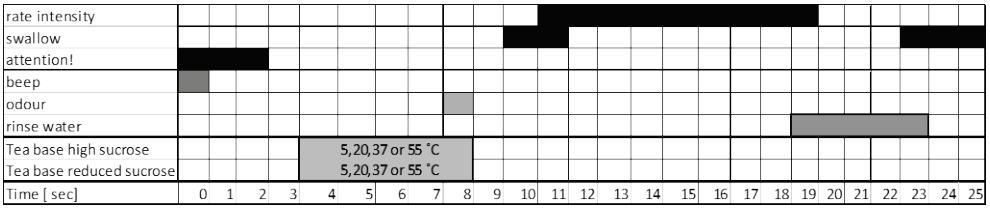

Figure 5-4: Stimulus events as occurring in time

Aside of the sweetness and saltiness also three other intensity questions were presented to the subjects to avoid halo dumping. These additional questions were selected based on their relevance to describe the apple flavoured tea or broth, respectively. Subjects scored bitterness, sourness and aroma intensity additionally to sweetness for the tea. The model broth was described using savouriness, saltiness, sourness and aroma intensity. Aside of avoiding halo dumping these questions provide additional information for the data interpretation as well.

Study 3: Block –wise sample temperatures

In this study 2.5 ml apple flavoured tea was presented with the gustometer (5 second pulse, flow 30ml/min). As mentioned before the stimuli were at 7°C, 25°C, 37°C and 50°C, however, the product was not presented completely randomised, but in a random block wise method. Thus all the different temperatures were presented in one block, but the different blocks were presented in random order. This would prevent the subjects from being distracted by the temperature, and to focus on the effect of the aroma. This resulted in a one session study with four blocks of 6 samples. Each block was separated by a five minute break. In between each sample 15 seconds resting time was imposed.

The stimuli presentation was kept equal to the previous study except for the stimulus pulse time which is increased from 100ms to 500ms, since this provided good results in previous [182]

Data Collection and Analysis

In all conditions data was collected using an automated scoring system (TIFN Score, Version 1.1, DNSmultimediafactory, Hamburg, Germany). Subjects scored the four attributes on a line scale rated 1-100, anchored "not sweet" (0) to "very sweet" (100) by moving a cursor on a vertical rating-bar on a computer screen. Subjects were asked to rate intensities via a computer screen.

Intensity ratings of all the four questions were averaged over the replicates (two or three replicates) and subjected to repeated measures ANOVA with sugar and NaCl concentration (2 levels), aroma (2 levels) and the four different temperatures (4 levels). All tests were performed at $\alpha=0.05$ and the statistical programs SenpaQ (QiStatistics, UK, 2008) and SPSS (Version 17, IBM, Chicago) were used for all statistical analyses. Pre-processing of the data was performed using Microsoft Excel (version 2010, Redmont, U.S.A).

Results

Study 1: Effects of temperature on taste perception and the magnitude of odour induced taste enhancement in apple flavoured tea as investigated by randomized temperature variation

As shown in Figure 5-5 at high sucrose concentrations (80g/L) no overall effect of the aroma on sweetness was found. No effect of aroma or temperature was observed for the tea low in sucrose concentration (56g/L).

This is peculiar since previous work reported an enhancing effect of the aroma at least at room temperature. An explanation for the lack of effect could be that the stimulus pulse of ethylhexanoate (100ms) was not long enough to have a clear enhancing effect. The short stimulus pulse was caused due to miscommunication within the research team. Because of this short stimulus presentation maybe not enough odour reached the subjects olfactory receptors to have an effect. To investigate whether an

influence of the stimulus presentation time could be the cause of the differences the odour presentation time was prolonged to 500 ms in study 3. However, as no effect was found in study 2, it is unlikely that the odour presentation time is the only factor to have influenced the results.

Table 5-3: Temperature and aroma conditions for sample groups (high and low sucrose)

Sample	T [°C]	Aroma
1	7	-
2	7	-
3	20	+
4	20	+
5	37	-
6	37	-
7	50	+
8	50	+

Talavera *et al.* [177] demonstrated an effect of temperature on the sweetness intensity. Therefore at least in the blanc (odourless) condition an overall temperature effect was expected. It could be that, in our case, the effect of temperature was not clear enough for the subjects, and large inter-individual differences level out possible effects.

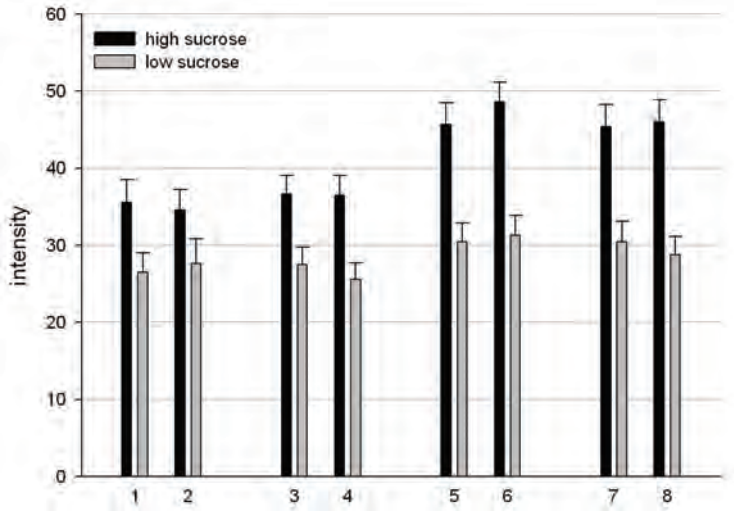

Figure 5-5: Sweetness ratings at different temperatures with and without ethylhexanoate in high [80g/L] and low [56g/L] sucrose tea

Post-hoc analysis showed an interaction between assessor and the temperature ($p<0.001$; $F=2.40$) and sucrose concentration ($p<0.001$; $F=3.06$). This means that it is dependent on the assessor whether there is a temperature or sucrose concentration effect. It cannot be excluded, that large inter-individual differences within the panel level out possible effects. This can be due to the inexperience of the panel as they are considered to be untrained. However in previous experiments by the authors similar untrained panels were used to evaluate the samples and enhancing effects of ethylhexanoate on sweetness have always been found. It is more likely that subjects are not used to rating the effects of temperature differences. Instead of just changing the perception of the taste modality, it might be that a change in the complete perception of products occurs, making ratings of sweetness a too complex task. This is supported by the fact that post-hoc analysis revealed almost always an assessor effect, which indicates large inter-individual differences. This could indicate that subjects had trouble indicating differences within the samples. The indecisiveness was also expressed by subjects themselves in personal communication during the experiments.

The importance of inter-individual differences are strengthened by the fact that trigeminal stimulation is very much seized by this, some subjects might be stimulated at much lower temperatures than others. Green and George [176] also found that not all people experience thermal taste, where about two out of every three subjects experience at least one taste modality. Sweetness is the most common thermal taste and saltiness the least common. Furthermore, individuals who experience thermal taste, give significantly higher ratings to chemical stimuli, often by a factor >2:1 to people that do not experience thermal taste.

Another explanation could be that subjects became distracted by the different temperatures they received. Probably the questionnaire did not offer them the possibility to express temperature differences and this led to distraction which as a consequence made subjects oblivious to answer the questions correctly. Because subjects did not get the opportunity to express their findings of temperature it is possible that halo dumping occurred, and subjects “dumped” these temperature expressions on one of the other questions an effect described by Clark and Lawless [156] or vd Klaauw and Frank [184].

Figure 5-6 shows that ethylhexanoate has no effect on the bitterness, sourness and aroma intensity at high sucrose concentration. As aroma intensity did not change with aroma presentation this could be an additional explanation for the lack of aroma effects on sweetness. One would expect that an increase in aroma intensity would be resulting in aroma induced taste enhancement. Hence, if the aroma intensity does not change also the sweetness cannot change.

At 37˚C and 50 ˚C the intensity of the aroma was found to be higher than at 7˚C and 20˚C ($p<0.05$; $F=3.67$). At 20˚C and 50˚C a trend in increased bitterness was observed, with bitterness increasing whenever the aroma was delivered. It seems that the addition of the aroma increased either bitter notes of the tea, or that the bitterness reflects the flowery/synthetic notes which are notorious off notes of ethylhexanoate as mentioned by some of the subjects and described in previous work by the authors[185]. Not being able to express such off-notes in the given context might influence assessors to express their opinion as bitterness, being the closest to undesired effectssuch as synthetic or flowery.

As shown in Figure 5-7 for the low concentration sucrose tea (56g/L) there is no effect of ethylhexanoate or temperature on the bitterness, sourness or the intensity of the aroma.

When comparing the high and low sucrose concentration tea, a significant decrease of aroma intensity was found in the low concentration sucrose tea ($p<0.001$; $F=31.80$). This shows that a decrease of sucrose in the product led to a decrease in aroma intensity. This corresponds with findings described in literature, where aside of the fact that odour can enhance taste also taste modalities enhance odour perception, thus in this case diminish odour perception [69, 70].

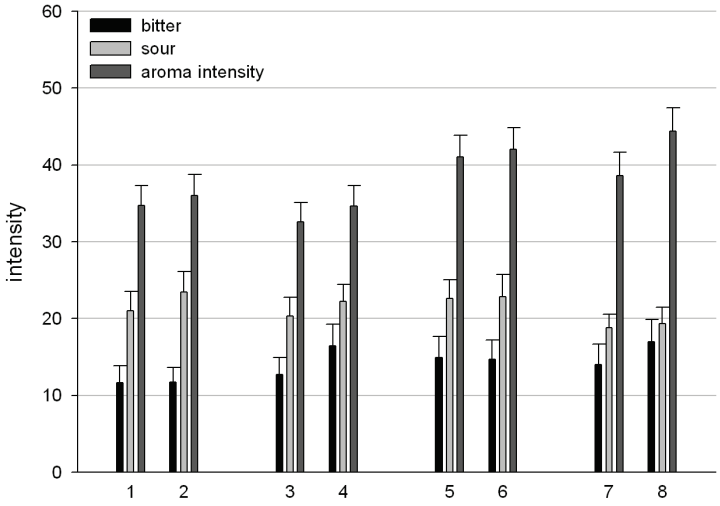

Figure 5-6: Bitterness, sourness and aroma intensity at different temperatures, with and without ethylhexanoate in high concentration sucrose tea [80g/L]

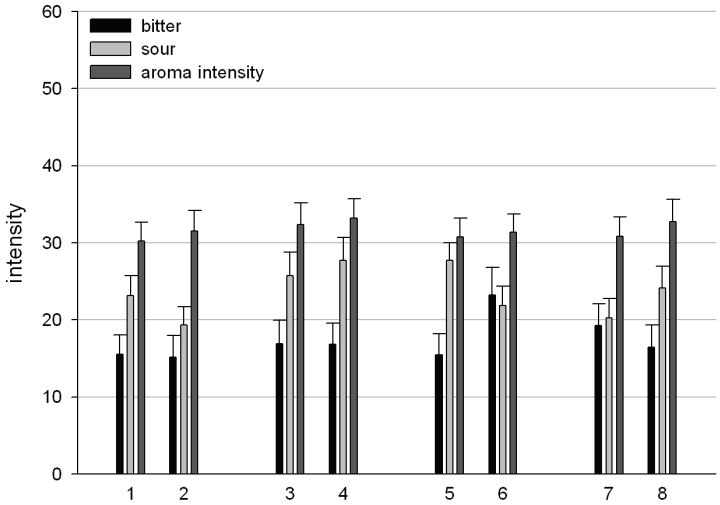

Figure 5-7: Bitterness, sourness and aroma intensity at different temperatures, with and without ethylhexanoate in low concentration sucrose tea

Study 2: Effects of temperature on taste perception and the magnitude of odour induced taste enhancement in a model broth as investigated by randomized temperature variation

As can be seen in Figure 5-8, no effect of aroma or temperature on perceived saltiness neither at high nor at low concentrations of NaCl was observed. Although not significant overall, at 7˚C an effect of the aroma on saltiness was observed. This effect cannot be explained by any other factor than the aroma effect. It has been described in literature that at a lower concentration of NaCl the effects of the aroma is more prominent [174, 186, 187]. However, this is not reflected in this work where only at high concentration of NaCl at 7˚C an aroma effect was seen. The found effect has therefore to be judged as random.

Table 5-4: Temperature and aroma conditions for sample groups (high and low NaCl)

Sample	T [°C]	NaCl [g/L]	Aroma
1	7	8,0	-
2	7	5,6	-
3	20	8,0	+
4	20	5,6	+
5	37	8,0	-
6	37	5,6	-
7	50	8,0	+
8	50	5,6	+

Since in this study the stimulus pulse time was increased from 100ms in the tea study to 500ms, the lack of effect of the aroma cannot be explained by the aroma stimulus pulse time. As for both systems, the ethylhexanoate/apple system as well as the sotolon/broth system, the enhancing effect has been proven [181, 182] a methodological mistake is most likely to be the cause of these results.

As mentioned before it is very likely that in both studies subjects were distracted by the different temperatures they receive, and because of that they are not focusing on the effect of the aroma or the differences in intensities over temperature. This is supported by the fact that the subjects had difficulties determining differences between the two concentrations of NaCl even though this is a 30% difference, which is

remarkable as normally this is a clearly perceivable difference. Althoug a trend can be seen no significant difference in saltiness was found between the concentrations. Our explanation is in line with a study of Mitchel [188] on the sensory sensitivity of subjects when they get distracted. Mitchel found that the subjects' sensitivity was lowered significantly even with small disturbances. Considerably more studies have been performed on the influence of noise and distraction on intellectual performance than

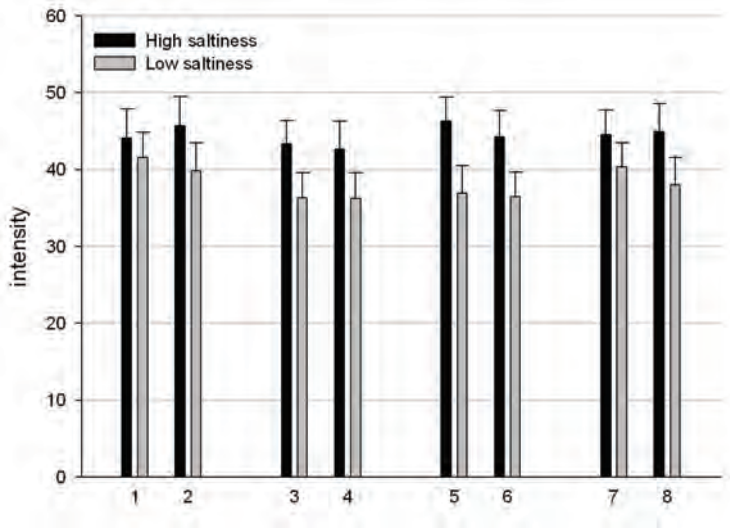

Figure 5-8: Saltiness score at different temperatures with and without Sotolon in high [8g/L] and low [5.6g/L] NaCl broth

on sensory perception. In a study by Simons et al. subjects were asked to count how many times basketball players were passing the ball, and subjects did not notice a monkey on the court. This shows that the subjects are then focussing on one thing and do not notice the other; the so called inattentional blindness [189]. Results are also comparable to studies where subjects were asked to identify separate components, in mixture products. The greater the number of components the subjects had to process, the greater the likelihood that identification of the separate components failed [190].

As well, it could be that subjects get a sensory overload at increased temperatures. When temperatures increase, excessive stimulation can occur and receptors can become overloaded. Because of the overload they might lose their ability to

discriminate. The receptor can no longer follow in synchrony (thus the effect of the odour on the taste perception), and the discontinuous stimuli are fused. This is also the case in the so called flicker fusion when we watch a movie [191]. Sensory overload is an overload of information for the brain to process as well [192]. Thus it could be that at higher temperatures the overload of sensory stimulation could limit the effect of odour induced taste enhancement. An explanation for the lack of temperature effects could be that the effects of temperature are levelled out by the overload that the brain has to process. Additionally, Green [193] found that the taste of food is probably not affected by thermal taste since the temperatures that provide thermal taste are rarely encountered during eating or drinking, and when they are, the chemical tastes of foods and beverages tend to mask thermal taste.

Another factor could have been the olfactometer research room, which was non-conditioned. Because the room could not be ventilated, or supplied with fresh air, the odours used were constantly prominently present in the room. Furthermore, there was a constant low concentration of sotolon present in the olfactometer flow. It could be that the subjects were already adapted to either the ethylhexanoate or the sotolon before the study started, and in that way the stimulus control suffered. Especially in the sotolon study this seems very likely since sotolon has a very low detection threshold, and the odour was extremely prominent in the room. This was also shown in the fact that one time the researchers, while preparing aroma stock solutions, became adapted within a couple of minutes to a degree that they were not able to smell the aroma even at extremely high concentrations. It indicates the potency of the aroma resulting in the subjects being adapted already before the study, and the aroma lost its potential. This corresponds with findings in literature where subjects lose their ability to discriminate effects when they are exposed to the stimulus longer exposure periods [194].

As presented in Figure:5-9 similar to the ethylhexanoate experiment, no effect of aroma or temperature on the savouriness or aroma intensity at a high concentration of NaCl was observed. Only at 7˚C the savouriness was scored lower when the aroma is presented. At 7˚C and 20˚C the addition of the aroma appears to enhance sourness, however, differences are too small to be significant.

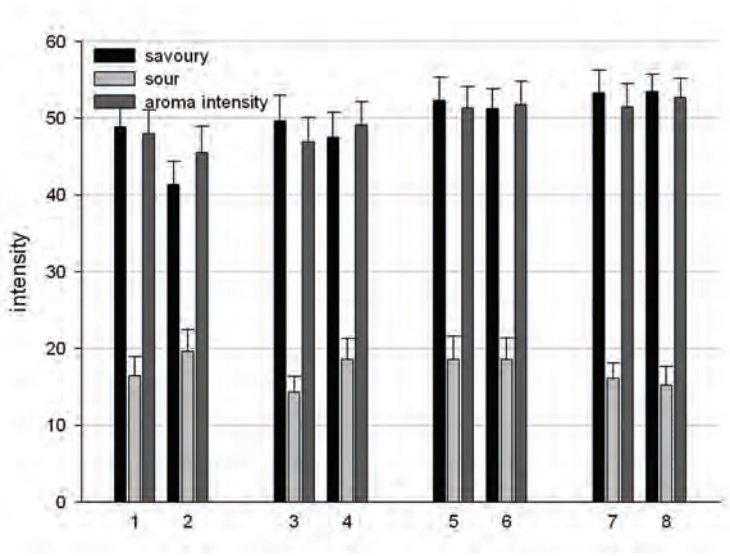

Figure:5-9: Savouriness, sourness and aroma intensity at different temperatures, with and without aroma in a high NaCl broth[8g/L]

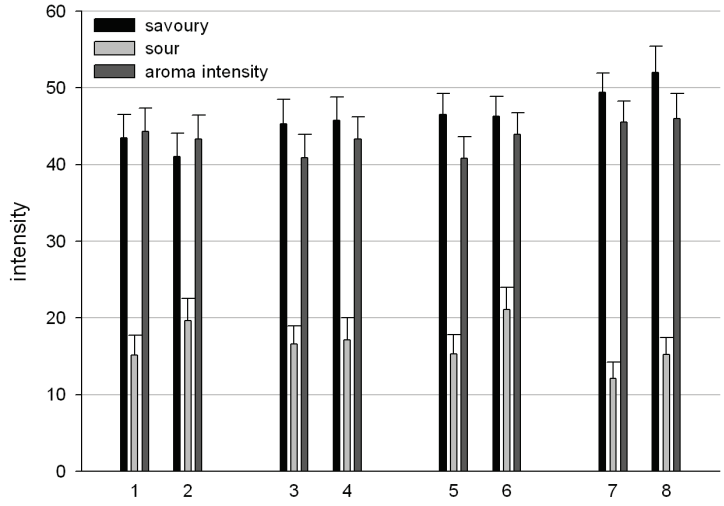

Figure 5-10: Savouriness, sourness and aroma intensity at different temperatures, with and without aroma in a reduced NaCl broth [5.6g/L]

As described in literature it is expected that for low concentrations NaCl, the effects of the aroma becomes more prominent [174, 186, 187], however, this does not show in this results, which indicate no effect of the aroma or temperature on the savouriness or aroma intensity (Figure 5-10). There does appear to be a slight effect of the aroma on the sourness at 7˚C, 37˚C and 50˚C. Since this trend was also visible at the high concentration of NaCl this initiates the idea that sotolon enhances sour notes in a broth. However, more research is necessary to describe overall changes in product quality induced by sotolon, as described for ethylhexanoate [185].

Study 3: Effects of temperature on taste perception in sweet formulations as investigated by block-wise temperature variation

As shown in Figure 5-11 there is no effect of aroma on the sweetness in this study, however, there are some temperature effects to be seen. Samples with and without aroma at 20˚C, 37˚C and 50˚C are all significantly more sweet than the samples at 7˚C (20˚C with aroma $p<0.05$; $F=2.40$) (20˚C without aroma $p<0.005$; $F=3.08$) (37˚C with aroma $p<0.05$; $F=2.40$) (37˚C without aroma $p<0.005$;$F=3.00$). (50˚C with aroma $p<0.01$; $F=2.60$). The increase in sweetness over temperature is supported by literature where the sensitivity of the receptor cells is highest around body temperature [177]. The highest sweetness at 50˚C could be explained by the higher volatility of the basic aroma compounds in the product (basic apple aroma), thus this might have an effect, in addition to the high temperature of the sample itself.

Table 5-5: Aroma, sucrose and temperature conditions for samples 1 – 8

Sample	T [°C]	Sucrose [g/L]	Aroma
1	7	80	-
2	7	56	-
3	20	80	+
4	20	56	+
5	37	80	-
6	37	56	-
7	50	80	+
8	50	56	+

A trend of an aroma effect on sweetness was observed, although not significant (Figure 5-12). At 7˚C, 20˚C and 50˚C the aroma does seem to enhance the sweetness of the apple flavoured tea.

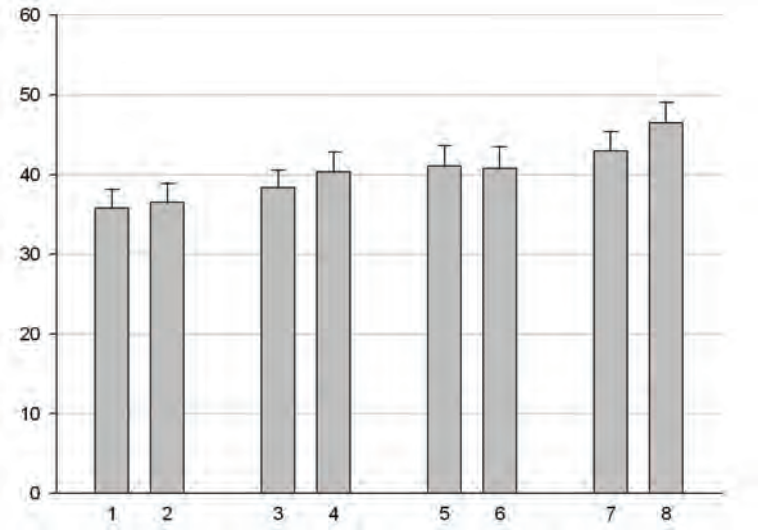

Figure 5-11: Sweetness scoring at different temperatures with and without ethylhexanoate

The fact that although the study design was changed, as in the other experiments no effect of the aroma on the sweetness was found, could be explained by the temperature still being dominant over the effect of aroma. In this study we do see an enhancing trend, thus you could argue that the block wise presentation did decrease the distracting effect of the aroma, however this seems to be not sufficient enough. It is expected that when each temperature is studied in different sessions the distraction will be brought to a minimum and effects will be seen.

As depicted in Figure 5-12 the aroma intensity is found to be higher for samples with aroma at 7˚C and 20˚C. At 37˚C and 50˚C no effect can be seen. Only at 20˚C the effect of the aroma on aroma intensity is significant ($p<0.05$; $F=2.08$).

As demonstrated in Figure 5-13 no effect of the aroma on bitterness and sourness was found. Some temperature effects were described such as an increase in bitterness at 20˚C compared to 7˚C with and without aroma (with aroma $p<0.001$; $F=3.50$, without aroma $p<0.005$; $F=2.80$).

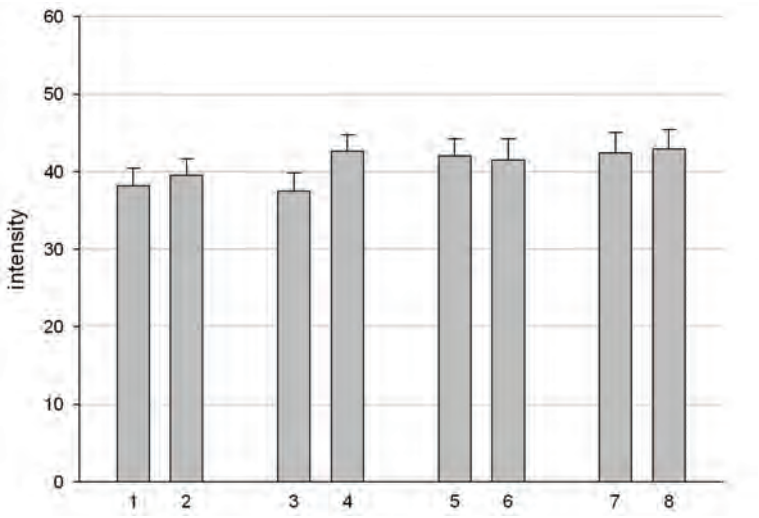

Figure 5-12: Aroma intensity at different temperatures with and without ethylhexanoate

For all temperatures a trend of increased bitterness over aroma presentation was observed, although not singificant. Since this effect was also observed in the randomised conditions, this initiates the idea that the addition of the aroma increases bitterness. However, the bitterness might reflect the flowery/synthetic off flavours that are identified in previous work with the use of ethylhexanoate [182].

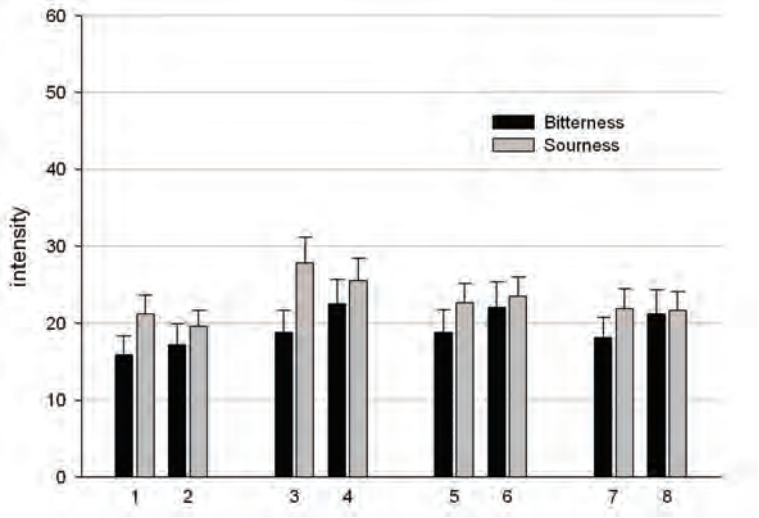

Figure 5-13: Bitterness and sourness scores at different temperatures with and without ethylhexanoate

Since the study in block-wise conditions was performed after the sotolon study, a persistent sotolon odour was present in the research room, even though there were three weeks in between the two studies. Because the subjects noticed the sotolon odour when they entered the research room it is possible that they were forming expectations regarding the experiment, which might have influenced the results. Furthermore, the sotolon present in the room might have had influence on the ethylhexanoate pulse presentation since it is possible that sotolon masks the stimulus, or changes the perception of the ethylhexanoate.

Discussion

As discussed in the previous chapters, the results seem uncertain. However, the reliability of the results can be seen as high, since in all three studies the results are comparable. However, the validity is low, since no effect of the aroma or temperature is found, although this effect was proven in previous work [177, 181, 182].

An explanation for the lack of temperature effects could be that the effects of temperature are levelled out by the overload that the brain has to process. However, since the effects of temperature on taste [172, 177, 195] and the effect of aroma on taste enhancement [181, 196], were proven for these specific systems before, it is likely that there are factors influencing the effect of temperature on taste and the magnitude of odour induced taste enhancement.

Randomised temperature presentation

As was discussed it seems that the subjects become distracted by temperature differences they receive, and therefore are focussing on this. This makes them unable to rate the samples properly, and score the questions accordingly.

The fact that subjects had difficulties determining the concentration differences of tastants in randomised temperature conditions, indicates that this method of presentation makes them less sensitive to rate tastant concentration differences. Previous work has shown that subjects are able to discriminate concentration differences as used in this study in controlled, non-randomised test set ups, but they seem to lose this ability when samples are presented in randomised temperature conditions.

Study environment

As described, the olfactometer research room was non-conditioned. Because the room could not be ventilated, or supplied with fresh air, the odours used were constantly prominent in the research room. Furthermore, there was a low concentration of the odour constantly present in the olfactometer air flow. It could be that the subjects were already adapted to the ethylhexanoate and sotolon before the study started, and in that way the stimulus control suffered from this.

Subjects and tasks

It is generally agreed upon that it is required to work with an untrained panel to study the effect of odour induced taste enhancement [181, 197, 198]. However, in an untrained panel the assessors performance is usually low, as in this study. Furthermore, subjects are not used to rating the effects of temperature differences. Since the temperature not necessarily changes one taste modality like for instance the sweetness, it still might change the complete perception of products and thus making ratings of sweetness and saltiness a maybe too complex task.

Conclusions

To summarize, the present work demonstrates that it is possible to accurately control the temperature of gustometer fluids with the use of the developed temperature controlling device.

The presented studies show that there is no overall effect of temperature or aroma on odour induced taste enhancement. There are slight effects of temperature observed, in cases where sweet taste increases over temperature at high sucrose concentrations. The concentration of NaCl and sucrose seems to influence the extent to which the temperature has effect.

The fact that subjects had difficulties determining differences between samples at randomised temperature conditions indicates that randomisation of temperatures delivered into the subjects mouth makes subjects less sensitive to NaCl or sucrose concentration differences.

Our results suggest that presentation in block-wise temperature conditions lead to more expressed aroma effects. It is likely that subjects are less distracted by

temperature differences presented in a block-wise design rather than in a randomised design.

Our experiments demonstrate no effect of stimulus delivery time on odour induced taste enhancement, although results seem doubtful.

Significant effects of the assessors were found, which demonstrates the individuality with which food is perceived. The results obtained in this study demonstrate the importance of study design in sensory research.

Since results from the presented study can be considered unclear, further research is needed to fully elaborate on the effects of temperature on taste and on the magnitude of odour induced taste enhancement. It is recommended to repeat the performed studies in a better controlled environment, so no adaptation of the subjects to the odour occurs. In order to study the effect of temperature on odour induced taste enhancement, it is suggested to execute the experiment with each temperature in a separate session to diminish the distracting effect of the different temperatures. Furthermore it is recommended to give the subjects the possibility to express temperature differences, when rating the samples to avoid dumping effects on other attributes.

More insight is needed in the effect of randomisation on the ability of subjects to discriminate taste differences at different temperature conditions. This direction seems worth pursuing, since its study set up appears to be more comparable to real life consumption settings.

Furthermore, an idea is to train subjects on temperature difference rating, without the addition of aroma. In this way they can get used to complex changes in taste over temperature, but they do not get trained on separating odour and taste perception, thus integration will still occur. This will most likely diminish the large inter-individual differences within the panel.

Acknowledgements

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Chapter 6

Temporal contrast of salt delivery in mouth increases salt perception

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Abstract

The impact of salt delivery in mouth on salt perception was investigated. It was hypothesized that fast concentration changes in the delivery to the receptor can reduce sensory adaptation, leading to an increased taste perception. Saltiness ratings were scored by a panel over time during various stimulation conditions involving relative changes in NaCl concentration of 20% and 38%. Changes in salt delivery profile had similar effect on saltiness perception when delivered either by a sipwise method or by a gustometer. The impact of concentration variations and frequency of concentration changes was further investigated with the gustometer method. Five second boosts and 2 s pulses were delivered during 3 sequential 10-s intervals, whereas the delivered total salt content was the same for all conditions. Two second pulses were found to increase saltiness perception, but only when the pulses were delivered during the first seconds of stimulation. Results suggest that the frequency, timing, and concentration differences of salt stimuli can affect saltiness. Specifically, a short and intense stimulus can increase salt perception, possibly through a reduction of adaptation.

Introduction

There is strong scientific evidence of the link between high sodium intake from food products and hypertension [199]. Furthermore, it has been demonstrated that significantly reduced sodium intake is an effective method to lower hypertension and associated risks on cardiovascular disease [200]. The WHO currently recommends a daily intake of 5 g of salt (NaCl) per day, instead of typical daily intakes of 9–12 g of salt (WHO 2007). A number of different approaches for salt reduction have been developed and reviewed, but these are primarily limited to reductions of up to 20–30% salt in products [201, 202]. Hence, there is a need for further methods that enable salt reduction in products while maintaining the same consumer acceptance. The study reported here was conducted in order to investigate the impact of salt delivery on perception.

It is generally accepted that receptors (for vision, temperature, taste, odor, etc.) are contrast detectors [203]. One can view the experiments of Linforth et al. [204] in this light. They compared in vivo aroma release and aroma perception in gels containing concentrated suspended droplets of aroma and the same amount of aroma compounds homogeneously distributed in the gel. Adding aroma compounds in droplets was found to increase both the maximum intensity of volatiles in the nasal cavity and the perceived aroma intensity. The conditions that delivered the largest in-nose contrast in concentration were perceived as more intense. In rats, it has been shown that the phasic portion of neural responses, that is, that part of the response that is transitional/adaptive, is influenced by the flow rate of salt solutions. The slower the flow, the smaller the maximums and the longer it takes to reach the peak of the phasic response [205-207]. This suggests that (at least in rats) the rate of taste molecules transported to the taste receptors can influence the receptor response.

Prolonged or repeated stimulation of receptors often leads to a gradual loss of the magnitude of the perceived intensity, which is called adaptation [208]. This loss of intensity is also often referred to as habituation [209], which is defined at the level of perception. Here we use the term adaptation, which is defined at the level of sensation. Sensory adaptation is a change over time in the responsiveness of the sensory system to a constant stimulus. Adaptation occurs at the level of nerve activity after stimulation of the receptor. Adaptation is observed for all senses [210]. Adaptation has been demonstrated in electrophysiological (e.g., [211]) and psychophysical

experiments (e.g., [212-214]). The time frame of the adaptation of sodium receptors is expected to be of the order of 100 ms (reaction time to salt stimuli) to seconds [215]. In the case of salty taste, sodium receptors have been shown to adapt to the sodium content of their surrounding medium, which can be Na+ from the saliva in the mouth or from previous stimulations [214]. Through the application of rapid stimuli to the receptor, adaptation may be reduced, which consequently may increase the resulting taste perception. We hypothesized that relevant parameters that may influence perception are the firing rate at the receptor and the time required to distinguish between input signals. Because of the fast receptor adaptation time [215], fast concentration change rates are required for a change in sensory adaptation. Such rates cannot be investigated using sipwise sampling with cups, for which the minimum sampling rate was foundtobearound15s.Recently, severalauthors reported a ''continuous'' flow delivery system to deliver solutions of different concentrations and compositions into the mouth [74, 160].

The aim of this paper was to investigate whether saltiness perception can be modified by changing the concentration and frequency of the salt stimulus delivered onto the tongue. Trained panellists were exposed to stimuli varying in concentration of salt over time, delivered via different methods (cups and continuous flow). Continuous flow delivery was achieved using a gustometer, a software controlled system with 8 pumps to deliver liquid stimuli into the mouth of panellists. Panellists scored saltiness intensity over time using timeintensity (TI) methodology. In a first experiment, the impact of salt variation on saltiness perception was investigated using a classical sipwise approach and a gustometer approach. The overall salt delivery profiles (concentration and frequency [15s]) were the same. In the second experiment, the role of salt concentration (high–in-salt vs. constant stimulations) and frequency of presentation (2 and 5 s) was further investigated.

Materials and Methods

Subjects

In experiment 1, 11 panellists (all females [39–62 years]) and 12 panellists (7 women and 5 men [20–47 years]) took part in the sipwise condition and gustometer condition, respectively. For experiment 2, the panel was composed of 5 women and

5 men (22–55 years). Assessors were selected using ranking tests and trained to score saltiness as a function of time (see below).

Stimuli

In experiment 1 (sipwise and gustometer), 3 stimuli of 80 ml each were provided to panellists. In the sipwise condition, the stimuli consisted of series of 8 samples of 10 ml each offered at a frequency of 15 s (stimulus duration 120 s). In the gustometer condition, each stimulus was offered under continuous flow; when different concentrations were delivered they were changed every 15 s. All stimuli had the same overall average concentration (6.3 g/l NaCl in demineralized water). The same amount of total salt was delivered in each stimulus. The stimuli were created as follow (Figure 6-1a): the salt concentration was either kept constant over the full delivery time (''constant'' stimulus; constant concentration 6.3 g/l) or varied: 5.6 and 7 g/l alternatively (20% concentration variation). For sequences varying in concentration, the sequence started either with the lower concentration (''Low–high'' stimulus) or with the higher concentration (''High–low'' stimulus; Figure 1a).

In experiment 2, the impact of salt concentration and frequency was further investigated. The average salt concentration was kept the same as in experiment 1 (6.3 g/l NaCl), but the frequency of concentration variation and the concentration levels were varied. Five stimuli of 40-s delivery were offered to panellists: a Constant stimulus and 4 stimuli varying in salt concentration (Figure 6-1b). A 20% concentration variation was used (5.6 and 7 g/l), and this was varied every 5 s. A 38% concentration difference (5.6 and 9.1 g/l) was also used, delivering a 2-s Pulse every 10 s. Hereafter ''Boost'' and ''Pulse'' refer to stimuli varying in concentrations every 5 and 2 s, respectively. Moreover, Low–high and High–low refer to the stimuli beginning with the low or high concentration, respectively. The sampling duration was 30 s, followed by a constant delivery of 6.3 g/l NaCl for 10 s for all conditions (Figure 6-1b).

All samples were provided at room temperature. Between stimuli, panellists were provided with mineral water and unsalted crackers as palate cleanser. The stimuli were offered to the panel members following a balanced Latin square design. In experiment 2, a dummy stimulus was given at the beginning of each session. All

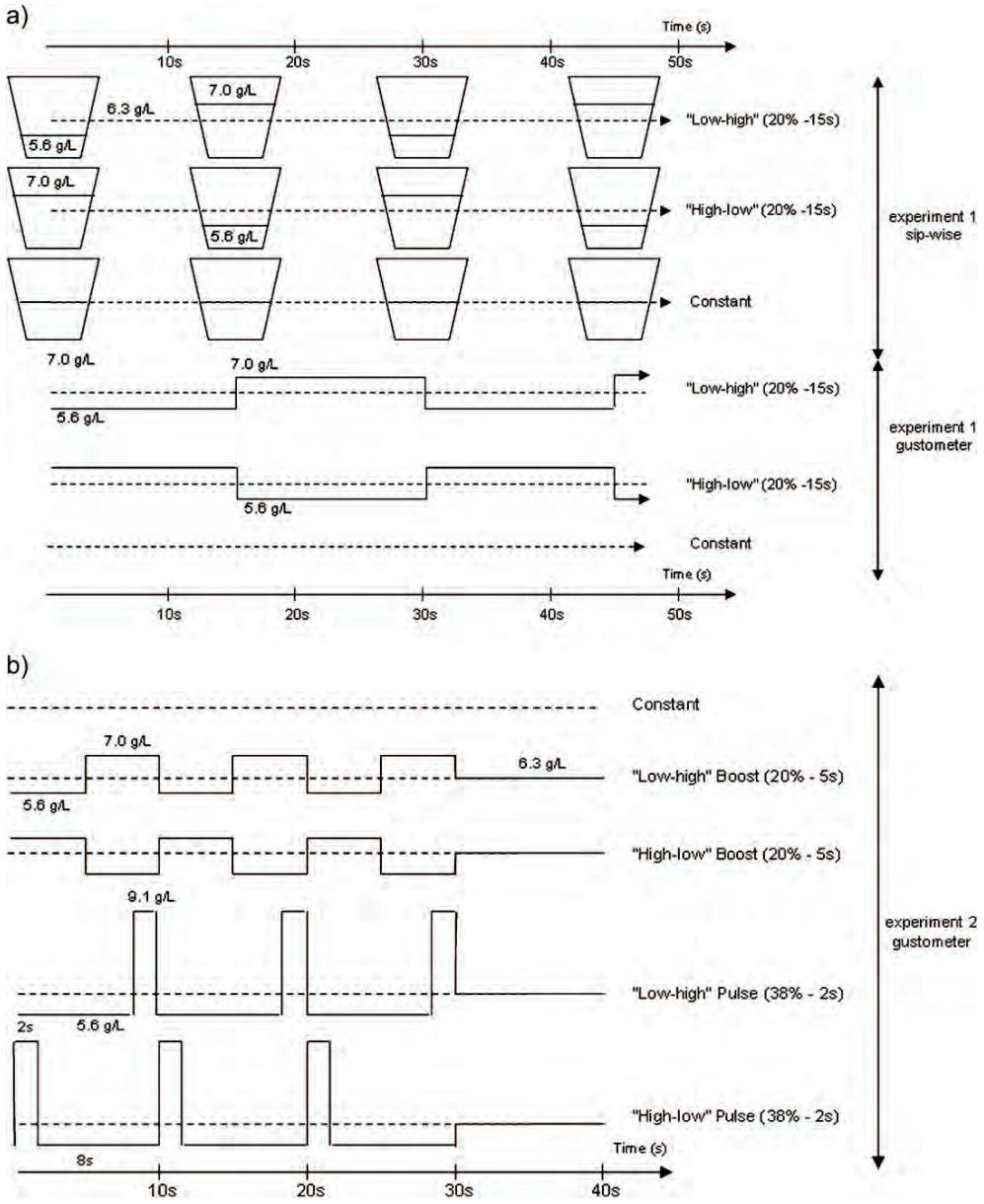

Figure 6-1: Overview of experiments 1 and 2. Schematic overview of the stimuli used in (a) experiment 1 (sipwise and gustometer) and (b) experiment 2 (gustometer). Salt concentrations and frequency as delivered in cups or via gustometer are indicated. Dashed line (---) indicates average salt concentration of 6.3 g/L for all conditions. For experiment 1, stimulus duration was 120s; data were collected for 240s. Experiment 2: stimulus duration, 40s; data collection 90s.

stimuli were evaluated in duplicate. Conductivity measurements were used to verify the actual delivery of salt solutions via the gustometer.

Sipwise delivery conditions

In experiment 1, sipwise conditions were chosen to be representative of the consumption conditions of a bouillon. Ten microliters of samples were provided in small cups. Every 15 s panellists were asked to put the full contents of a cup into the mouth and to swallow 2–3 s later. FIZZ 2.0 (Biosystems, Couternon, France) was used to indicate the actual timing of sipping the samples.

Gustometer delivery conditions

The gustometer was constructed from 8 pumps, a central control unit, a computer with dedicated software, and a manifold [160]. Eight identical membrane liquid pumps (KNF Stepdos FEM03.18RC, KNF Verder, Vleuten, The Netherlands; 0.030–30.0 ml/min) were connected via Teflon tubing (1.6-mm inner diameter) to an 8-channel input–1-channel output manifold (Inacom, Veenendaal, The Netherlands). Each input channel was fitted with an in-line check valve and mixing took place in the manifold. From the manifold, an approximately 10 cm long Teflon tube leads to the mouth of an assessor. Pump flow rates can be adjusted at any time from the central control unit (terminal eliminator plus, Black Box, Lawrence, PA), using the dedicated software [160]. In experiments 1 and 2, the flow rate of all stimuli wasfixed at 40 ml/min (same overall frequency as in the sipwise setup). The flow rates of individual pumps were varied in order to deliver different concentrations into the mouth of the panellist by combining 1% (w/w) salt solutions and water. During the sessions of experiment 2, panellists wore headphones to restrict possible impact of pump noise on panelist evaluation. The effect of wearing headphones to restrict sound was further investigated in an extra session. Results (not presented here) showed that the minor sound that panelists could hear did not significantly impact their scoring.

TI measurements

TI measurements were used to investigate saltiness perception over time. For each experiment, panel members were trained during at least 2 sessions. For the sipwise experiments, special attention was given to taking in the samples at exactly the right time, the actual perception of the taste intensity, and continuously scoring on the scale of the perceived intensity. For the gustometer experiments, the training of panel

members focused on the familiarization with the gustometer method, with continuous sample delivery and on TI scoring, using representative delivery profiles.

TI data of the sipwise experiment were collected using FIZZ 2.0 (Biosystems, Couternon, France). For the gustometer experiments, in-house Time-intensity (TI) software (Visual Basic.NET) was used. In all experiments, the scale was set from 0 to 100. In the gustometer experiments, panel members received a reference (10 g/l salt solution) before each sequence was delivered. This reference was given a fixed score of 100 and 80 in gustometer experiments 1 and 2, respectively. Data were collected during sample delivery (120 and 40 s) and afterwards (aftertaste) (120 s and 50 s) for experiments 1 and 2, respectively.

Data Analysis

The TI measurements produced data with multiple peaks, for which traditional analysis of TI data was not suitable. For all TI curves, obtained from experiments 1 and 2, area under the curves (AUC) were analyzed, which are highly correlated with the perception of taste at a given point (e.g., [162, 216]). For each individual ‘‘panellist–replicate’’ curve obtainedfor each method (sipwise or gustometer) and each stimulus (Constant, Boost, Pulses, Low–high, and High–low; Figure 6-1), the following parameters were extracted: Taste AUC (AUC corresponding to the tasting time) and Aftertaste AUC (AUC corresponding to the aftertaste of the sequence of samples; the aftertaste started after delivery of the last salt sample into the mouth). Extracted parameters were analyzed using an analysis of variance (ANOVA). Data for experiment 1 were analyzed using a 3-wayANOVA(panellist within method, method, and stimulus; with panellists within method as random factor). Data from experiment 2 were submitted to a 3-way ANOVA (panellist, stimulus, and replicate). When the stimulus effect was significant ($p < 0.05$), an LSMEANS post hoc comparison test was performed. Statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc., Cary, NC).

Results

Experiment 1: Impact of salt variation on saltiness perception using two different delivery methods

Average TI curves from the sipwise condition are presented in Figure 6-2a. The intake of 8 samples every 15 s induced TI profiles with multiple peaks. Whereas the stimulus with constant salt concentration produced 8 peaks with small amplitude, stimuli with alternating salt concentrations show 4 peaks of higher amplitude. These peaks can be

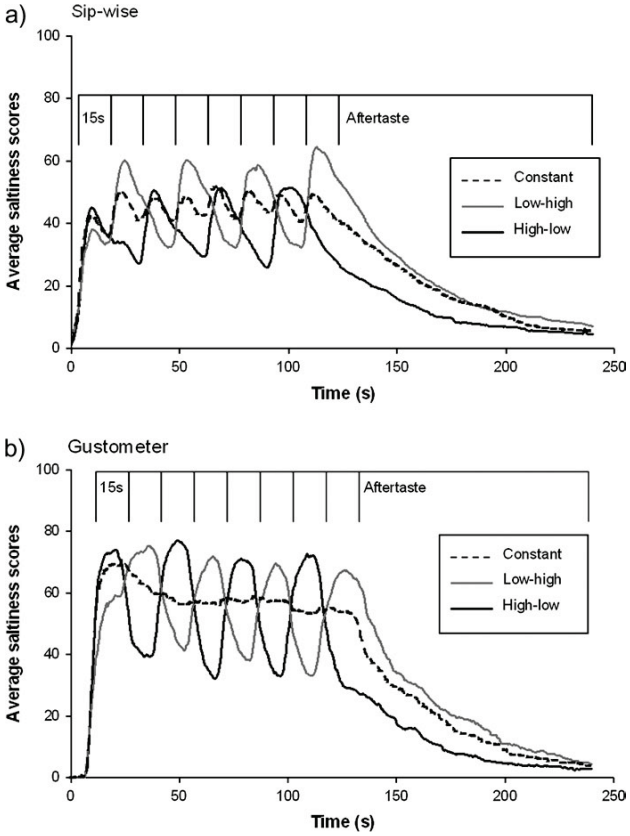

Figure 6-2: Average TI curves of experiment 1. Average salt concentration is 6.3 g/L; salt concentration difference is 20% (7 and 5.6 g/L). Vertical lines indicate concentration changes every 15 s (in-mouth) and the aftertaste interval. (a) Sipwise condition and (b) gustometer condition

related to the actual delivered salt concentrations. TI profiles from the continuous flow condition (gustometer) are shown in Figure 6-2b. For the constant concentration

curve, a peak is observed at the start, followed by a more or less constant lower intensity. For High–low and Low–high sequences, TI profiles consisted of 4 peaks in a regular pattern. Both in the sipwise and in the gustometer experiments, the alternating sequence starting with a low concentration (Low–high sequence) shows a lower maximum for the first peak. Furthermore, for both delivery methods, the sequence ending with a high concentration (Low–high sequence) displays increased saltiness intensity compared with the sequence ending with the low concentration. This effect extends into the aftertaste interval. Upon closer inspection of the shape of the TI profiles resulting from concentration variations, differences can be observed between the 2 delivery methods. The relative position of the curves and where they cross each other is different. For the sipwise experiment, the positions of the minimums and maximums mirror each other for the ''opposite'' stimuli, but their shapes are skewed. The minimums and maximums of the 2 opposite sequences of the gustometer profiles have similar values, and the profiles cross each other on the constant concentration curve. The relative size of the amplitude of minimum and maximum peaks for the alternating salt concentrations was of comparable magnitude, 55% and 68% for sipwise and gustometer delivery, respectively.

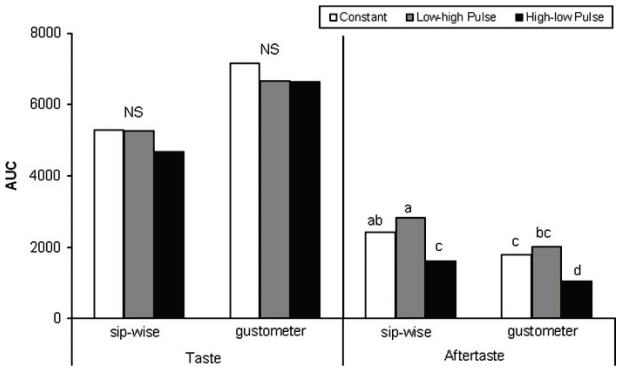

Figure 6-3: Areas under the curve for experiment 1. Taste and Aftertaste AUC for sipwise and gustometer conditions. Mean values associated with the same letter are not significantly different ($\alpha = 0.05$). NS, not significantly different

As shown in Figure 6-3, cup-wise delivery elicited a weaker taste (judged by Taste AUC) than delivery by gustometer ($F(1,21) = 13.07$, $P < 0.01$). For each delivery method, data from individual panellists differed significantly ($F(21,111) = 4.10$, $P < 0.001$). However, the way by which NaCl was presented in time (Constant, or with a

20% concentration variation) did not affect taste significantly for both methods used. With regards to the perception of the aftertaste (judged by the aftertaste AUC; Figure 6-3), the method used to deliver the stimuli did not significantly affect the perception. For the cupwise and gustometer delivery methods, data from individual panellists differed significantly ($F(21,111) = 6.70$, $P < 0.001$). The salt delivery profile affected the aftertaste ($F(2,111) = 16.15$, $P < 0.001$). As shown in Figure 6-3, the High–low 20% variation stimulus induced a weaker aftertaste than the Low–high and Constant stimuli, whatever the delivery method used.

Experiment 2: Impact of different salt delivery profiles on saltiness perception

In experiment 2, the gustometer was used to deliver salt concentrations with a faster frequency than in experiment 1. Boost and Pulse stimuli (see Materials and methods and Figure 6-1) with high–in-salt concentration delivery of 5 and 2 s, respectively, were provided to panellists. Stimuli were shortened to 30 s and a 10-s delivery of 6.3 g/l salt was added to all stimuli (from 30 to 40 s) because a large impact of the last concentration on aftertaste was observed in experiment 1. This 10-s supplementary delivery was added to all stimuli to ensure that all sequences ended with the same salt concentration. All stimuli had the same average salt concentration. Average TI curves are presented in Figure 6-4. For the Constant stimulus, saltiness scores stay almost constant over the 40 s of delivery (Figure 6-4a and b). Concerning the Boost and Pulse stimuli, TI curves present multiple peaks that can be related to salt concentration delivery. From these curves, it can be assumed that panellists were able to discriminate between salt concentrations.

It can be noticed that all stimuli showed an increase in saltiness with time. This seems less apparent for the Constant condition. This effect is more apparent under the pulsed conditions (Figure 6-4b), which suggests that the Pulse stimuli have been perceived as saltier than the other stimuli, the effect being more obvious for the High–low Pulse. The latter stimulus also shows a high increase in saltiness score around 45 s that is not observed for the other stimuli. Concerning the 3 remaining stimuli (Boost and Constant; Figure 6-4a), the curves present a similar average saltiness TI score, the Constant stimulus lying mainly in between the up and down parts of the Boost curves.

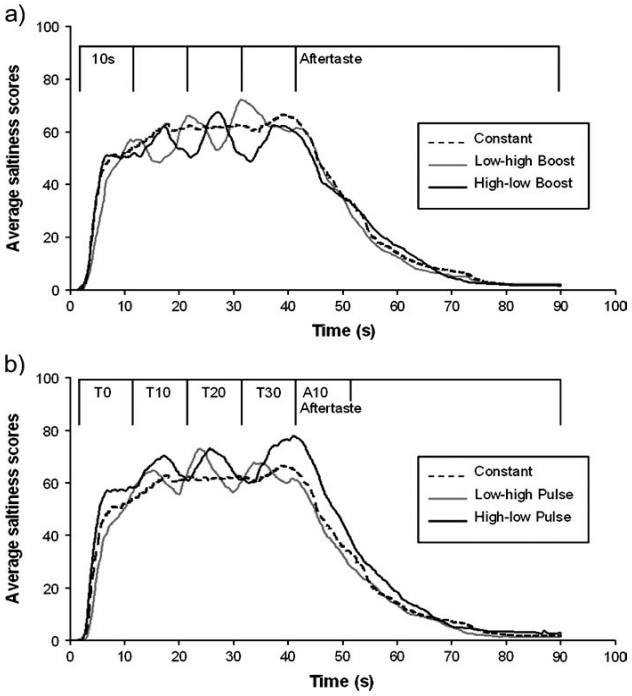

Figure 6-4: Average TI curves of experiment 2 (gustometer condition). Average salt concentration is 6.3 g/L. Constant condition is displayed in (a and b). (a) Salt concentration difference is 20% (7 and 5.6 g/L); high-in-salt boosts of every 5 s every 10 s were delivered 3 times, followed by a 10-s delivery of 6.3 g/L. Vertical lines indicate concentration changes every 10 s (in-mouth) and the aftertaste interval. (b) Salt concentration difference is 38% (9.1 and 5.6 g/L); high-in-salt pulses of every 2 s every 10 s were delivered 3 times, followed by a 10-s delivery of 6.3 g/L. 10-s epochs are indicated: T0 corresponds to the taste interval between 0 and 10 s, T10 corresponds to the taste interval between 10 and 20 s, etc. A10 corre sponds to the first 10 s of the aftertaste interval (the interval between 40 and 50 s)

For taste and aftertaste, data from individual panelists (judged by Taste and Aftertaste AUCs) differed significantly ($F(9,36) = 18.55$, $P < 0.001$ and $F(9,36) = 39.52$, $P < 0.001$, respectively). There was no significant replicate effect. As shown in Figure 5, the way by which NaCl was presented in time affected taste ($F(4,36) = 3.22$, $P < 0.05$) and aftertaste ($F(4,36) = 2.69$, $P < 0.05$).TheHigh–lowPulse stimulus elicited stronger taste and aftertaste than other stimuli. As observed from the curves (Figure 6-4), saltiness intensity of the Low–high and High–low Boost stimuli and the Constant stimulus were not found to be significantly different.

The effect of salt delivery on saltiness perception over time was further investigated by examining the evolution of AUC every 10 s (corresponding to the repetitive

element of each stimulus). Each individual TI curve was divided into epochs of 10-s intervals, and the AUC was calculated for each 10-s epoch (T0, T10, T20, T30, and A10, corresponding to the first 10 s of the aftertaste). For each stimulus, average epoch AUCs increased from T0 to T10 and decreased after salt delivery from T30 to A10 (Figure 6-6; results are shown for Constant and Pulse conditions only). No evidence of an adaptation effect (a decrease in perceived saltiness intensity upon prolonged stimulation) was observed between 10- and 30-s stimulation, as based on simple examination of the evolution of epochs over time (Figure 6-6).

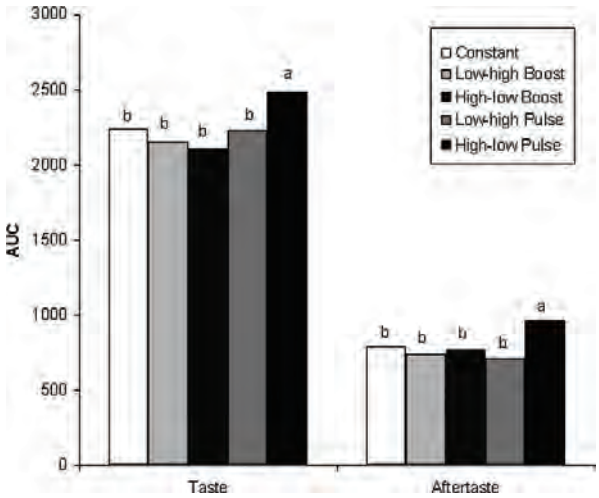

Figure 6-5: Areas under the curve (AUC) for experiment 2 (gustometer condition). Taste and aftertaste AUC for all 5 conditions. Mean values associated with the same letter are not significantly different ($\alpha = 0.05$).

Moreover, from Figure 6-6 it can be observed that for each 10-s interval the AUC is higher for the High–low Pulse (Figure 6-6). Such effect was not observed for the delivery profile of the Low–high Pulse. The main differences between the 2 Pulse sequences consisted in the timing of the high–in-salt Pulses: these Pulses are presented first for the High–low Pulse and only after 8 s for the other stimulus.

The results reported here show that saltiness perception may be modified by changing the salt delivery profile. A pulsatile profile (2-s Pulses) starting with a high–in-salt Pulse resulted in higher saltiness scores. The data suggest that panelists are specifically influenced by the concentration of salt during the 2 first seconds of delivery.

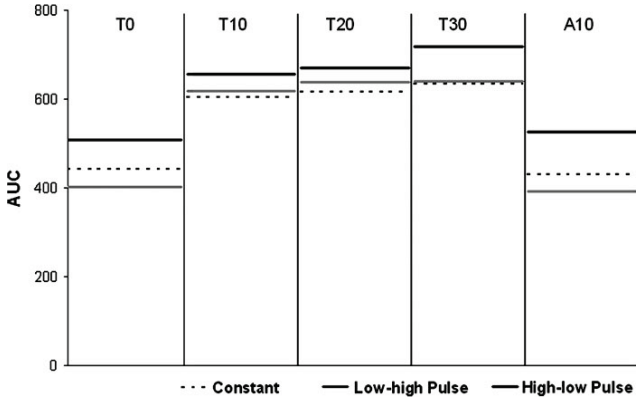

Figure 6-6: Epoch analysis of experiment 2 (gustometer condition). AUCs for 10-s intervals for the Constant, Low-high Pulse, and High-low Pulse condition. T0 corrsponds to the taste interval between 0 and 10s; T10 corresponds to the taste interval between 10 and 20s, etc. A10 corresponds to the first 10s of the aftertaste interval (the interval between 40 and 50s)

Discussion

In experiment 1, salt solutions of constant and alternating salt concentrations were delivered into the mouth of panelists during 120 s via 2 methods. The overall delivery conditions had been matched. In the sipwise condition with constant concentration, the saltiness score was reduced somewhat during each 15-s interval. This may be due to dilution with saliva, swallowing, and adaptation. The beginning of the next sip of the same concentration was consequently perceived as slightly higher due to contrast. Hence, constant concentrations assessed under sipwise conditions produced 8 small peaks, which are in accordance with studies by Guinard et al. [217], Bornstein et al. [218], and Schiffman et al. [219, 220], who obtained similar curve shapes for such experiments. In the constant concentration condition, delivered under continuous flow with the gustometer, a constant flow of liquid was delivered in mouth and after an initial peak a constant perception level was reached.

Variation of the concentration in both delivery methods (experiment 1) produced similar but not identical TI profiles comprising of 4 peaks, which can be attributed to the contrast experienced with the preceding 15-s sample of different concentration. This effect has been defined as successive cumulative contrast: solutions preceded by a high concentration level are judged to be significantly less intense than solutions proceeded by a low concentration level [221].

Delivery conditions for the 2 methods have been matched, that is, same concentrations and overall the same sampling frequency of 10 ml per 15 s have been applied. However, there is intrinsically a different temporal delivery profile associated with each method. With the cups, 10 ml is placed in the mouth at once, and this is repeated every 15 s (''batchwise''). With the gustometer, 10 ml is delivered over a period of 15 s, and this is repeated without a pause with the same or different concentrations (continuous). These differences impact the evolution and timing of the TI profiles. The TI profiles obtained with the gustometer display stable shapes. This may be attributed to highly controlled sample delivery into the mouth (concentration, flow, and timing) for the gustometer setup, whereas for the sipwise condition, only the intake time of each cup and its volume and concentration can be controlled. In-mouth processing (mouth movements and timing of swallowing) was not controlled in the 2 conditions. The panel composition has not been the same in the 2 conditions due to availability of panel members, but all panel members were trained. Despite significant differences in AUC observed between the 2 studies—conducted with different delivery methods and different panellists—reported in experiment 1, similar effects of salt concentration on saltiness perception were observed, whatever the delivery method used.

No increase in perception was observed upon concentration variations for both delivery methods. Both contrast and adaptation effects are assumed to play a role upon sequential delivery of concentration variations, and these effects appear to have counteracted each other. Rather, a faster stimulation frequency might be needed to reduce adaptation and hence increase perception. The impact of faster frequency delivery rates on perception was assessed using the gustometer in experiment 2.

In experiment 2, analysis of the evolution of AUC over time (10-s intervals) suggested that there was no adaptation effect. This is in line with results of Meiselman and Halpern [222], who reported absence of adaptation under conditions designed to simulate drinking, with pulsatile delivery of salt and water stimuli onto the tongue [223]. Hence, the results presented here suggest that fast concentration changes can lead to increased perception, which is attributed to a reduction of the adaptation experienced at the receptor. Such effects at the receptor have been reported for studies involving rats [205-207].

An increase in saltiness perception (as represented by AUC) was observed for the Pulse condition with the high–in-salt concentration delivered at the beginning of the sequential 10-s intervals. The difference in saltiness between this stimulus and other stimuli (especially the Low–high Pulse) can possibly be attributed to different parameters of the delivery design. It can be hypothesized that the first 2-s interval of the stimulus is a relevant parameter influencing the overall saltiness rating (from the beginning and until the aftertaste). The effect could be due to a perceptual effect (a strong saltiness at the beginning of a stimulus inducing a stronger overall saltiness perception) and/or to a scoring effect (the higher scores rated at the beginning of the scoring inducing an overall higher intensity score over time, via continuous rating on the scale). The fixed concentration at the end of the stimulus delivery is another parameter possibly influencing the saltiness rating of the High–low Pulse. It was observed that for this condition, there was a relatively large increase in saltiness perception during the 30- to 40-s interval. There may be a contribution in increased saltiness perception from the fixed salt concentration of 6.3 g/l at this interval, which has been an increase as compared with the concentration delivered prior to it. Such effects on aftertaste have been observed in experiment 1. However, it should be noted that such an effect was not observed for the High–low Boost condition.

As a conclusion of this study, a significantly increased perception for 2-s high–in-salt Pulses was shown, when starting the sequence with a Pulse. It has been primarily suggested that this could be attributed to a reduction of adaptation as induced by fast concentration changes delivered in mouth. Analysis of the results also suggests other parameters that could impact on saltiness rating. This preliminary study, in which samples were delivered with a gustometer and the effects on saltiness perception were investigated using TI, would need further validation. Further work is recommended in order to understand if saltiness perception is more influenced by the frequency (2 s or less) or the salt concentration differences of short salt stimuli and to understand the importance of timing in the sequence of the Pulse. Furthermore, it is recommended to measure the overall salt perception (and not over time) and during shorter stimuli (e.g., 10 s) with varied salt delivery profiles within the stimulus. This would be more representative of real consumption conditions of products.

The food matrix or microstructure affects the temporal release profile of tastants and aroma, which in turn has an impact on perception [224-228]. Studies with the gustometer,

such as the current study, can be very useful for the definition of design rules for release profiles that lead to a higher perception with the same tastant or aroma composition. Ultimately, this should provide input for product design.

Acknowledgements

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Chapter 7

Sweet taste intensity is enhanced by temporal fluctuation of taste and aroma and depends on phase shift

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Abstract

Pulsatile stimulation enhances taste intensity compared to continuous stimulation with stimuli of the same net tastant concentration. In the present work, we studied the effects of pulsatile delivery of aroma and taste on their combined contribution to taste intensity. Effects on taste perception were evaluated for aroma and taste pulsation and the aroma pulse–taste pulse phase shift. High-concentration sucrose pulses were alternated with water rinses every 2.5 s. Four different aroma (isoamyl acetate) versions were presented: (1) no aroma, (2) continuous aroma (3) aroma pulses in-phase and (4) aroma pulses out-of-phase with taste pulses. Aroma–taste combinations were evaluated for sweetness intensity by a 15-member trained panel using time-intensity analysis. Sweetness intensity was enhanced by pulsatile stimulation of sucrose or isoamyl acetate. In addition, taste enhancement by aroma and tastant pulses was additive if both were presented out-of-phase which resulted a sweetness intensity enhancement by more than 35% compared to a continuous sucrose reference of the same net sucrose concentration. Aroma-induced sweetness enhancement can be explained by cross-modal aroma-taste integration. Amplification of aroma-taste integration by pulsatile stimulation may be attributed to a potentiated afferent input of aroma and taste information prior to aroma-taste integration. Alternative mechanisms include the importance of swallowing on aroma-taste integration.

Introduction

Tastant delivery in the form of high-concentration pulses that are embedded in a continuous tasteless solvent enhances taste intensity compared to a reference solution of the same average concentration where the tastant level remains constant over time. This so called "taste enhancement by pulsatile stimulation" has been demonstrated for salt taste [161, 222] and sweet taste [229]. Different factors that contribute to enhancement have been reported. First, enhancement by pulsatile stimulation was attributed to contrast effects that may be introduced by the continuous alternation of high-concentration taste pulses with water [161, 229]. Moreover, the enhancing effect was found to be independent of whether subjects consciously perceived pulsation or not, suggesting that the observed enhancement originates at preconscious levels of information processing and is not affected by subsequent conscious pulse perception.

A second mechanism attributes the observed enhancement to a reduction in adaptation by pulsed stimulation. Continuous exposure of the peripheral taste infrastructure—receptors and neural relays in the afferent system—causes a gradually decreasing responsiveness to a stimulus, with correspondingly lower perceived intensities [230, 231]. This could be overcome by the alternation of taste stimulation with water rinses in pulsed applications. Adaptation reduction may be valid for experiments where tongue movements are constrained and the stimulus not swallowed [222, 232]. However, as shown in a previous study, reduction of adaptation could not explain taste intensity enhancement by pulsatile stimulation. At settings close to real food consumption (free tongue movement and swallowing at will), taste intensity increased upon stimulation with sweet solutions over 40 s for both pulsed stimuli and the continuous reference [229].

A third mechanistic explanation for taste enhancement by pulsatile stimulation was derived from vision: at rates slightly below the frequency at which subsequent flickers fuse into a continuous percept (critical flicker fusion frequency [CFF]), a flickering stimulus appears brighter than a continuous stimulus of equal mean luminance, the so called "Brück–Bartley effect" [233]. This enhancement coincides with an increased burst count of afferent fibers for sequences of transient responses to pulsating stimuli compared to the lower steady state response for a continuous stimulus [234]. Similarly, in rats, transient chorda tympani bursts are observed after short (2.5 s) interruptions of taste presentations [235, 236]. For sufficiently short interruptions, the net burst count is

enhanced due to repeated phasic responses of the chorda tympani. A similar neurophysiological explanation of pulsation-induced taste enhancement may be valid in humans. Here, taste enhancement was pulsation rate dependent and peaked at a pulsation periods of 4–6 s. Furthermore, this range coincided with the median "taste fusion period" of 5.1 s where taste pulses fuse into a continuous percept [229].

An alternative way to enhance taste intensity is the addition of odorants [237]. Aroma-induced taste enhancement has been reported for sweet taste [26, 49, 71, 162] and salt taste [122, 237]. Adding an aroma that is commonly experienced in a sweet product to a sucrose solution often results in a mixture that is perceived as sweeter than the sucrose solution by itself [100]. This phenomenon has been attributed to a cross-modal integration of olfaction with gustation allowing an elevation of sweet taste by the "sweet" aroma [27, 49]. Indeed, functional magnetic resonance imaging (fMRI) revealed that secondary sensory projection areas in the orbitofrontal cortex (OFC) are activated by aroma as well as taste [238] and that the activation of secondary sensory projection areas in the OFC and amygdala by combined taste and smell exceeded the summed activity of taste and smell alone [239].

According to the "unity assumption", sensory inputs will be more likely integrated into a unitary percept if they are highly consistent in one or more directions (e.g. time, space, temporal patterning; [240, 241]). The importance of temporal synchrony, for example, has been demonstrated for integration of auditory and visual stimuli [242, 243] and for integration of aroma and texture stimuli [83, 160]. Temporal aspects were also studied for aroma–taste presentations: asynchronous delivery of a sweet smelling aroma (benzaldehyde) and sweet taste (saccharin) decreased aroma–taste integration compared to synchronous stimulation [71]. These results suggest that, besides space continuity, temporal synchrony of receptor activation may be of importance for aroma–taste integration.

In the present work, we studied the effect of pulsatile stimulation of taste and aroma on cross-modal aroma–taste integration. We hypothesized that, as observed for vision and taste, pulsed delivery will also enhance aroma intensity and, as a result, amplify aroma–taste integration. Secondly, we tested whether the synchrony of aroma–taste pulses was of importance for the extent of cross-modal aroma–taste interaction. This was tested for sweet taste (sucrose) and banana aroma (isoamyl acetate, congruent

with sweet taste) stimuli that were delivered by a Gustometer [160]. The following questions were addressed: (1) Does aroma pulsation amplify aroma-induced taste enhancement? (2) If so, does aroma-induced taste enhancement depend on the timing of taste and aroma pulses?

Materials and Methods

Stimuli

An eight-channel computer controlled Gustometer [160] optimized for high frequency pulse applications was used to deliver welldefined, alternating taste–aroma concentrations embedded in a continuous flow. Stimuli were produced at desired concentrations by running four pumps in parallel, mixing a sucrose solution (150 g/L) and an aroma solution (isoamyl acetate; 1 g/L) with water at predefined ratios resulting in eight different aroma–taste profiles (Table 7-1).

Table 7-1: Taste-aroma Gustometer stimuli; sucrose (S) and isoamyl acetat (A) were delivered in continuous (c) or pulsed (p) mode; A0: no A present; in pulsed stimuli, S- and A-pulses (2.5 s) were intermitted by water rinses (2.5 s) to yield the same average (av) concentration (C) than continuous stimuli (S: 60 g/L, A: 0.067 g/L [if A present]); in Sc7A0 the average S-concentration was 70 g/L; if SpAp, S- and A-pulses were given in-phase [i] or out-of-phase [o]; the total flow of each stimulus was 15 mL/min

Stimulus	Sucrose			Aroma		
	C [g/L]	Mode	C_{av} [g/L]	C [g/L]	Mode	C_{av} [g/L]
ScA_0	60	c	60	-	-	-
SpA_0	120	p	60	-	-	-
ScA_C	60	c	60	0.067	c	0.067
SpA_C	120	p	60	0.067	c	0.067
ScAp	60	c	60	0.134	p	0.067
SpAp[i]	120	p	60	0.134	p	0.067
SpAp[o]	120	p	60	0.134	p	0.067
Sc_7A_0	70	c	70	-	-	-

Sucrose was delivered in a continuous (Sc) or pulsed (Sp) mode. In the pulsed mode, high-concentration sucrose pulses (120 g/L; 2.5 s) were continuously alternated with water rinses (2.5 s). All stimuli, pulsed and continuous, yielded an average sucrose

concentration of 60 g/L, except for the continuous reference stimulus Sc7A0, which had a sucrose concentration of 70 g/L.

Sucrose pulsation conditions were presented without accompanying aroma (A0), in combination with a continuous aroma (Ac; Cisoamyl acetate: 0.067 g/L) or in combination with a pulsating aroma (Ap; Cisoamyl acetate: 0.134 g/L; 2.5 s on–2.5 s off), in a full-factorial (2×3) fashion. In addition, in case of pulsating sucrose solutions, aromas were pulsated either an in-phase or out-of-phase with the sucrose pulsation. As such, four different aroma conditions are defined: A0, Ac, and Ap in-phase with sucrose pulses (Ap[i]) and Ap out-of-phase with sucrose pulses (Ap[o]). The average isoamyl acetate concentration in Ac and Ap samples was 0.067 g/L. The total oral stimulus flow was kept constant at 15 mL/min and the stimulus duration was 40 s each.

To verify that each stimulus yielded the nominal net sucrose concentration (60 g/L or 70 g/L respectively), each profile's eluent was collected over 40 s. The sucrose concentration was analyzed by refractometry in triplicate (PAL-1, ATAGO U.S.A., Inc., Bellevue/USA).

Subjects

15 subjects (age: 22–52, 5 male) were recruited. Some of the subjects (n=6) participated in an earlier taste pulsation study [229]. All subjects were trained on the Gustometer taste-delivery method. Subjects were not allowed to consume anything except for water during the hour prior to the test. Materials and methods used did not require medical ethical approval under Dutch regulations (retail ingredients, oral delivery). Subjects gave written informed consent.

Method

Subjects kept a mouth-piece (Teflon-tube; inner diameter: 0.24 cm) between their central incisors. While stimuli were pumped into their mouths, subjects rated sweetness intensity over time (timeintensity; scale: 0–100; anchored “not sweet”-“very sweet”) by moving a cursor on a vertical rating-bar on a computer screen. Subjects swallowed at will; tongue movements were not restrained. Each subject evaluated all 8 stimuli 4 times in a randomized order over 2 sessions on separate days. In one session, 16 stimuli were presented in 4 sets of 4 stimuli each, separated

by 5-minute breaks. Each stimulus set was preceded by the reference "ScA0 " for selfcalibration ("ScA0" was also included as blind). Stimulus Sc7A0 was included as a second reference to quantify sweetness enhancement in terms of sucrose equivalence. Between samples, at least 1 min was given to rinse the mouth with water. At the beginning of each session, subjects received two additional warm-up stimuli.

Data Analysis

The area under the sweetness–intensity curve (AUSC) from 0 to 40 s was treated as the dependent variable. To evaluate the effects of taste pulsation and aroma presence/pulsation and their interaction on AUSC, a multifactor Taste Pulse (fixed factor: continuous or pulsating)×Aroma (fixed factor: not present, continuous, pulsating) ANOVA was performed in which also Replicate (4 replicates) and Subjects were included as fixed and random factors, respectively (SPSS, Chicago, version 17). The ANOVA was full-factorial with the exception of the four-way interaction, to accommodate for the lack of degrees of freedom in the dataset. In this analysis, only 60 g/L sucrose samples were compared and from the samples in which both aroma and taste were pulsed, only SpAp[o] was used. To test for differences between the aroma in-phase and out-of-phase conditions, AUSC were subjected to a separate, independent ANOVA testing the Phase effect (ApSp[i] vs. ApSp[o]), again including the factors Replicate and Subjects as fixed and random factors, respectively. All tests were at α=0.05.

Results

Sucrose concentration

The net average sucrose concentration delivered over 40 s by the Gustometer as measured by refractometry was 6.1%±0.1% (M±SD) for the reference sample ScA0 and 7.2%±0.1% for profile Sc7A0. For the remaining profiles, the overall mean sucrose concentration was 6.1%±0.2%.

Psychophysical results

AUSC differed over stimuli ANOVA revealed significant main effects of Aroma [F(2,28)=21.53; pb0.001] and Taste Pulse [F (1,14)=16.02; pb0.001] on AUSC indicating that both the presence of aroma (here: isoamyl acetate) and pulsatile sucrose stimulation affected sweetness intensity. Figure 7-1 shows that aroma presence enhances AUSC and that pulsation of both taste and aroma further enhances AUSC. No significant Subject and Replicate effects were observed. Of all tested

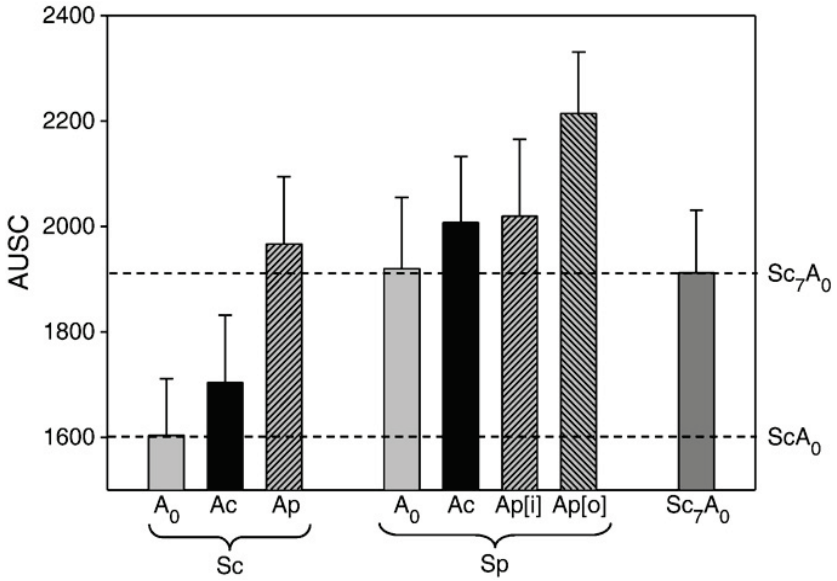

Figure 7-1: Area under sweetness curve (AUSC) averaged over 15 subjects (4 replicates) for Gustometer profiles shown in this figure; ScA0: continuous sucrose stimulation; SpA0: pulsed sucrose stimulation (each 60 g/L sucrose); Sc7A0: continuous sucrose stimulation (70 g/L sucrose); aroma (isoamyl acetat; A) was given in four categories together with sucrose stimuli: no aroma (A0); continuous aroma (Ac); pulsed aroma (Ap) in-phase (Ap[i]) or out-of-phase (Ap[o]) with sucrose pulses; dashed lines represent sweetness intensity of ScA0 and Sc7A0.

interactions, the Taste Pulse×Subject interaction was significant [F(14,12771)=3.61; p=0.014] indicating that the magnitude of the pulsation-induced intensity enhancement differed between subjects. The only other significant interaction observed was a Replicate×Aroma×Subject interaction [F(84,84)=1.70; p=0.008], which can be described as different subjects showing different relative contributions of aroma to AUSC compared between replicates. No other significant main effects or interactions were observed. Inspection of Figure 7-1 shows that pulsed sucrose and pulsed aroma delivery increased sweetness intensity in an additive fashion for the

out-of-phase condition, which is reflected in the absence of Aroma×Taste Pulse interaction (Figure 7-2).

When pulsating both aroma and taste, AUSC differed significantly between the in-phase and out-of-phase conditions [F(1,14)=11.6; p=0.004]. The Subjects effect for this test was also significant [F(14,17070)=4.57; p=0.002] and the only observed

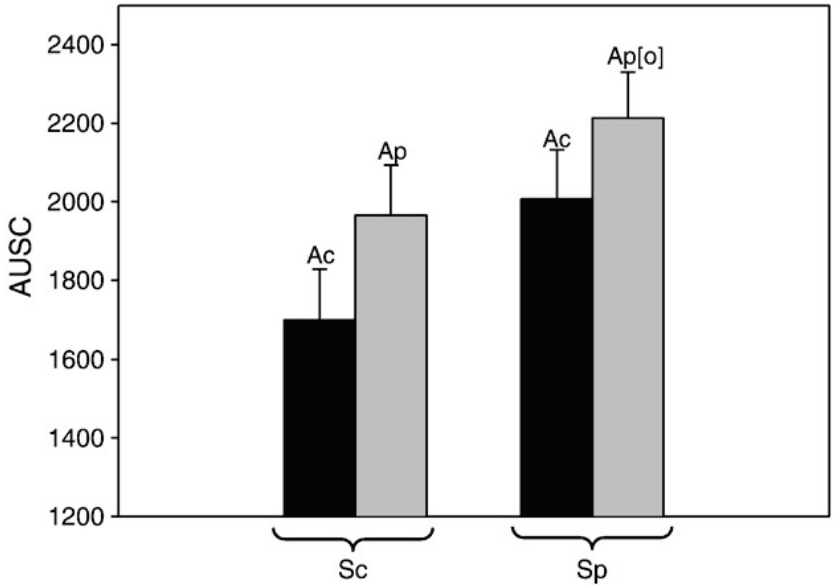

Figure 7-2: Effect of pulsed delivery on aroma-taste interaction; AUSC: area under sweetness curve representing overall sweetness intensity; taste (sucrose; S) and aroma (isoamyl acetat; A) were delivered in continuous (Sc/Ac) or pulsed (Sp/Ap) mode; Ap[o]: aroma pulses were delivered out-of-phase with sucrose pulses

significant interaction was a Subject×Replicate interaction, indicating that AUSC drifted over replicates for some of the subjects. The main effect of Phase is explained by the fact that the in-phase stimulus resulted in lower AUSC than the out-of-phase stimulus, which remained at the same AUSC as SpAc, the stimulus with pulsating sucrose but continuous aroma (Figure 7-1). It is worth noting that stimulus SpAp[o] was perceived more than 35% sweeter than stimulus ScA0 (Figure 7-1) although both contained the same net sucrose concentration.

Discussion

The present study demonstrates that sweetness intensity can be enhanced by 3 factors without changing the net sucrose concentration: (1) aroma–taste integration,

(2) pulsed delivery of aroma or taste, and (3) optimizing of the time phase-shift between taste and aroma pulses. In addition, aroma pulsation and taste pulsation enhanced sweetness intensity in an additive fashion if aroma and taste were pulsed out-of-phase.

Intensity enhancement by pulsatile stimulation has been found for taste [161, 222, 229] and vision [233, 244]. Our data demonstrates that pulsation of a taste-enhancing aroma eventually leads to a further taste enhancement. It is not clear whether this is due to an initial aroma intensity enhancement by pulsation or mere enhancement upon cross-modal integration. In vision, flickering stimulation at the CFF induces a higher accumulated receptor response compared to non-pulsating stimulation [234]. Similarly, in rats, short interruptions of taste presentations yield transient receptor bursts. At sufficiently short interruptions, the net burst count is enhanced due to a repeated phasic response of chorda tympani [235, 236]. Likewise it is possible that pulsatile aroma stimulation induces a higher average receptor response, that, upon integration with taste, amplifies the output of aroma–taste integration. This hypothesis should be confirmed in electrophysiological studies.

In a previous study, the importance of the pulsation period on sweet taste enhancement was investigated [229]. The pulsation period was identified as an important factor modulating sweet taste enhancement. Pulsation periods that produced the most enhancement centered around TFP, with remarkable difference between individuals. It was suggested that alternating stimulation with taste pulses and water rinses may allow a partial recovery of transient receptor responses as was observed earlier for multi-unit response recordings in the rat [245]. Differences in TFP were attributed to differences in individual recovery times. Because fixed pulsation periods were used in the present study, the observed Taste Pulse×Subject interaction observed may reflect similar individual differences in taste pulse processing and/or recovery times.

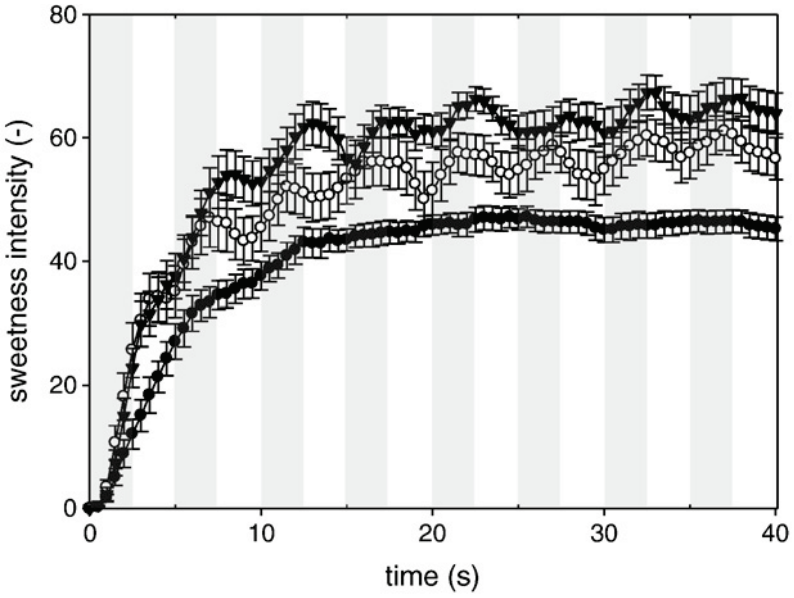

Figure 7-3: Time-intensity curves for "sweetness intensity"averaged over 15 subjects (4 replicates); ---●---: continuous sucrose delivery (ScA_0); ---○---: sucrose and isoamyl acetat pulsed in-phase (SpAp[i]); ---▼---: sucrose and isoamyl acetat pulsed out-of-phase (SpAp[o]); sucrose pulses (2.5 s duration) are represented by grey bars; the net sucrose concentration was 60 g/L for each sample; error: standard error

Time–intensity ratings for stimuli SpAp[i] and SpAp[o] showed similar pulsed patterns over time which followed the Gustometer sucrose delivery (Figure 7-3). Nevertheless, sample SpAp[o] was perceived as sweeter than sample SpAp[i]. This rather unexpected outcome indicates that synchrony of aroma–taste pulses was important for the amount of enhancement induced by the odorant pulses, and optimal when taste and aroma pulses were delivered asynchronously in the mouth. The "unity assumption" describes that sensory inputs should be more likely integrated into a unitary percept if they are consistent in one or more domains (e.g. time and space [240, 241]). Hence, aromainduced taste enhancement is expected to be larger if stimuli are presented in a synchronous rather than asynchronous fashion, as was demonstrated for integration of saccharin and benzaldehyde signals at sub-threshold levels [71]. In the present study, aroma stimuli were delivered orally. Consequently, the aroma had to be swallowed for volatiles to be transported from the back of the oral cavity via the nasopharynx to arrive retronasally at the olfactory epithelium [91, 246]. Therefore, retronasal aroma delivery is affected by different factors such as mastication and swallowing patterns [204, 226], and differences in the topographical adsorption of aromas

to the oral and pharyngeal mucosa and the olfactory epithelium [91]. As a result, swallowing induces aroma–taste asynchrony, with the aroma being perceived somewhat after the taste. It is very well possible that the out-of-phase presentation of aroma and taste stimuli at the pulsation frequency used may have compensated for the temporal delay induced by swallowing. In this case, aroma and taste stimuli are processed in sufficient temporal proximity to fulfill the unity assumption. Equally, swallowing may have resulted in an asynchronous perception of aroma-taste signals for the in-phase aroma-taste pulsation mode to reduce the magnitude of cross-modal integration. Alternatively, because taste stimulation precedes aroma stimulation under normal eating conditions [225], human perception may actually be tuned towards this desynchronization. If the natural taste precedence over aroma is compensated for neurologically, this could also explain why sweetness enhancement is highest when taste and aroma pulses are delivered out-of-phase. These hypotheses can be tested by delivering both taste and smell stimuli with a high time resolution on the tongue and at the olfactory epithelium, respectively. Current technology allows for such a test [83].

Aroma–taste integration is facilitated if the aroma appears to originate from the mouth while consuming the food [247], which happens when the aroma arrives at the olfactory epithelium through the retronasal pathway. This is supported by the observation that perceptual aroma–texture interactions occur only when the aroma is presented retronasally [248]. Additional support comes from neurophysiological studies showing super additive responses to combined taste and retronasal aroma stimulation in regions processing aroma and taste information, whereas the same regions reveal deactivation if the aroma is delivered in orthonasal fashion [248]. In the present study, isoamyl acetate was delivered retronasally and resulted in aromainduced sweetness enhancement. Literature suggests that the observed enhancement would have been less pronounced upon orthonasal aroma delivery.

Earlier suggestions that taste enhancement by pulsed taste stimulation can be explained by a reduction in adaptation [161, 222, 249] were contradicted in a previous study [229]. There, we showed that both prolonged, continuous taste stimulation and pulsated taste stimulation over 40 s did not yield a decrease in intensity ratings, as is expected had adaptation occurred [231, 250]. Results of the present study, performed under similar conditions, further support the idea that adaptation reduction does not explain taste enhancement by taste pulsation (see also Figure 7-3). In fact, under the current tasting

protocol, allowing for free tongue movements and swallowing of the stimulus, the occurrence of taste adaptation is expected to be minimal [232].

In summary, the present work supports recent studies on taste enhancement via pulsed stimulation. Moreover, this effect was found to amplify the contribution of isoamyl acetate to the sweetness of sucrose. Enhancement by pulsatile stimulation may originate at early stages of sensory processing, as is the case for vision, where discontinuous, pulsed stimuli invoke serial phasic receptor responses resulting in an overall higher afferent input [234]. Elevated aroma signals from the periphery would then explain the enhanced aroma contribution to sweetness. Sweetness intensity increased in an additive fashion when aroma and taste stimuli were given out-ofphase. This observation requires further investigation but may be explained by delaying factors in retronasal aroma processing (e.g. swallowing) that result in a temporal re-alignment of taste and aroma signals upon cross-modal integration. Individual differences in the magnitude of sweetness enhancement by pulsatile simulation may be attributed to genetic variation in the taste system, that, besides taste sensitivity, may also be expressed in temporal aspects in taste processing (e.g. TFP). This is currently under study.

Chapter 8

Combinatory effects of texture and aroma modification on taste perception

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Abstract

In this study additive and/or cumulative effects of texture modification and aroma induced sweetness enhancement were systematically investigated in apple flavoured semi-solid Na-caseinate gels. Gels containing apple juice as basic flavour were developed differing in stiffness, brittleness and serum release (texture modification), aroma and sugar concentration (flavour modification). In a full factorial design (2x2x2) eight samples were evaluated by a sensory consumer panel on ten attributes (five texture, five flavour). Sweetness was enhanced significantly by either modification of texture, aroma or sugar concentration. Texture modification was found to be by far the greatest contributor to overall sweetness, while aroma and sugar concentration changes resulted in minor sweetness enhancement. An additive, but no synergistic effect of texture modification and aroma induced sweetness enhancement was found. It can be concluded that texture modification is a valid tool to enhance taste intensity. Hence, texture modification can compensate for a loss of sweet taste intensity induced by sugar reduction, while aroma induced sweetness enhancement can contribute to further taste enhancement in order to achieve healthier products.

Introduction

Consumers can perceive small variations in sweetness intensity of products such as cookies and sugar solutions [251-255]. The taste of the product is affected by the reduction of sugar when developing calorie reduced foods, which can lead to repercussions on the choice of the consumer. Several strategies have been proposed to reduce sugar content while maintaining the taste of the reformulated product unvaried.

One strategy to enhance sweetness intensity in semi-solid foods involves the modification of textural properties of the food matrix. Three approaches have been described, each of which involves the modification of a specific textural property, to achieve taste intensity enhancement by (a) the reduction of the stiffness of the food matrix, (b) an increase of the brittleness of the food matrix and (c) an increase of the serum release from the food matrix. The three approaches are summarized briefly in the following:

(a) Clarck [256]reported an increase in taste intensity with decreasing stiffness for soft solid foods. Flavour intensity was shown to be enhanced upon reduction of stiffness and thickness of a variety of semi-solid gels prepared from different biopolymers [257]. An enhancement of sweetness intensity with decreasing polymer concentration was also reported for k-carrageenan and gellan gum gels containing sucrose and aspartame as tastants [258]. The reduction of k-carrageenan and gellan gum concentration lead to a decrease in Young's modulus and fracture stress (i.e. a decrease in stiffness and toughness) of the gel. In addition, it was reported for liquid foods that taste intensity increased upon reduction of viscosity. The magnitude of change in taste intensity seems to depend on hydrocolloid concentration and is hydrocolloid specific [82, 259-262]. It should be noted that literature is not univocal on the relationship between stiffness of semi-solid gels and taste intensity. While it is often observed that the extent of taste intensity enhancement in gelled systems depends on the stiffness, perceptible differences were reported in sweetness intensity between different gels with similar stiffness [263, 264].

(b) Morris et al. [265, 266] demonstrated that for gels with similar firmness but higher brittleness the taste intensity was higher. They concluded that taste intensity of gels does not only depend on stiffness, but on brittleness. They suggested that upon chewing in the oral cavity the gel breaks down into a larger number of smaller

fragments. The gel break down is accompanied by an increase of total gel surface since new surfaces are generated due to the fragmentation process. They hypothesised that the increase in gel surface facilitates more contacts between tastant containing gel fragments and the taste buds leading to an enhancement of taste intensity.

(c) The effect of serum release on sweetness intensity in semi-solid gels has been reported. Serum release has been defined as the mass of the exuded serum which is released upon mechanical compression from a specific mass of the food matrix. In those studies the serum consisted of an aqueous solution of sugars and polysaccharides which was exuded upon mechanical compression of the gel. Serum release has been demonstrated to strongly influence taste intensity and texture perception of mixed protein/polysaccharide gels [267-270]. It has been suggested that serum release can be related to the juiciness perception in fruits, vegetables and meat products. Serum release in semi-solid gels can be induced by modifications of the microstructure, whereby gels with bicontinuous microstructures reveal the highest amount of serum release upon mechanical compression. Tastants like salt and sugar need to be dissolved so that they are perceived by the taste buds. Serum release is a strategy to optimize the delivery and availability of tastants since the release of the tastant from the gel matrix is increased. Sala et al. [267] used semi-solid mixed protein/polysaccharide gels with constant large deformation properties but varying in serum release to demonstrate a significant boost of sweetness intensity with increasing amount of serum release. The sweetness intensity of gels with 12% serum release and 3wt% fructose was very similar to gels with 2% serum release and 4wt% fructose. They concluded that serum release is a strategy which allows modifying the textural properties of the food matrix to boost taste intensity.

An alternative strategy to reduce sugar content in foods while maintaining sweetness is offered by the use of multimodal sensory integration processes. Whenever eating or drinking, different sensory modalities such as taste, smell, sound, vision and touch (mouth feel) contribute to the overall perception of food. Multimodal sensory integration can lead to taste intensity enhancement by aromas [53, 101, 124] provided that aroma and taste qualities match in a natural way. Such perceptually similar stimulus combinations are referred to as “congruent” [102]. The occurrence of aroma-induced sweetness enhancement highly depends on the aroma used. The sweetness of sucrose solutions was enhanced by strawberry aroma but not by ham aroma. Schifferstein and

Verlegh [102] also showed that the degree of congruency does not correlate with the degree of taste enhancement. An aroma can have a high sweetness enhancement capacity even though it might not be the most congruent aroma to that specific taste aspect of the food. Knoop et al. [124, 155] demonstrated that aroma induced taste enhancement can be used as a strategy to enhance sweetness of a complex food product (apple juice). They reported that sweetness enhancement can be obtained through addition of ethylhexanoate, a naturally in apples occurring aroma component which is congruent with the apple juice. Next to the aroma induced sweetness enhancement, which allows for reduction of sugar content by 15% without decreasing sweetness, the addition of ethylhexanoate introduced an undesired off-flavour to the apple juice indicated by an increase of flowery and synthetic notes [124]. The off-flavor can be masked by addition of a combination of ethylhexanoate with ethylbutanoate and ethyl-2-methyl-butanoate to the apple juice while maintaining the aroma induced sweetness enhancement. By those means an apple juice with a balanced flavor profile was obtained [155].

The aim of this study was to determine whether combinatory effects of the modification of food texture to enhance taste intensity and aroma induced taste enhancement lead to a cumulative enhancement of taste intensity.

Materials and Methods

To test the hypothesis we used Na-caseinate gels sweetened with sugar and flavored with apple juice and apple aroma. The gels were supposed to simulate the texture matrix of semi-solid foods and provide the natural, hence complex, flavour of apple juice. A 2x2x2 full factorial design was used for the gel preparation (Table 8-1). Two texture conditions were used with the objective to obtain one texture which is perceived sweeter than the other. The “low” sweetness texture condition should include gels with high stiffness, low brittleness and low serum release. The “high” sweetness texture condition should included gels with low stiffness, high brittleness and high serum release. Differences in texture were obtained by lowering the Na-caseinate concentration and addition of gellan gum to induced microphase separation. Two aroma conditions were employed with the objective of obtaining one aroma condition perceived sweeter (aroma induced taste enhancement) than the other (no aroma induced taste enhancement). This was realised for the “high” sweetness aroma

condition by the addition of ethylhexanoate to the apple flavoured gels which is known to enhance sweetness in apple juice. It should be noted that the apple aroma used to prepare all gels did not include ethylhexanoate. Two sugar conditions were used (sweetness condition sugar) by varying the total sugar concentration of the gels. All gels were analyzed for their mechanical properties using uni-axial compression tests. A sensory study with naive panelists was carried out to determine perceptual combinatory effects of the variation in texture, aroma and sugar concentration.

Table 8-1: Overview of the 2x2x2 factorial design of the study and composition of the gels. For the sweetness condition texture "Low" corresponds to gels with high stiffness, low brittleness and low serum release and "High" corresponds to gels with low stiffness, high brittleness and high serum release. For the sweetness condition aroma "Low" corresponds to gels without added ethylhexanonate (no aroma induced sweetness enhancement) and "High" to gels with added ethylhexanoate (aroma induced sweetness enhancement). For the sweetness condition sugar "Low" corresponds to a total sugar concentration of 6%, and "High" to a total sugar concentration of 8%. Na-caseinate, gellan, fructose, glucose, total sugar and GDL concentrations are expressed as wt% on total gel. All gels contained 2wt% apple juice, 50 ppm apple aroma and had a pH of 4.61 ± 0.02.

Sample	Sweetness condition			Na-Caseinate (%)	Gellan (%)	Ethylhexanoate (ppm)	Fructose (%)	Glucose (%)	Total sugar (%)	GDL (%)
	Texture	Aroma	Sugar							
1	Low	Low	Low	6.5	0	0	4	2	6	0.975
2	Low	Low	High	6.5	0	0	5.3	2.7	8	0.975
3	Low	High	Low	6.5	0	15	4	2	6	0.975
4	Low	High	High	6.5	0	15	5.3	2.7	8	0.975
5	High	Low	Low	5	0.04	0	4	2	6	0.750
6	High	Low	High	5	0.04	0	5.3	2.7	8	0.750
7	High	High	Low	5	0.04	15	4	2	6	0.750
8	High	High	High	5	0.04	15	5.3	2.7	8	0.750

Materials

Sodium-caseinate (EM07) was obtained from DMV International (Veghel, The Netherlands). Gellan gum (low acyl, Kelcogel F) was kindly provided by CP Kelco Inc. (Lille Skensved, Denmark). Glucono-δ-lactone (GDL) was kindly donated by Jungbunzlauer S.A. (Marckolshein, France).Glucose and fructose were obtained from

local shops. All materials were used without further purification. All samples were prepared with demineralised water. Apple juice concentrate, very low in aroma content was used (Royal Friesland Campina, Ede, The Netherlands). Apple aroma (IFF, Hilversum, The Netherlands) was used to restore apple aroma qualities. The basic apple aroma did not include ethylhexanoate. Ethylhexanoate (purity: 98%, pro analysis) was used (IFF, Hilversum, The Netherlands).

Gel Preparation

Mixed Na-caseinate/gellan gum gels were prepared by acid-induced cold gelation. Na-caseinate solutions were prepared at a concentration of 11 wt% protein. After adding the Na-caseinate powder to water, the mixtures were stirred for 2.5 hours at room temperature. Stock solutions of gellan gum (0.6 wt%) were prepared by stirring the polysaccharide in water for 2 hours and subsequently heating at 80°C for 30 minutes under constant stirring. After heating, the polymer solution was cooled to approximately 18°C under running tap water. The Na-caseinate solution was mixed with varying amounts of gellan gum solution, different sugars (glucose, fructose), apple juice concentrate, apple juice aroma and ethylhexanoate (Table 8-1). The pre-gel solutions were diluted with water to different final protein concentrations. To induce cold gelation GDL was added. An incubation at 25°C for 17 hours followed. The final pH of all gels was 4.61 ± 0.02. The composition of all samples with is reported in Table 8-1.

pH Measurements

The pH of all gels was measured with a Knick Portamess 911 pH pH-meter (Knick Elektronische Messgeräte, GmbH & Co. KG, Berlin, Germany) by inserting the electrode at room temperature directly into the gel and waiting until a constant value was reached.

Compression Measurements

Uni-axial compression tests were performed approximately 20 hours after preparation on cylindrical gel pieces of 25 mm height and 25 mm diameter. An Instron 5543 machine (Instron International Ldt., Edegem , Belgium) equipped with a plate-plate geometry was used. In order to prevent friction between the plates and the samples, the plates were lubricated with a thin layer of paraffin oil. The measurements were performed at room temperature, at a constant deformation speed of 1 mm/s and

up to a compression strain of 80%. The true strain (ε_H), i.e. the absolute deformation of the specimen, and the true stress (σ_t), i.e. the overall stress acting on the sample during deformation, were calculated according to Sala et al [267]. For each sample at least 5 gel specimens were analysed.

Determination of serum release

The serum release was measured as described by van den Berg et al. (2007) using uni-axial compression tests with cylindrical gel specimens of 25 mm height and 25 mm diameter. The compression tests were performed with an Instron 5543. A strain rate of 0.004 s^{-1} was applied in all cases. For these measurements, no paraffin oil was used to lubricate the Instron plates. The gel specimens were placed in a Petri dish in order to collect the exuded serum. The collected serum was weighted and the serum release was calculated as follows:

$$SR = \frac{M_s}{M_g} \qquad (1)$$

where M_s is the mass of the exuded serum and M_g is the mass of the gel before compression. The measurements were performed at least in duplicate.

Sensory Evaluation

Assesors and sensory attributes

Thirty female, paid subjects (age 22-58) participated in the study. The subjects were tested for normal sense of smell and taste, prior to the study. The subjects were naïve with respect to the experimental conditions and purposes. However, some took part in earlier food sensory evaluations. All subjects gave informed written consent prior to the sensory evaluations.

Samples were evaluated on ten attributes of which five described the products flavor (sweet, sour, fruity, synthetic, apple flavour) and five the mouthfeel (stiff, elastic, crumbly, brittle, watery) (Table 8-2). The attributes were generated by a descriptive panel prior to the experiment and were selected to fully describe the product with respect to relevance in the given study context.

Evaluation Method

A 2x2x2 full factorial design was choosen to investigate all aspects of interactions between texture modification, aroma addition and sugar concentration (Table 8-1).

Samples were evaluated by the 32 assessors in duplicate and presented to the panel in two sessions of each 45 minutes. Both sessions were conducted on one day with a thirty minutes break in between. The samples were presented in fully randomized order and fully balanced over both sessions. Subjects were given a description of the attributes in oral and written form, prior to the experiment.

The subjects were instructed not to smell on the amples after opening the containers. They were asked to take a spoonful of the sample and place it into their mouth. They were asked to chew on the sample and swallow the bolus. Subjects were free to chew as they wished. Following swallowing the subjects scored the attribute on linescales from 0 – 100, anchored at 0 'not at all'and 100 'very'. Subjects were free to take another spoonful, if necessary.

Table 8-2: Sensory attributes generated by the sensory panel.

Group	Attribute	Description
Flavour	sweet	sugar like, sweet
	sour	citrus-like
	apple taste	taste of apples
	fruity	fruit taste
	synthetic	non-natural
Mouthfeel	stiff	stiff, effort to compress, compact
	elastic	elastic, degree of spring back
	crumbly	(small) pieces in the mouth
	brittle	how the sample spreads between tongue and palate
	watery	water released upon compression in the mouth

Data Collection and Analysis

Intensity ratings were collected using an automated data collection system (FIZZ 2.30C, Biosystems, Couternon, France). Subjects evaluated samples on 0 -100 line scales anchored at 0 'not at all' at the left end and 100 'very' at the right end.

Repeated measures multi-factor-ANOVA testing of intensity ratings for main effects of samples (8), texture modification (2), ethylhexanoate addition (2) and sugar concentartion (2) as a fixed factor and subjects as a random factor (32) was performed with Senpaq 4.7, (QIStatistics, UK, 2008). Post-Hoc analysis was performed using Tuckey-HSD. To investigate dependencies between attributes, Pearsons correlation coefficients were calculated (SPSS 17, Chicago, USA). Average intensity ratings were considered as significantly different, when $p < 0.05$.

Results

Mechanical properties of the gels

The presence of gellan gum in Na-caseinate gels induced phase separation phenomena at microscopic scale resulting in macroscopic homogenous gels with the similar microstructural features observed previously in whey protein isolate/ polysacchariode gels [267] (data not shown). For Na-caseinate gels, the presence of these microstructural features caused by addition of gellan gum was

Table 8-3: Large deformation properties and serum release of the Na-caseinate gels. The standard deviation of the parameters as obatined from 8 replicate measurements is reported.

Sample	Modulus (kPa)	Fracture stress (kPa)	Fracture strain (mm/mm)	Serum release (%)
1	5.90 ± 0.46	6.43 ± 0.19	0.772 ± 0.03	8.42 ± 0.52
2	6.90 ± 0.41	6.96 ± 0.6)	0.763 ± 0.01	7.18 ± 2.38
3	5.90 ± 0.47	6.64 ± 0.33	0.776 ± 0.02	9.87 ± 1.40
4	7.10 ± 0.35	7.28 ± 0.54	0.751 ± 0.03	9.37 ± 1.54
5	4.96 ± 0.87	3.62 ± 0.39	0.579 ± 0.06	18.07 ± 0.01
6	4.70 ± 0.59	3.83 ± 0.17	0.631 ± 0.07	15.06 ± 0.71
7	4.30 ± 0.17	3.51 ± 0.21	0.607 ± 0.05	16.68 ± 1.44
8	3.20 ± 0.25	2.90 ± 0.16	0.388 ± 0.02	17.15 ± 2.11

related to an increased release of serum upon gel compression (Table 8-3 and Table 8-4). The serum release of Na-caseinate gels containing gellan gum was twice as high compared to Na-caseinate gels without polysaccharide. In the Na-caseinate gels the presence of gellan gum caused an increase of Young's modulus and fracture stress accompanied by a decrease of fracture strain (Table 8-3 and Table 8-4). The Young's

modulus represents a measure of the stiffness of the gel, the fracture stress a measure of the strength and the fracture strain a measure of the brittleness.

Table 8-4: Average large deformation properties and serum release of two groups of Na-caseinate gels corresponding to the "Low" and "High" sweetness condition for texture. The standard deviation of the parameters is reported.

Sample	Sweetness condition Texture	Average Modulus (kPa)	Average fracture stress (kPa)	Average fracture strain (mm/mm)	Average serum release (%)
1-4	Low: High stiffness Low brittleness Low serum release	6.45 ± 0.64	6.83 ± 0.37	0.77 ± 0.01	8.71 ± 1.18
5-8	High: Low stiffness High brittleness High serum release	4.29 ± 0.78	3.47 ± 0.40	0.55 ± 0.11	16.74 ± 1.26

Effects of texture, aroma and sugar concentration on sensory properties and sweetness perception

To identify effects of texture modification, aroma addition and sugar concentration on attribute ratings an ANOVA was carried out comparing either the two texture, aroma or sugar categories. Results of comparisons between both texture categories are shown in Figure 8-1. Texture modification did influence the firmness [$F(1, 434) = 84.32$; $p < 0.0001$], wateriness [$F(1, 434) = 62.18$; $p < 0.0001$] and elasticity [$F(1,434) = 15.19$; $p < 0.0005$] of the samples significantly. Post-Hoc analysis revealed a decrease in firmness and elasticity and an increase in wateriness for texture category 2 as compared to texture category 1. Crumbliness and brittleness of the samples was not affected by the two different texture conditions. Sweetness was changed significantly by texture modification [$F(1, 434) = 19.57$; $p < 0.0001$], while sourness did not change with changes in the texture of the samples. Apple flavour was significantly changed by texture modification [$F(1, 434) = 10.12$; $p < 0.005$] as was fruitiness [$F(1, 434) = 4.38$; $p < 0.05$], however synthetic notes did not change with texture.

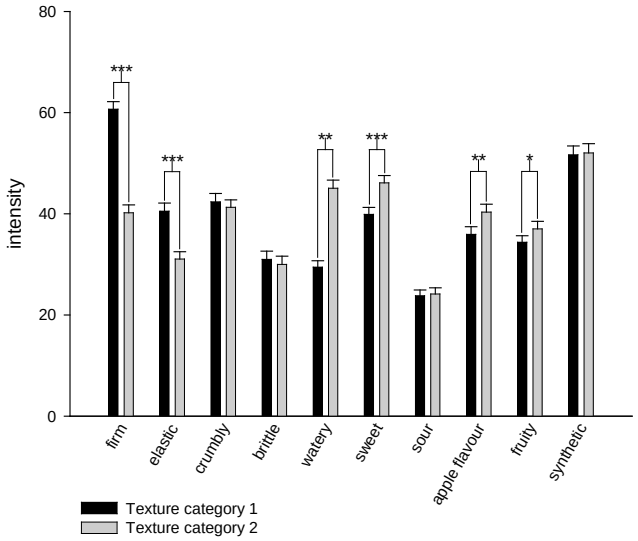

Figure 8-1: Intensity ratings for both texture categories. Texture category 1 includes averaged scores of samples 1-4 texture category 2 includes averaged scores of samples 5-8. Significance levels are indicated as * = p < 0.05; ** = p < 0.01; *** = p < 0.001. Error bars indicate the standard error.

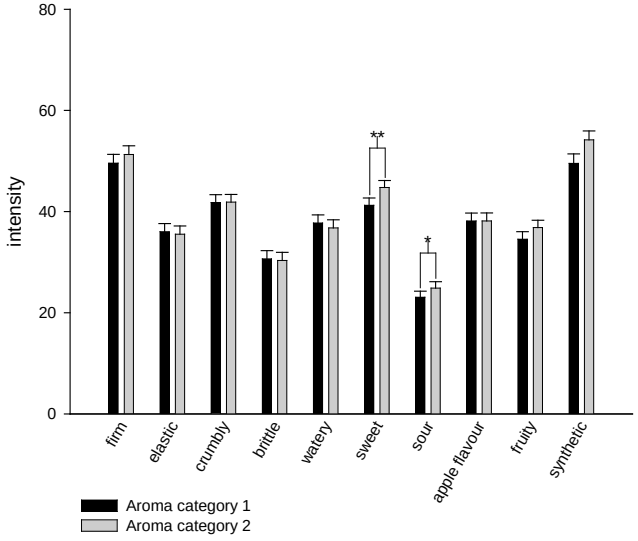

Figure 8-2: Intensity ratings for both aroma categories. Aroma category 1 includes averaged scores of samples 1, 2, 5and 6, aroma category 2 includes averaged scores of samples 3, 4, . Significance levels are indicated as * = p < 0.05; ** = p < 0.01; *** = p < 0.001. Error bars indicate the standard error.

Figure 8-2 presents the comparisons of the two aroma categories, with and without added ethylhexanoate. None of the texture related attributes were changed upon aroma addition. Sweetness was influenced significantly when ethylhexanoate was added [F (1, 434) = 7.04; p < 0.01] with higher ratings achieved with added aroma. Sourness was decreased upon aroma addition [F (1, 434) = 4.38; p < 0.05]. The three other flavour related attributes apple flavour, fruity and synthetic were not significantly influenced with aroma addition.

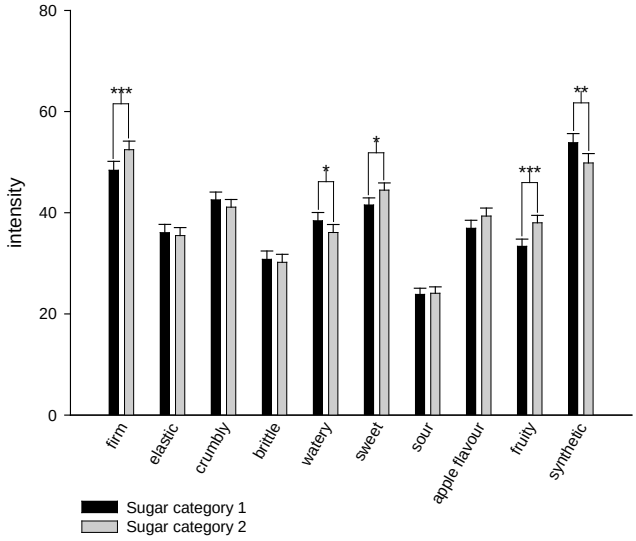

Figure 8-3: Intensity ratings for both sugar concentration categories. Sugar category 1 includes averaged scores of samples 1, 3, 5 and 7; sugar category 2 includes averaged scores of samples 2, 4, 6 and 8. Significance levels are indicated as * = p < 0.05; ** = p < 0.01; *** = p < 0.001. Error bars indicate the standard error.

The third category to be analyzed was the sugar concentration. Figure 8-3 shows an increase in firmness with an increase in sugars added to the gels that was found to be significant [F (1, 434) = 14.25; p < 0.005]. Wateriness was also influenced significantly [F (1, 434) = 6.10; p < 0.05], but with a decreases of wateriness with added sugars. Elasticity, crumbliness and brittleness were not affected by sugar concentration. A significant increase of sweetness with increasing sugar concentration was found [F (1, 434) = 8.11, p < 0.01], while sourness was not changed. Apple flavour and syntheticness was significantly increased after sugar addition (apple flavour: [F

(1,434) = 28.82; p < 0.0001]; syntheticness [F (1, 434) = 10.37; p < 0.005], while fruitiness ratings remained the same.

Combinatory effects of texture modification and aroma on sensory properties and sweetness perception

In this section the samples are looked at independent of the category (texture, aroma or taste) in order to gain further insight in cumulative or combinatory effects of aroma induced taste enhancement and texture modification. Table 8-5 shows average ratings

Table 8-5: Average intensity scores of all attributes generated by the sensory panel of all samples. Averages in the same row followed by the same letter are not significantly different at a level of 5% significance.

Attribute	Sample							
	1	2	3	4	5	6	7	8
Sweet	37.9 cd	37.1 d	39.5 cd	45.4 ab	42.5 bc	47.6 a	46.4 ab	48.1 a
Sour	20.9 b	23.1 ab	25.5 a	26.0 a	25.1 a	23.5 ab	24.3 ab	23.9 ab
Apple taste	33.4 c	37.9 bc	36.6 bc	36.2 bc	37.8 bc	43.9 a	40.3 ab	39.7 ab
Fruity	29.0 c	36.0 ab	35.3 ab	37.5 ab	33.7 bc	39.8 a	35.7 ab	39.1 a
Synthetic	49.9 bc	47.7 c	54.5 ab	54.8 ab	53.9 ab	47.1 c	57.6 a	50.2 bc
Stiff	58.2 a	61.7 a	60.4 a	62.7 a	36.7 c	42.0 bc	38.6 bc	43.7 b
Elastic	42.0 a	38.2 ab	40.9 a	41.2 a	31.5 c	32.7 bc	30.1 c	30.2 c
Crumbly	41.5 a	42.3 a	43.0 a	43.1 a	42.0 a	41.6 a	43.8 a	37.8 a
Brittle	31.2 a	32.2 a	30.5 a	30.5 a	31.1 a	28.5 a	30.7 a	30.1 a
Watery	26.9 c	28.9 c	32.0 c	30.1 c	49.8 a	45.6 a	45.1 ab	40.0 b

and post-hoc analysis results for all samples and attributes. The panel was able to perceive a difference in gel stiffness [F (7, 248) = 32.69; p < 0.0001] and elasticity [F (7, 248) = 6.15; p < 0.001]. The samples without gellan gum were perceived significantly stiffer (p < 0.001) and more elastic (p < 0.005). No differences in crumbliness and brittleness among samples were detected by the sensory panel. Samples were evaluated significantly different in wateriness [F (7, 248) = 20.75; p < 0.0001]. Post-hoc analysis results show that the samples containing gellan gum were perceived significantly more watery than the samples without polysaccharide

(Table 8-5) ($p < 0.01$). Out of the flavour related attributes significant differences were observed for 'sweet' [$F(7, 248) = 6.20$; $p < 0.001$], 'apple flavour' [$F(7, 248) = 2.35$; $p < 0.05$], 'fruity' [$F(7, 248) = 3.64$; $p < 0.001$] and 'synthetic' [$F(7,248) = 3.33$; $p < 0.01$]. Samples containing gellan gum were perceived significantly sweeter than the gels without polysaccharide ($p < 0.01$) (Table 8-5). Neither aroma addition, nor sugar concentration showed a clear trend of influencing sweetness ratings. The ratings for the flavour attribute apple taste were higher for the gels with higher serum release ($p < 0.01$). Furthermore, a positive effect of the serum release was observed for the attribute fruity ($p < 0.05$).

Discussion

Mechanical properties

All Na-caseinate gels without gellan gum (sample 1-4) were stiffer (higher Young's modulus), stronger (higher fracture stress) and less brittle (higher fracture strain) than the samples containing the polysaccharide (sample 5-8). The sugar concentration and the addition of ethylhexanoate did not affect the large deformation properties of the gels significantly. It can be concluded that two groups of gels were obtained with distinctively different mechanical properties resembling two texture conditions. Samples 1-4 are characterized by high stiffness, low brittleness and low serum release. Samples 5-8 are characterized by low stiffness, high brittleness and high serum release (Table 8-1). It has been shown for semi-solid foods that the lower the stiffness, the higher the brittleness and the higher the serum release becomes, the higher becomes the taste intensity [258, 263, 267-269]. Hence, each modification of the mechanical properties is expected to lead to an enhancement of taste intensity. Therefore, samples 1-4 are referred to as the "low" sweetness texture condition and samples 5-8 as the "high" sweetness texture condition.

General and combinatory effects of texture, aroma and sugar concentration on sensory properties and sweetness perception

The significant difference found for the attribute stiffness and elasticity by the sensory panel clearly indicates that the differences in Young's modulus and fracture strain (Table 8-4) are perceivable. The difference in fracture strain between samples with

and without gellan gum did not affect the sensory attributes crumbly and brittle, as ANOVA results did not reveal any significant differences among samples for those attributes. A possible explanation for this could be difficulties of the untrained panel to evaluate and score those attributes which are supposed to resemble the deformation at which the gels start to break down in the mouth. This is not a texture property most consumers pay particular attention to while eating. Therefore these might have been too difficult attributes for the panelists to rate. In addition, the definition given to the panellists might have been insufficient or unclear. Furthermore the differences in the mechanical properties were found to be small and might have been too little to be perceivable.

It was shown that samples with and without gellan gum were perceived significantly different in wateriness. Perceived wateriness and an increase in serum release were positevly correlated ($r = .310$; $N = 496$; $p < 0.001$). This observation strongly demonstrates that the difference in serum release between the samples is sufficient to deliver not only a physically measurable, but also perceptual increase of water phase in mouth. This is also reflected in post-hoc analysis results that show that the samples containing gellan gum were perceived significantly more watery than the samples without polysaccharide (Table 8-5). We conclude that the addition of gellan gum leads to perceivable differences of the water phase.

The samples containing gellan gum, i.e. with changed textural properties, were perceived significantly sweeter than the gels without polysaccharide (Table 8-5). Sweetness ratings for this attribute were up to 25% higher for the samples with gellan gum. The main contributor to sweetness enhancement by change of textural properties seems to be the serum release. As reported a correlation between serum release and perceived wateriness was found among samples. This correlation also holds for the measured serum release and the sweetness perception ($r = .371$; $n = 496$; $p < 0.01$). This result corresponds with previous work presented by Sala et al. [267]. In order to transport tastant molecules to the receptor in the mouth it is necessary that the tastants are dissolved. This can either be in the saliva or in the serum released from the food, hence in strong taste enhancement upon an increased serum release. While changes in texture did strongly contribute to the overall found sweetness increase, contributions of aroma addition and sugar concentration were found to be much smaller. Although aroma and sugar addition led to significant sweetness

enhancement, the effect was found to be much smaller than that found for texture modification. One reason for the small effect on sweetness by ester addition could be the concentration of ethylhexanoate. A strong dependence of concentration on the magnitude of ethylhexanoate induced sweetness enhancement was reported earlier [124]. The aroma was added in a concentration of 15 ppm in this study. Although a concentration of 1 ppm was previously found to be sufficient to induce significant sweetness enhancement in apple juice, in gelled systems the concentration required, seems to be even higher than 15 ppm. Aroma release in products is reportedly decreased with increasing gel firmness [57, 58]. Therefore the increase might not have been sufficient and the aroma concentration might have been too low. Ratings for the attribute 'synthetic' furthermore support this hypothesis. Whenever sweetness was enhanced by ethylhexanoate synthetic notes increased significantly as well. In this study no influence of ester addition on synthetic ratings were observed [124]. That synthetic notes were not increased upon ester addition gives a strong indication that the concentration of the ester might have been too low to be consciously perceived. Although subconscious sweetness enhancement by odours has been reported [71, 99], in the case of the odour/flavour pair ethylhexanoate/ apple juice previous studies showed that conscious odour perception was necessary to induce sweetness enhancement [155, 271]. It is therefore assumed that one reason for the relatively little contribution of aroma to sweetness enhancement is caused by the low ester concentration. However, this is contradicted by the observation in this study, that sourness was significantly decreased with odour addition. Neither texture modification, nor sugar concentration did affect sourness significantly. Sourness reduction is known to be an effect of increased sweetness [100, 120]. It is therefore concluded that low odour concentration might have been one reason for the low effect of the aroma on sweetness, however this cannot be the only reason. Another possible explanation for this observation could be the plain sweetness of the samples. Previous work (data not shown) by the authors showed that the magnitude of ethylhexanoate induced sweetness enhancement does not only depend on the ethylhexanoate concentration, but also on the basic sweetness of the sample itself, with a higher magnitude of enhancement found for less sweet samples. The 10% sugar containing and less serum release sample was overall judged to be the least sweet. This sample showed highest sweetness enhancement compared to the same non-odour condition among all non-odour/odour pairs. Hence, for this sample ethylhexanoate could

produce its highest effect. This observation supports the theory that the sweetness of the sample itself influences sweetness enhacement. Surprisingly little influence was observed for sugar concentration on sweetness of the samples. In previous studies a clear difference in sweetnesss was found for 8% sugar and 10% sugar containing gels [267]. Although significant, compared to the effect observed for the texture of the samples, a higher sugar concentration contributed little to the sweetness of the samples. A possible explanation for this observation could be the composition of the gels. The gels contain apple juice and apple aroma and can be considered perceptually more complex than previous gels much blender in flavour. The complexity of the gels introduces taste/taste and aroma/taste interaction to the gels which are new to the system and have not been studied before. Especially the introduction of sourness to the gels, but also the overall flavour changes might have overpowered mere sugar concentration effects on sweetness. Interactions between different tastes and odour and taste have been widely described in literature [50, 100, 120] and might have led to less sweetness enhancement upon an increase in sugar concentration. Furthermore in contrast to previous studies, where a well trained QDA panel evaluated the samples, in this study a consumer panel was used. This was done to avoid training effects that would lead to disintegration between taste and smell by the assessors. Training of panels was reported to counteract odour/taste integration [152]. However, a consumer panel might have been confused by the complexity of the samples and therefore might have been less sensitive to changes than previous QDA panels.

The ratings for the flavour attribute apple taste were higher for the gels with higher serum release. A positive effect of the serum release has also been observed for the attribute fruity, but in this case the statistical significance was lower than for the attribute apple flavour. This is probably due to the higher level of abstraction of the attribute fruity, which makes the evaluation of the parameter more difficult. The scores for the taste attribute sour were similar for all samples. This demonstrates that the enhancement of the taste perception related to the level of serum release is not the same for all tastes. No clear effect of the serum release on syntheticness of the samples was observed, but the addition of sugar increased synthetic notes significantly.

Generally, texture modification results in strong sweetness enhancement and and overall stronger flavour impressions of the product. Those strong effects overpower

the aroma effect and therefore, although positively influencing each other, just a slight additive but no cumultative effect of both strategies was found.

Conclusions

The variation of sugar and aroma concentration allowed to systematically investigate all aspects of cumulative effects of texture modification and aroma induced taste enhancement to boost sweetness intensity of apple juice containing model gels. No synergistic sweetness enhancement was found. The texture modification (decrease of gel stiffness accompanied by increase of gel brittleness and serum release) appears to boost taste perception more than the increase of the concentration of aroma components from natural foods. It was demonstrated that the magnitude of taste enhancement caused by texture modification is not the same for all tastes, as clear differences were observed for sweetness and sourness of the apple juice gels. When using different approaches to increase taste intensity, the overall effect is additive and not synergistic. It can therefore be concluded, that, when combining different strategies to reduce sugar concentrations in perceptual and structural complex food matrices it is possible to achieve additive effects. It cannot be excluded, that synergism of different cross-modal integration strategies exists, however this study only proved cumulative effects.

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Chapter 9

Aroma influences texture perception of cheeses and dairy model gels

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Abstract

The influence of texture on aroma perception in food has widely been described. Little is known about the influence of aromas on texture perception.In this study the effect of butter aroma on texture perception of dairy model gels was studied. The gel stiffness was varied by changing casein concentration and pH.At high pH a decrease in perceived firmness with increasing butter aroma concentration was observed. At low pH no effect of butter aroma on firmness perception was found. At high pH the gels were described as 'cream-cheese-like', while at low pH samples were perceived as 'yoghurt-like'. Buttery notes are more congruent with cream cheese taste rather than with yoghurt taste. Therefore, butter aroma could influence only the perceived texture of the high pH gels. The results show that the concept of congruency as described for aroma/taste-interactions also applies for aroma/texture-interactions.

Introduction

Todays food industry is determined to find ways to produce healthier foods reduced in sodium salt, sugar and fat content to meet nutritional recommendations such as the World Health Organisation guidelines [199, 272]. Reduction of those components often leads to large changes of the sensory properties of the foods [273, 274]. For example, fat reduction results in changes of the texture of a product influencing taste and aroma perception. Texture perception of dairy products strongly depends on the fat content of the product [275, 276]. As cheese is considered one of the products with a high fat content in western diets, the food industry is determined to lower the fat content of cheeses. However, the acceptability of low fat cheeses by consumers is often limited due to undesired changes of the texture of the cheese. Ratings of sensory attributes generally considered as desirable in cheese such as creaminess or smoothness, are often correlated with high fat contents, whereas low fat cheeses are often described with negative sensory attributes such as chewy and rubbery [275, 277]. Therefore, it is necessary to compensate for changes in the texture of a cheese induced by reduction of fat content. Usually, fat replacers are used as structure breakers and fracture initiators to achieve a less rubbery and more acceptable texture of low-fat cheeses. In this study, we describe an alternative approach to influence the texture perception of cheeses and dairy gels using aromas.

Food aromas are volatiles that are released from the food matrix and perceived during consumption. It has been shown that food aromas can effect taste perception and vice versa [26, 74, 152]. In addition, it has been described that the texture of foods influences the perception of aroma qualities and intensity [58, 82, 160, 278] and vice versa [160]. Those interactions of different sensory modalities are often refered to as cross modalities [279]. Among the many textural parameters that can influence aroma perception, viscosity has received by far most attention in liquid model foods. In general enhancing the viscosity of liquid foods leads to a decrease in aroma and taste perception [57, 82, 279-283], but other factors such as binding of aroma and taste molecules, mouth coating of oils and fats and fresh surface generation during consumption can also play a role. In model gels, an *in-vivo* aroma release study by Weel and co-workers [57] did not find an effect of texture on the release. However, they found that the perceived intensity of food aromas depends less on the aroma release profile than on the products texture as such. Contrary to these observations, a change of aroma release and a corresponding

change in aroma intensity was described by Gierczynski et al. [284] for fresh model cheeses. They observed a more intense aroma for harder products. Later they confirmed their findings reporting a change in aroma release profile in combination with an increased perceived aroma intensity while chewing on firmer gels [81]. An explanation for those contradictory results could lay in the fact that both studies employed different gelling agents. Lethuaut et al. [56] studied the effect of textural agents on aroma and taste perception. They showed that aroma perception can depend on the type of gelling agent. Furthermore it has been shown that not only the gelling agent itself influences perceived aroma intensity, but also the concentration of the gelling agent with an increase in gelling agent resulting in a decrease of perceived aroma intensity [227, 285-288]. More recently, the influence of textural factors in solid foods on aroma perception has been considered [289] . In general, it was shown that the overall perceived aroma intensity and the aroma release in solid foods are lower when the strength of the gel matrix is increased.

Both physicochemical factors and cognitive, cross modal factors can be at the origins of the interaction between texture and aroma perception. Physicochemical factors include the physical or chemical binding of aroma components to the food matrix influencing the release of volatile aromas from the food matrix. Cognitive factors include congruency between various stimuli and expectation and attention given to a stimulus [237, 279]. Several studies showed the importance of congruency between stimuli when introducing multisensory perception of foods. Congruency can be defined as the qualitative similarity and familiarity between two or more sensory modalities such as the taste, aroma or texture [102].

Little is known about the effect of aromas on texture perception. De Wijk et al. [290] described a positive effect of creamy aroma on creamy texture perception in custards. Their study focused on food intake rather than on texture perception. We hypothesize that aromas can influence texture perception in semi-solid food products. In this paper we first study whether native food aromas have an effect on texture and taste perception in cheeses. In the second study we investigate in more detail the influence of butter aroma on the perceived texture of casein based model gels. This approach allows us to control textural and taste aspects of the gels in such a way that perceived aroma induced texture variations can be studied as a function of aroma, texture and taste combined.

Materials and Methods

Study 1: Aroma-Texture interactions in cheeses

Materials

Six commercial cheeses were obtained from a local cheese retailer. The cheeses were selected to offer a broad variety of texture and aroma. The cheeses included

Table 9-1: Fat and Sodium contents in different varieties of cheeses [291].

Cheese	*Fat [%]*	*Na+[g/100g dm]*
Gouda, young	29.2	0.7
Gouda, matured	30.8	0.9
Gouda, old	31.6	1.0
Grana Padano	25.8	0.7
Leerdammer	29.7	0.4
Gorgonzola	31.2	0.8

Gouda (Royal Friesland Campina, The Netherlands) at three ripening stages 'young' (ripening time 4-6 weeks), 'matured' (ripening time 2-3 months), 'old' (ripening time 6-12 months), Grana Padano (Galbani, Italy), Gorgonzola (Galbani, Italy) and Leerdammer (Bel Nederland BV, The Netherlands). Fat and sodium contents of those types cheeses are shown in Table 9-1.

Method

Sample Preparation

The cheeses were cut into cubes (1cm x 1cm x 1cm) using a customized cheese harp (string distance 1 cm). To assure equal amounts of cheese for each cube, only solid parts of the cheeses were selected avoiding parts that contained holes or unequal distribution of blue mold. Three cubes of each sample were placed in 5cm round PET containers (DUNI GmbH & Co KG, Bramsche, Germany) and closed with a lid. The samples were stored in the fridge at 5°C. Samples were taken out of the fridge two hours before the sensory evaluation to reach room temperature.

Sensory Evaluation

Twenty seven subjects (age 21 - 59; 8 ♂, 21 ♀), naïve with respect to the study objectives, evaluated the cheeses on ten attributes. The attributes were generated by a descriptive panel prior to the study. An overview of the descriptors and its explanations is shown in Table 9-2. The samples were evaluated with and without nose-clips in standard sensory booths (ISO 8589). To avoid undesired effects of visual properties of the cheeses on sensory ratings, samples were presented under black light conditions. Samples were evaluated on 0-100 line scales, anchored on the left (0) 'not at all X' and on the right (100) 'very X', with 'X' being the respective attributes.

Table 9-2: List of sensory attributes and description used in study 1 and 2. The description represents the written explanation of the attributes as given to the subjects.

Study	*Attribute*	*Category*	*Description*
1	Salty	Taste	Salty taste as in NaCl
	Sweet	Taste	Sweet taste as in sucrose
	Sour	Taste	Sour/Acidic taste as in citric acid
	Bitter	Taste	Bitter as in quinine drinks
	Savoury	Taste	Taste of a broth/ Umami taste
	Aroma Intensity	Aroma	Overall intensity of products aroma
	Butter Aroma	Aroma	Buttery notes as if smelling melted butter
	Herbal	Aroma	Aroma of freshly cut herb mixtures
	Firm	Texture	Resistance while chewing
	Creamy	Texture	Mouth coating and melting behaviour in mouth
2	Sour	Taste	Sour/Acidic Taste
	Aroma Intensity	Aroma	Overall intensity of product aroma
	Butter Aroma	Aroma	Buttery notes as if smelling melted butter
	Firm	Texture	Resistance while chewing
	Creamy	Texture	Mouth coating and melting behaviour in mouth
	Brittle	Texture	Breakdown of samples in mouth

Samples were evaluated in two 45-minutes sessions, separated by a 15-minutes break. In each session, seven samples were presented to the subjects. The first sample was a dummy sample of young Gouda cheese, results for this sample were not evaluated, followed by the six sample cheeses in randomized order. In the first session, subjects closed their nostrils with a nose clip, then opened the container,

placed one cube into their mouth, chewed and swallowed it and then rated attribute intensities. They were allowed to evaluate a second, identical cube, if necessary. After each sample subjects were asked to rinse their mouth with water and wait two minutes before taking the next sample. For comfort, subjects were allowed to take the nose-clip off during the two minutes waiting period. The second session followed the same protocol as the first session, but subjects now evaluated the samples without a nose-clip.

Study 2: Aroma-Texture interactions in dairy model gels

Materials

Medium-heat spray skimmed milk powder was obtained from Friesland Foods Butter (Promex, Lochem, The Netherlands). Sodium-caseinate (EM07) was obtained from DMV International, (Veghel, The Netherlands). Pasteurized and homogenized cream (fat content 35 wt%) was obtained from a local retailer. Butter flavour (UBF-TW-352-CAST, Volla 20) was kindly donated by Unilever (Vlaardingen, The Netherlands). Food grade glucono-delta-lactone (GLD) (F2500) was kindly donated by Jungbunzlauer S.A (Marckolsheim, France). All solutions were prepared with demineralised water.

Method

Sample preparation of dairy gels

Milk gels with added Na-caseinate and cream were prepared by acid-induced cold gelation. Gels with different textural and aroma release properties were prepared by systematic variation of Na-caseinate concentration, acidifier content (Glucono delta-lactone or GDL) and aroma concentration. An overview of the composition of all dairy gels is given in Table 9-3. Solutions of 18 wt% skimmed milk powder containing Na-caseinate (4, 5 or 6 wt%) were heated at 80°C for 30 minutes. After cooling the milk/ caseinate solutions with tap water to approximately 18°C, 14.3% cream containing butter aroma (0, 0.5, 1 wt%) was added (fat concentration in the gels: 5 wt%). The aroma was homogenized into the cream using an Ultra Turrax (Polytron, Kinematica AG, Lucerne, Switzerland). GDL at concentrations depending on the protein content and on the desired final pH was added to induce gelation. An incubation at 25°C for 17

hours followed. The samples used for the determination of the large deformation properties were allowed to set in 60 ml plastic syringes (internal diameter 26.4 mm) coated with a thin film of paraffin oil. The samples used for sensory analysis were prepared in round plastic cups with a volume of 25 ml.

Table 9-3: Sample composition of dairy model gels as used in study 2.

Sample No.	$c_{milkpowder}$ *[%]*	$c_{caseinate}$ *[%]*	*GDL content*	*pH*	c_{fat} *[%]*	c_{aroma}*[ppm]*
1	18	4	Low	4.7	5	0
2	18	5	Low	4.7	5	0
3	18	6	Low	4.7	5	0
4	18	4	Low	4.7	5	0.5
5	18	5	Low	4.7	5	0.5
6	18	6	Low	4.7	5	0.5
7	18	4	Low	4.7	5	1
8	18	5	Low	4.7	5	1
9	18	6	Low	4.7	5	1
10	18	4	High	4.2	5	0
11	18	5	High	4.2	5	0
12	18	6	High	4.2	5	0
13	18	4	High	4.2	5	0.5
14	18	5	High	4.2	5	0.5
15	18	6	High	4.2	5	0.5
16	18	4	High	4.2	5	1
17	18	5	High	4.2	5	1
18	18	6	High	4.2	5	1

pH measurements

A Knick Portamess 911 pH pH-meter (Knick ElektronischeMessgeräte, GmbH & Co. KG, Berlin, Germany) was used to determine the pH of all gels. The electrode of the pH-meter was immerged into the sample specimen at room temperature until the pH reading was constant [292].

Determination of the large deformation properties

An Instron universal testing machine (Model 5543, Instron International Ldt., Edegem, Belgium) was employed to carry out uni-axial compression measurements. Cylindrical gel specimen (25 mm height)to which one to two droplets of paraffin oil were added

to minimize friction between gel specimen and plate were compressed between two flat plates (plate-plate geometry). Gel specimen were compressed at a compression speed of 1mm $s^{-1}$to a strain of 80%. The absolute deformation (true strain (ε_H)) and the overall stress acting on the sample (true stress (σ_t))were calculated according to Sala et al. [293, 294].

Confocal laser scanning microscopy (CLSM)

Rhodamine B (0.2 wt% solution; 10 µL per mL sample) was added to the gel specimen to stain the protein phase of the gel. A Confocal Laser Scanning Microscope (CLSM; Leica Microsystems (CMS) GmbH, Mannheim, Germany; Model LEICA TCS SP5 with an inverted microscope (Leica DMI6000) and an objective lens (HCX PL APO 63x/1.20 W CORR CS, 63x magnification)) with an excitation wavelength of 488 nmwas employed at room temperature to determine the microstructure of the gel specimen. Micrographs represent an image size of 0.159 mm x 0.159 mm and were obtained with a resolution of 1024 x 1024 pixels [293, 294].

Sensory Evaluation

Samples were evaluated by a sensory panel. Subjects were not trained specifically on the gels used in this study but were used to evaluate food products on a regular basis. The panel consisted of 25 subjects (age 26 – 60, 23 ♀, 2 ♂). The eighteen samples were presented in duplicates in fully-balanced randomized order over 3 sessions, each 45 minutes. In each of the sessions, two dummy samples were presented to the subjects up-front. Samples were presented at 20°C. Subjects were instructed to take one spoonful of the product into their mouth, chew on it and swallow. Directly after swallowing they were asked to rate the intensity of six sensory attributes describing taste and odor qualities (Table 9-1) by clicking on 150-mm linescales presented on a TFT computer screen. The scales were anchored to the left (corresponding score = 0) 'not at all X' and to the right (corresponding score = 100) 'very X', with X denoting the six attributes, respectively. Subjects were allowed to take a second spoonful if desired.

Statistical data analysis

Data was collected via an automated data collection system, FIZZ 2.30C (Biosystems, Couternon, France) for both studies.

Statistical data analysis for study 1: Aroma-texture interactions in cheeses

Attribute intensity ratings were evaluated by multi-factor ANOVA for main effects of Sample (fixed within-subject factor; 12 cheeses-nose clip combinations) and Subjects (27) as random factor using SENPAQ 4.7 (QiStatistics, UK, 2008) with Tuckey-Kramer HSD as post-hoc analysis. Average intensity ratings were considered as significantly different when $p < 0.05$.

Statistical data analysis for study 2: Aroma-Texture interactions in dairy model gels

Multivariate analysis was performed using repeated measures ANOVA on the results of 25 subjects using SPSS (Version 17, Chicago, USA) testing for main effects of aroma (0, 0.5, 1.0 ppm), casein concentration (4, 5 or 6 wt%) and pH (4.2 and 4.7) as fixed within-subject factors. The fixed factors were evaluated in a full-factorial design. Post-Hoc analysis was performed with Tuckey-Kramer-HSD correction for multiple comparisons. To investigate dependencies between attributes, Pearsons correlation coefficient was calculated (SPSS 17, Chicago, USA). Average intensity ratings were considered as significantly different when $p < 0.05$.

Results and Discussion

Study 1: Aroma-Texture interactions in cheeses

In study 1 subjects evaluated cheeses under two conditions: with and without nose clips resulting in no aroma perception with noseclip and ortho- and retronasal aroma stimulation without nose-clip, respectively. By tasting cheeses with and without wearing a nose-clip, effects of exposure to the cheese aroma on the perception of taste and aroma characteristics can be studied effectively. ANOVA results revealed a significant difference between samples for aroma intensity [$F(11, 324) = 29.36$; $p < 0.001$], sweetness [$F(11, 324) = 10.00$; $p < 0.01$], creaminess [$F(11, 324) = 26.02$; $p < 0.001$], butter aroma [$F(11, 324) = 10.69$; $p < 0.01$] and firmness [$F(11, 324) = 55.52$; $p < 0.001$]. Furthermore, results show, that saltiness increased significantly when aroma was perceived in the without nose-clip conditions [$F(1, 324) = 25.26$; $p < 0.001$]. The largest differences were observed when comparing same cheeses under

with and without nose-clip conditions in post-hoc analysis. For example post–hoc analysis revealed, that the largest effect of

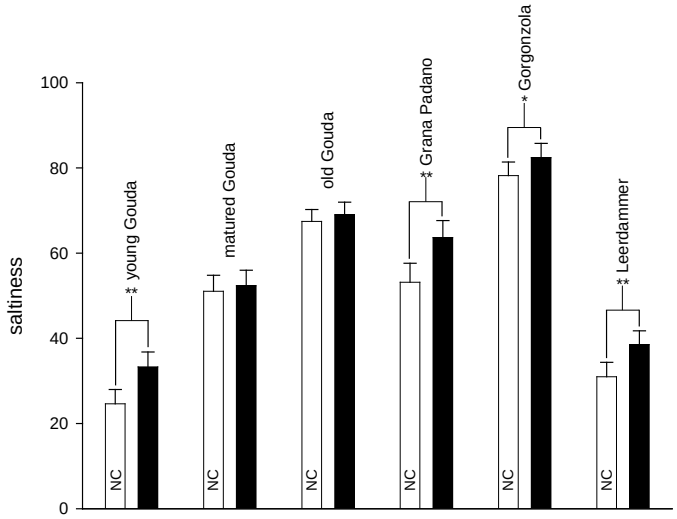

Figure 9-1: Saltiness of six commercial cheeses, evaluated with and without noseclip. The left bar in each pair represents the nose-clip condition = □, the right bar in each pair the without nose-clip condition = ■ Significances are indicated as: * = $p < 0.05$; ** = $p < 0.01$, error bars indicate the standard error.

aroma on saltiness was observed for less matured, and therefore, less salty cheeses such as young Gouda cheese ($p < 0.001$)and Leerdammer ($p < 0.001$), while cheeses already high in salt concentration such as Gorgonzola showed only slight increases in saltiness when tasted without nose clip ($p < 0.05$) (Figure 9-1). It can be concluded that the overall perception of the cheese was enhanced with the aroma present. An enhancing effect of certain aromas on perceptual qualities such as taste has been described before and was to be expected. Knoop et al. [124] described the enhancement of sweetness in apple juice by single aroma component ethylhexanoate, while Lawrence et al. [237] showed aroma induced saltiness enhancement in low-salt solutions. This study demonstrates that such aroma-taste interactions not only occur in liquid foods, but also in solid foods like cheeses. As a result, odour-induced taste enhancement could be used to reduce salt contents in cheese. Considering the strict legislation related to the cheese production process, achieving the desired aroma composition in cheeses for saltiness enhancement would require interventions in the

natural ripening process by, for instance, culture modifications. This would require further investigation.

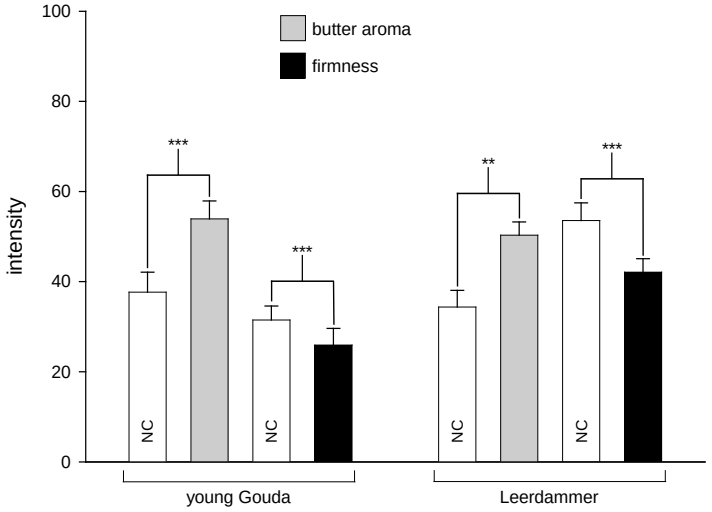

Figure 9-2: Intensity of butter aroma and firmness in young Gouda and Leerdammer cheese tested with nose-clip (NC) and without nose-clip. Significances are indicated as: * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$, error bars indicate the standard error.

The main objective of study 1 was to determine the impact of cheese-born aroma on the texture perception of six commercial cheeses. ANOVA results showed that under nose-clip conditions overall aroma intensity was significantly less than in the without nose-clip conditions [$F(1, 324) = 20.60$; $p < 0.001$] (Figure 9-2). This is expected as wearing a nose-clip prevents aroma perception. The same change was observed for buttery notes [$F(1, 324) = 5.94$; $p < 0.05$]. Post-hoc analysis revealed

significant positive contributions of aroma exposure to buttery notes for young Gouda cheese ($p < 0.01$) and Leerdammer cheese ($p < 0.001$), whereas the buttery notes of the other four cheeses did not differ significantly between nose-clip conditions. While aroma exposure increased the perception of buttery notes for young Gouda and Leerdammer cheeses, their perceived firmness actually decreased when tasted without a nose clip (Figure 9-2). This decrease was found to be significant in both cases (young Gouda: $p < 0.005$; Leerdammer $p < 0.001$). Buttery notes are naturally

associated with texturally softer products like actual butter, but are also characteristic of other dairy products like cheese. This cognitive association regarding textural properties might have contributed to the observed firmness decrease of those two cheeses. Although this interaction might have occurred, other factors such as chewing or breathing behavior might have contributed to the observed firmness suppression by aromas, in case subjects had adopted unique chewing or breathing patterns for particular cheeses. Subjects were not instructed to observe chewing or breathing protocols during the evaluation, as those protocols require training and training might have led to learning effects prior to the study. Busch an co-workers [152] demonstrated that extensive training desensitizes subjects with respect to odor-taste interactions in the evaluation of salted soups. Hence, an influence of the chewing or breathing behavior on product perception cannot be excluded. Additionally, wearing a nose-clip is uncomfortable and might have been perceived as artificial by the subjects, which might have influenced their scores. However, the second texture related attribute creaminess was not affected by the two different conditions which indicate that those factors might not have influenced the subjects scoring behavior.

To the authors knowledge a change of texture perception due to specific aroma stimulation in solid foods has yet not been described. To further investigate whether aroma can impact the texture perception of solid foods, a fully stimuli controlled study is needed. Therefore, we study it in more detail (study 2) by using dairy based model gels of well-controlled and independently adjusted mechanical properties, aroma compositions and acidity (either "congruent" high- or incongruent low pH when combined with the cheese-like texture and buttery aroma).

Study 2: Aroma-Texture interactions in dairy model gels

Mechanical properties and microstructure of Na-Caseinate gels

The Na-caseinate gels that were acidified with Glucono delta-lactone at low or high pH (4.2 and 4.7) at three Na-caseinate levels showed an increase of Young's modulus with increasing Na-caseinate concentration (Figure 9-3). This is expected as increasing the Na-caseinate concentration contributes to a higher density of the protein network.

The gels with the lower pH showed a higher Young's modulus than the gels with higher pH (Figure 9-4). This is in line with expectations since the increase of

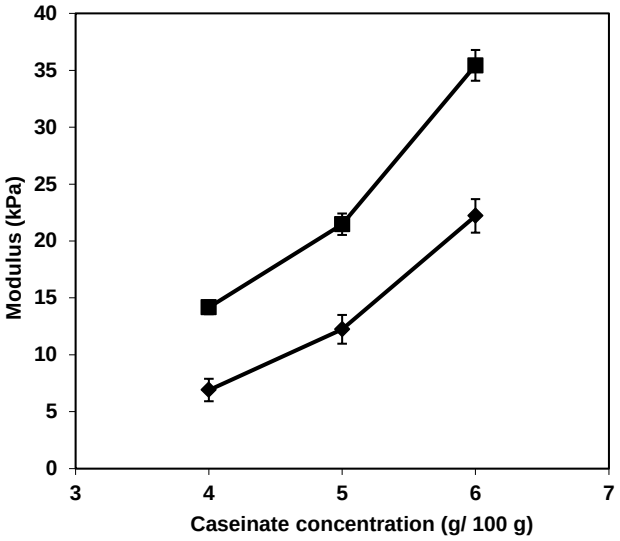

Figure 9-3: Effect of Na-caseinate concentration on Young's modulus of two sets of Na-Caseinate gels with different pH [(♦: pH 4.7; ■: pH 4.2); see table pH values].

hydrophobic interactions between casein molecules due to decreasing pH increases the Young's modulus of milk gels above the isoelectric point of casein (4.6). However, below the isoelectric point of casein this relation is reversed as the Young's modulus decreases at lower pH values [295]. This was explained by the occurrence of electrostatic repulsions between casein molecules at pH values below 4.6. Nonetheless, the gels in study 2 also show a higher Young's modulus at pH 4.2 than at pH 4.7. This may be due to a shift of the pH at which electrostatic repulsions start to affect the gels Young's modulus negatively. This shift could be caused by the high concentrations of milk powder in the gels, resulting in a high concentration of calcium phosphate in the system and, finally, in a higher buffering capacity of the milk powder/ Na-caseinate solution as compared to natural milk. The gels with the low pH (4.2) also had lower fracture strains (i.e. were more brittle). The fracture strain of the gels was not affected by the Na-caseinate concentration (Figure 9-4). For the studied systems fracture strain appears to be mainly controlled by the milk powder concentration, i.e. by the

presence of micellar casein, than by the total protein concentration. The increase of Young's modulus observed with increasing milk powder concentrations was exclusively related to an increase in gel strength.

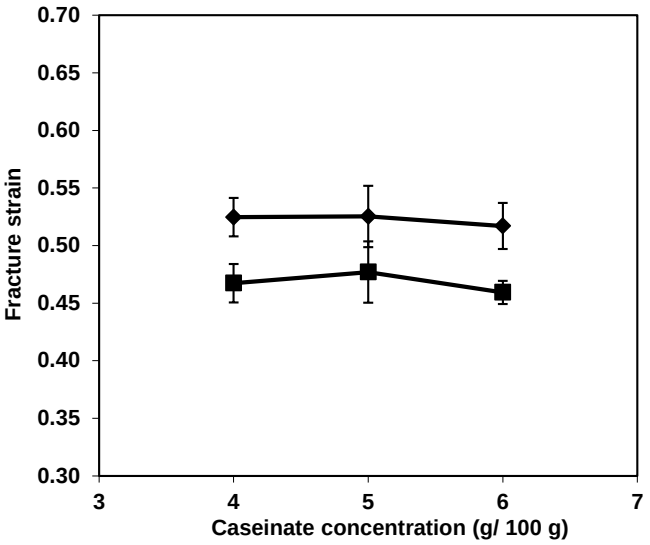

Figure 9-4: Effect of Na-caseinate concentration on fracture strain of two sets of milk gels with different pH (♦: pH 4.6-4.8; ■: pH 4.3-4.4).

The Na-caseinate concentration strongly affected the viscosity of the milk solutions before gelation. The higher the Na-caseinate concentration was, the higher the viscosity (data not shown). This had repercussions on the microstructure of the gels. In gels with higher Na-caseinate concentration the milk fat globules were homogeneously dispersed throughout the entire volume of the gel (Figure 9-5). In gels with lower Na-caseinate concentration macroscopic phase separation was observed during acid induced cold gelation. For these systems, the viscosity of the pre-gel solutions was not sufficient to restrain creaming of the fat globules. As a consequence, the milk fat globules were mainly present in the top layers of the gel specimens. Nonetheless, creaming of the milk fat globules did not affect the breakdown properties of the gels. In Figure 9-6 the fracture curves of 4 specimens coming from different positions of one single tube are shown. No differences in fracture behavior were observed between these specimens. The fracture behavior was found to be

independent of the position of the sample in the syringe, although the microstructure differed significantly (see Figure 9-5). Milk fat globules represent a weakening element within the casein network of the gels. Therefore, it can be expected that they

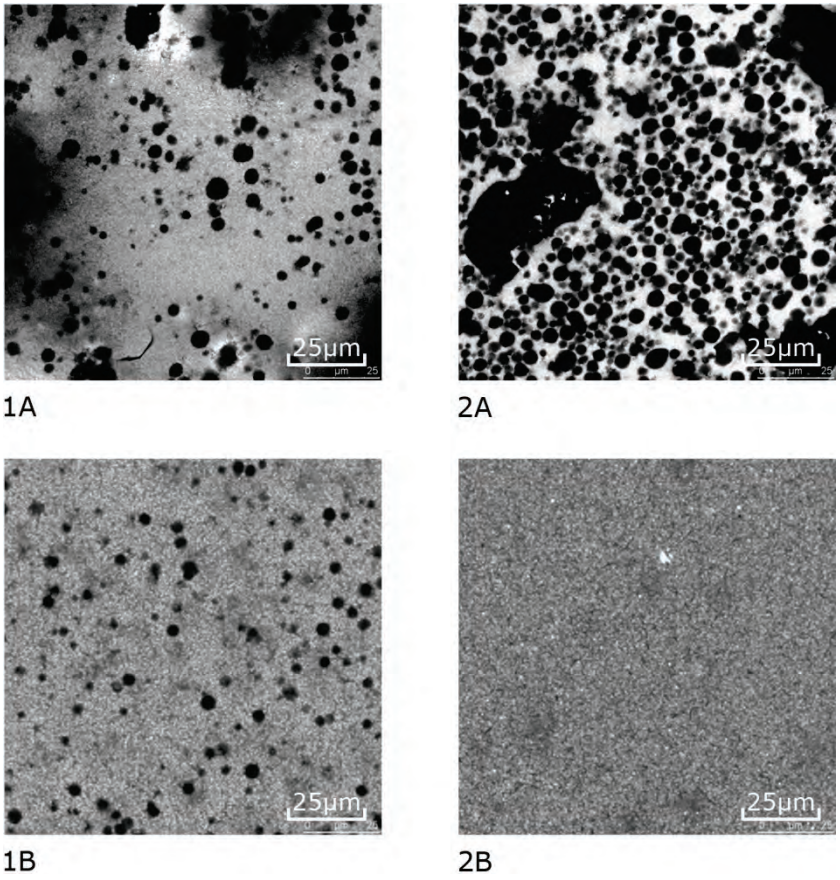

Figure 9-5: CLSM images of gels with different caseinate concentrations (1: 6 wt% Na-caseinate; 2: 4 wt% Na-caseinate) and sampled from the first centimeter at the top (A) and the last centimeter at the bottom (B) of the tubes in which they were prepared (image size 159 X 159 µm).

act as fracture initiators and their presence should result in a decrease of the fracture strain of the gels. The absence of fracture behavior differences between specimens therefore suggests that the fracture behavior of the gels was mainly determined by the protein matrix, partly due the low fat concentration (5%). In conclusion, the observed phase separation does not affect the fracture behavior. This means that the mechanical properties of the gel are independent of the position in the tube from

which they are obtained. Therefore, the sensory properties of the gels were independent of the way of sampling used by the panel members.

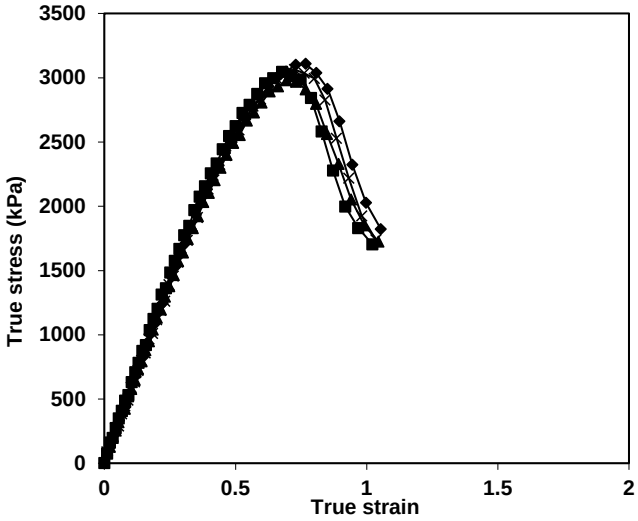

Figure 9-6: Fracture curves of four different gel specimens obtained from one tube.

Effects of butter aroma on the texture perception of Na-Caseinate gels

Aroma intensity ratings were significantly influenced by aroma concentration [$F(2, 45) = 3.64$; $p < 0.05$] and casein concentration [$F(2, 45) = 5.22$; $p < 0.01$] but not by pH, whereas the perception of butter aroma was affected significantly by all three factors (pH [$F(1, 46) = 26.33$; $p < 0.01$], aroma [$F(2, 45) = 15.10$; $p < 0.01$], casein [$F(2, 45) = 14{,}28$; $p < 0.01$]). This indicates that the variation of butter aroma concentration was sufficient to be perceived. Sourness was strongly influenced by pH [$F(1,46) = 186.10$; $p < 0.0001$] demonstrating that despite the little difference in pH value (4.2 vs. 4.7) the samples clearly tasted different in sourness. The sensory panel

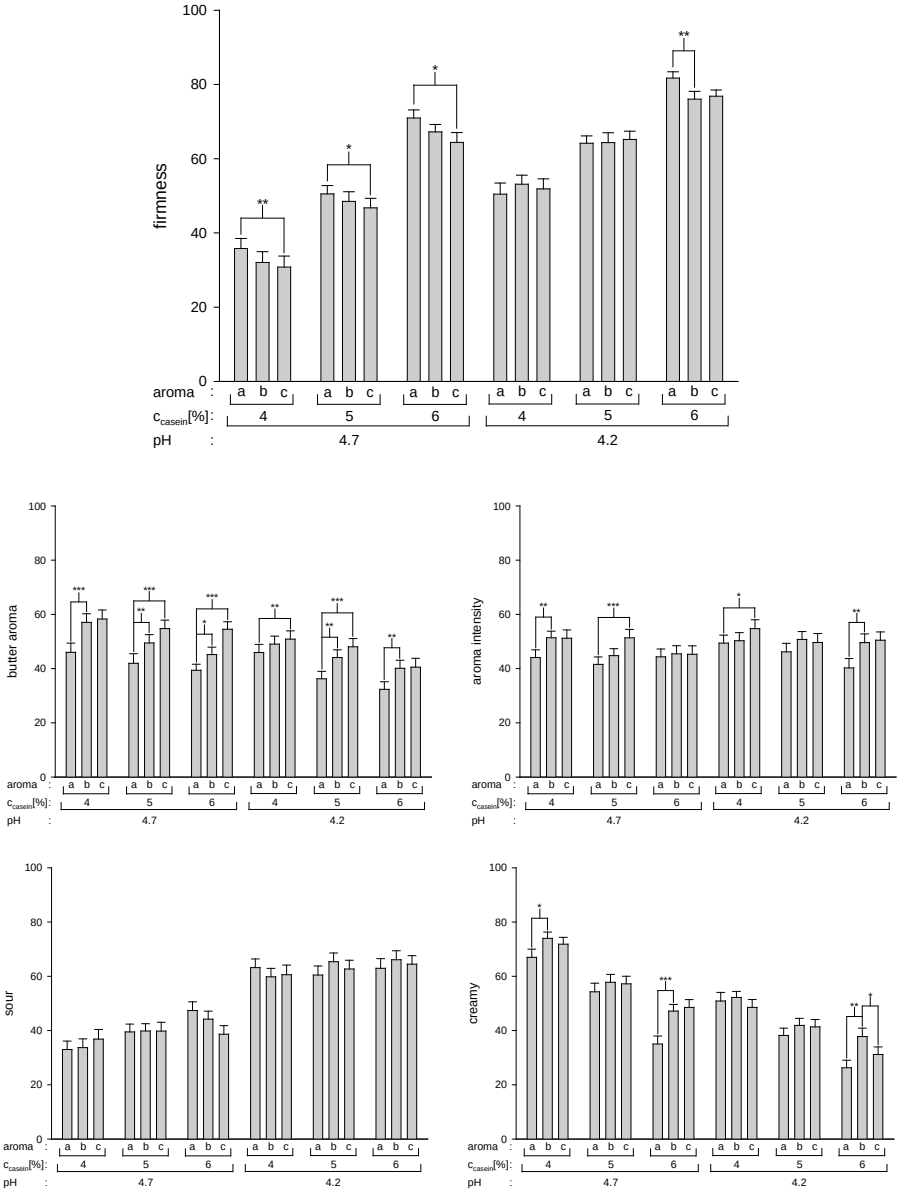

Figure 9-7: Intensitiy ratings for attributes firm, butter aroma, aroma intensity, sour and creamy. Letters a (0 ppm); b (0.5 ppm) and c (1 ppm) indicate aroma concentrations. Casein concentrations and pH values are indicated underneath the graphs. Significance levels are indicated as * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$, error bars indicate the standard error.

described the high pH samples (pH 4.7) as “cream cheese”-like, while the low pH samples (pH 4.2) were described as yoghurt-like, probably due to their sour taste. The pH of the samples used in study 2 represent the pH of the mentioned products with an average pH of cream cheese around 4.7 (4.4 – 5.0) and yoghurt around 4.2 (3.8 – 4.4)[296, 297]. From this and the panels description of the products it can be concluded that the model systems of study 2 closely resemble commercial dairy products, such as cream cheese and yoghurt and that conclusions made from this study might be applicable to commercial products.

Significant changes in perceived firmness were induced by the variation of casein concentration [$F(2; 45) = 142{,}63$; $p < 0.0001$] and aroma concentration [$F(2, 45) = 148{,}84$; $p < 0.0001$], but not by pH. A similar result was observed for brittleness (casein concentration [$F(2; 45) = 102{,}51$; $p < 0.001$]; aroma concentration [$F(2, 45) = 83{,}62$; $p < 0.001$], data not shown). Firmness and brittleness tend to be confused by panels and results indicate that the panel was not able to discriminate between those two attributes, despite the difference in description of the attribute (Table 9-2). Brittleness was positively influenced by caseinate addition in the sensory study which is contradicted by the measured mechanical properties. Figure 9-4 shows that fracture strain is independent of caseinate concentration while perceived brittleness clearly increases with increasing caseinate concentration. Figure 9-4 also shows, that, at high pH, samples were lower in fracture strain. Fracture strain has been shown to correlate with sensory perceived brittleness [298, 299]. It can be concluded, that the untrained panel was probably not able to separate between the attributes firmness and brittleness and therefore evaluated brittleness similar to firmness. Aroma addition did not influence the brittleness of the samples neither at higher, nor at lower pH. Creaminess was influenced significantly by all three factors (pH [$F(1, 46) = 130.50$; $p < 0.0001$], aroma [$F(2, 45) = 82.50$; $p < 0.0001$] and casein [$F(2, 45) = 8.54$; $p < 0.01$]).

As mentioned before, increasing aroma concentrations invoked a significant increase of perceived butter aroma notes and a decrease of perceived firmness for all samples (Figure 9-7). Post-Hoc analysis revealed that especially at higher pH (4.7), increasing the buttery aroma caused a significant decrease of perceived firmness ($p < 0.01$). At lower pH (4.2) no influence of aroma concentration on firmness was observed (Figure 9-7). As reported earlier the pH did not influence the overall aroma intensity, but was closely linked to the perception of the butter aroma. This shows that the perception of

butter aroma reduces the perceived firmness although mechanical properties are similar. This can only be explained by cognitive integration. Food perception was shown to be multimodal instead of unimodal [300-302], meaning all senses are involved in the perception of food and all impressions collected by our senses influence each other. This study shows that those cross modal integration processes can lead to texture modification without actual texture manipulation.

A decrease in perceived firmness was just found for the low pH samples, but not for the high pH samples. A reason for this observation could be the congruency between texture, taste and aroma as described for aroma and taste by Schifferstein and Verlegh [102] and Knoop et al. [124]. They showed that odour and taste congruency is crucial to achieve odour induced taste enhancement. Butter aroma is associated with cream cheese, but not with yoghurt. In a wide range of products such as cheese cake, bread spreads and dipping sauces, butter and cream cheese appear together as ingredients in western diets. Therefore the butter aroma is expected to be more congruent with the flavour of the high pH samples (samples 1 - 9). In contrast, butter aromas are uncommon in yoghurt or yoghurt like products, so the aroma is not congruent with the taste of the low pH samples (number 10 - 18). We conclude that this likely explains why a decrease in firmness due to the butter aroma was only observed for the higher pH samples. In addition at higher pH (4.7), with increasing butter aroma concentration, creaminess perception increased ($p < 0.05$), while at lower pH(4.2), butter aroma concentration had no effect on creaminess(Figure 6). This is opposite to the results reported for firmness. Statistical analysis revealed a negative correlation of firmness and creaminess ($r = -.359$, $N = 1022$, $p < 0.01$). Buttery notes significantly enhanced creaminess at higher pH ($p > 0.05$). The highest effect on creaminess was found for high pH and low caseinate conditions ($p > 0.001$). At lower pH no significant effect on the creaminess of the samples induced by the aroma could be observed.

Conclusions

Both studies demonstrate that the perceived texture of cheeses and model dairy gels is influenced by aromas. Study 1 demonstrated that the aroma of a cheese is crucial for its overall perception including texture and taste. Saltiness was enhanced significantly with increased aroma perception. The decrease of perceived firmness observed for more buttery perceived cheeses led to our hypothesis that butter aroma has the

ability to decrease perceived firmness of dairy model gels under controlled texture conditions. Study 2 showed that butter aroma can decrease perceived firmness of dairy model gels with similar mechanical properties. The congruency of aroma, texture and taste of the product is a crucial factor for cross modal interactions to occur. For the first time it is reported that an increase in aroma can decrease a products firmness. This allows texture modification by aromas without altering mechanical properties. This could be a tool in the development of low fat or low salt dairy products by soothing texture through aroma addition while maintaining mechanical properties.

Acknowledgements

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Chapter 10

General Discussion

In the light of increasing rates of obesity, diabetes, high blood pressure, stroke and other diseases linked to malnutrition it is necessary to reduce sugar, salt and fat concentrations in industrial produced food [199, 272]. Reduction of those ingredients results in a decrease of the flavour of the food and consequently in rejection by the consumer. It is therefore necessary to find alternative ways to compensate for a loss in flavour in order to achieve low salt, sugar and fat products that are accepted by the consumer. Sugar, salt and fat do not only contribute to the flavour of a product, but have further functionalities in food. Salt and sugar have important preservative functions and, in certain, mainly high, concentrations, inhibit microbiological spoilage [303-306]. Furthermore, salt has a high impact on the structure and therefore texture of food by, for example, strongly influencing the formation of protein networks [307-310]. Fat contributes to the flavour formation in cheese [23, 273], but also breaks the structure of cheeses, making cheese appear less firm and rubbery. Thus fat reduction in cheese leads to changes in the texture of the cheese and as a consequence is often rejected by the consumer [311]. A strategy to overcome this problem by using aroma is described in chapter 9. However, effects of salt, sugar and fat reduction other than flavour related ones are outside the scope of this thesis.

At the start of the PhD project (September 2007) it was widely known that certain aromas have the capacity to enhance taste perception [25-27, 48, 74, 99, 101]. However, up to then most studies focused on food model systems of very limited complexity such as solutions of sucrose or NaCl in water with one aroma component or mixture. The complexity of food makes it difficult to transfer findings obtained in model systems to real food. This resulted in very limited knowledge on the possibility to use odour induced taste enhancement to reduce sugar and salt contents in food production. It was not known whether it is possible to predict changes induced by aromas with sweetness or saltiness enhancing capacity on the products overall flavour profile, which would be necessary in order to use odour induced taste enhancement on an industrial scale. Furthermore, very limited knowledge was available on the conditions under which odour induced taste enhancement is most effective or how stable possible effects are when combined with other sugar and salt reduction strategies.

The aim of this thesis was to investigate whether odour induced taste enhancement can be applied to complex food matrices and whether it is a tool for sugar and salt reduction in industrial food production (chapter 2, 3 and 9). Sugar reduction by

odours was studied in an apple juice system, whereas salt and fat reduction by odours was investigated in cheese and Na-caseinate gels. Cheese and apple juice are products very common in the Dutch diet. Both products highly contribute to the sugar and salt intake. Apple juice and cheese were therefore chosen to represent complex sweet and savoury products.

The impact of a change in the concentration of aroma components on the overall flavour of the product was studied (chapter 3). Furthermore it was investigated what the optimal conditions are under which odour induced taste enhancement should be applied and how stable found effects are (chapter 2, 4, 5, 7, 8 and 9). Subsequently, mechanisms underlying odour induced taste enhancement were objective to this research (chapter 4 and 10). The effect of temporal contrasts in tastant delivery, as well as the effect of temporal fluctuation of aroma and taste on taste intensity was studied (chapter 6 and 7)

In addition it has been studied whether aroma can affect the texture perception of cheese and Na-caseinate gels in order to improve the perceived texture of low-fat cheeses (chapter 9).

Aroma induced taste enhancement in complex food matrices

Aromas can be used to enhance taste intensity in a large variety of complex foods including apple juice and cheese (chapter 2 and chapter 9). An increase in the concentration of aroma component ethylhexanoate significantly enhances sweetness in apple juice (chapter 2 and 3). Cheese own aromas enhances saltiness (chapter 9). However, not all aroma components are able to enhance taste perception. Out of the four esters (ethylbutanoate, methylbutanoate, ethyl-2-methylbutanoate and ethylhexanoate) tested for their sweetness enhancing capacity in apple juice, only ethylhexanoate showed significant sweetness enhancement. In literature, all four esters were found to be key components in the aroma of an apple [113-115, 312-315] and all four have been described as sweet smelling by sensory panels [110, 316]. Consequently, all four esters are congruent to sweet taste and apple juice as defined by Schifferstein and Verlegh [102]. Hence, if congruency and co-occurrence of flavour and aroma component would be the only factors leading to taste enhancement, all four esters should have enhanced sweet taste in apple juice. Only ethylhexanoate showed significant

sweetness enhancement. This demonstrates that in addition to congruency and co-occurence other factors are important when applying odour induced taste enhancement to complex products such as apple juice and cheese. Ethylhexanoate is described as fruity and sweet smelling. Increased fruitiness was found in samples containing ethylhexanoate (chapter 3). This increase in fruitiness might have contributed to ethylhexanoate's sweetness enhancing capacity. However, ethylhexanoate has only been proven to enhance sweetness in apple juice. Whether or not ethylhexanoate is able to enhance sweetness in other products or juices remains unknown. It is likely, that ethylhexanoate is able to enhance sweetness in other fruit juices such as melon or apricot, where it is part of the natural flavor of the juice.

Not only sweetness was enhanced by ethylhexanoate. Off-notes, such as synthetic and flowery, were also introduced to the apple juice by ethylhexanoate addition. Those undesired side effects of ethylhexanoate induced sweetness enhancement needed to be compensated for in order to achieve a balanced, acceptable product. This was achieved by combining ethylhexanoate with two esters (ethylbutanoate and ethyl-2-methylbutanoate). All possible combinations of the four esters used in the work described in chapter 2 and 3 had to be tested in order to obtain a balanced product while maintaining ethylhexanoate induced sweetness enhancement. Currently, it cannot be predicted in which direction the addition of odorous components change a product. Findings described in this work are supported by literature, where it has been shown, that the addition of an odour components to an odour mixture can lead to changes in odour quality that is not resembled by any of the involved odorants [136, 138, 140].

In order to use odour induced taste enhancement in future food production, it will be necessary to test for each product individually which aroma or aroma component is suitable to achieve the desired taste enhancing effect. This thesis demonstrates that it is possible to use odours in order to enhance taste perception in complex food. The reduction of sugar and salt predicted to be achieved by applying odour induced taste enhancement based on the results from studies presented in this thesis is approximately 15%. This is supported by estimations presented by Busch, who calculated a possible contribution of multisensory principles to salt reduction of up to 20% [317]. With a potential of 15 – 20% reduction, multisensory approaches remain a comparable small contributor to tastant reduction. It was shown that flavour

enhancers can compensated up to 40% tastant reduction, while tastant replacers compensated for approximately 25%, combined a compensation for more than 50% salt reduction was found without the sensory panel detecting a difference in saltiness [317, 318]. Those strategies, as shown for odour induced taste enhancement (chapter3), have undesired side effects. Flavour enhancers such as mono-sodium-glutamate (MSG) and protein hydrolysates, as well as tastant replacers such as KCl can induce off-flavours. Additionally the use of flavour enhancers is not accepted by the consumer. Odours might help to mask off-flavours whilst contributing themselves to taste enhancement. Combinations of odours to induce taste enhancement with other tastant compensation strategies need further investigations.

Potentially taste enhancing odours have to be identified for each food item individually, as no general rules for taste enhancement by odours can be applied. One odour that enhances taste in one specific product, like ethylhexanoate in apple juice, might be less effective in another product. Likewise an odour that does not enhance taste in one product could be very effective in another. Furthermore, undesired side effects when increasing the concentration of certain aroma components have to be expected (chapter 3). That odours in odour mixtures lead to changes in the odour quality that are not a resemblance of their individual odour qualities is described in literature [136-140]. This is referred to as synthetic mixture perception, in contrast to analytical mixture perception [27, 141]. The way olfactory receptor neurons (ORN) are activated could offer an explanation for those observations. Olfactory receptors (OR) are sensitive to a wide range of odorant molecules. Different odour components in a mixture bind to the same receptor. Duchamp-Viret et al. [142] as well as Rospars et al. [143] showed that in binary odour mixtures the activation patterns of olfactory receptor neurons (ORN) differed strongly from the activation caused by the single odours. Both studies showed that ORN activation by odour mixtures are not a mere addition of the activation patterns caused by single components but resulted in a non-additive change in the temporal response patterns. Rospars et al. concluded that natural odours that are very complex it is not possible to predict changes in activation as too many interactions between the odours take place. Their results are supported on a perceptual basis by findings presented in this thesis. Unpredictable flavour changes occur, if adding one aroma component to an odour mixture, such as apple juice aroma. More systematic investigations are needed to better understand and as a consequence predict which interactions occur in the perception of odour mixtures. It has therefore

carefully to be tested, whether certain odours change the whole profile of the product and whether possible changes might lead to rejection of the product by the consumer. In this thesis it has been shown that odour induced taste enhancement can be used in a sweet product such as apple juice. The strong contribution of the aroma phase on the saltiness perception of cheese (chapter 9) and work done by Batenburg et al. [319, 320] indicate that odour induced taste enhancement can equally well be transferred to savoury products.

It can be concluded, that odour induced taste enhancement can contribute to tastant reduction, however, to achieve reductions as recommended by health authorities it needs to be combined with other sugar and salt replacing strategies. It needs to be noted, that the application of odour induced taste enhancement to industrial produced food remains challenging.

Optimization of aroma delivery conditions to induce maximum taste enhancement

In chapter X it has been shown that an increase in aroma concentration resulted in an increase in the magnitude of odour induced taste enhancement. Conscious odour perception was necessary in order to achieve maximum taste enhancement. This is contrary to results presented by Labbé et al. [99] who found sweetness enhancement at subthreshold level. Labbé et al. studied simple sucrose/water solutions of limited complexity. The sensitivity towards odour effects on taste perception seems to decrease with increasing complexity of the food. It is likely that in complex foods suprathreshold concentrations of the odour are needed in order to achieve taste enhancement. In order for the taste enhancing aroma component to become active conscious changes in the flavour seem to be necessary. A reason for this could be that the perceptual complexity of the flavour in odour mixtures can overpower the delicate changes induced by subthreshold aroma addition. Moreover, in experiments reported in this thesis, the taste enhancing aroma component ethylhexanoate is already naturally present above threshold in apples. Although our basic apple aroma did not include ethylhexanoate as such, the consumer would expect ethylhexanoate to be present, even though not consciously identified as such. Therefore changes in the aroma need to be above threshold. This hypothesis is supported by results described in chapter 9, which show that the texture of cheese can be influenced by buttery aroma notes. It was necessary for the odour to be consciously perceived to obtain

effects on the texture of the cheese. When the buttery aroma notes were not perceived no effect on the texture of the cheese was observed.

The timing of odour presentation was found to be crucial in order to achieve large taste enhancement (chapter 4 and chapter 6). Especially odour presentation time with respect to the moment of swallow determines the magnitude of taste enhancement. Odour presentation 2.5 seconds before or after the moment of swallow is most effective in order to achieve maximum taste enhancement, whereas odour presentation at the moment of swallow leads to less taste enhancement. Results presented in chapter 6 support those findings, as it was shown that out-of-phase pulsation of aroma and taste has the highest effect on sweet taste perception. Thus, asynchronous delivery of taste (sucrose) and aroma (isoamyl acetat) led to highest perceived sweetness. This contradicts findings obtained by Pfeiffer et al. [71] who found highest enhancement at the moment of swallow, thus synchronous presentation of odour and taste. The experimental conditions in Pfeiffers work were not fully controlled with respect to the moment of swallow. It is likely that the moment of swallow strongly differs in their study between different conditions. The importance to correct for the real moment of swallow (RMS) has been demostrated in chapter 4. In this study odour was presented retro-nasally before, at and after the moment of swallow, to investigate whether mechanical movement (swallowing) has an impact on the magnitude of odour induced taste enhancement. Inter-individual and intra-individual differences in swallowing behavior led to strong variations in odour presentation time around the RMS. That no effect on the magnitude of odour induced taste enhancement was observed when conditions were not corrected for the real moment of swallow shows the importance of correction. When corrected for real moment of swallow, strong differences in the magnitude of odour induced taste enhancement were found among odour presentation conditions. On a first glance it seems counterintuitive that the odour needs to be presented asynchronous with respect to the moment of swallowing (2.5 s before or after swallow) in order to achieve maximum taste enhancement. However, as the nasopharynx is closed off at the exact moment of swallow, usually no odour reaches the olfactory receptors during swallowing [164]. Odours are released shortly after swallowing [90, 157]. It is hypothesized that the transmission of olfactory receptor neuron signals (ORNs) is delayed until after the moment of swallow. This is supported by the fact that taste perception normally precedes aroma perception under regular eating conditions [225]. This might

have led humans to be drawn towards desynchronization in food perception, with highest potential of odour and taste integration with asynchronous stimulation. Further investigation of this hypothesis was conducted using functional magnet resonance imaging (fMRI) to determine whether brain activation patterns change during the moment of swallow with respect to areas in the brain involved in aroma influenced taste perception. Preliminary results indicate that signal transmission is indeed delayed at the exact moment of swallow, resulting in lower taste enhancement at swallowing. Activation in the orbito frontal cortex was found to be higher when the aroma was presented asynchronous with swallowing. The orbito frontal cortex is known to be involved in odour taste integration processes [153, 238, 321-323]. The data analysis of the fMRI study was still in progress at the time of completing the PhD thesis. Hence, final conclusions cannot be drawn yet.

On the bases of the work shown it is recommended to introduce a delay of approximately 2.5 s between the swallowing of the food and aroma presentation in order to optimize taste enhancement induced by aromas. The optimal use of odour induced taste enhancement would result in fewer odour needed to introduce taste enhancement effects. This sequentially results in fewer problems with off-notes whose magnitude have been shown to depend on odour concentration (chapter 2). However, the transfer of the presented results into delivery optimized industrial food products remains challenging.

Aroma and texture interactions

It is known that texture influences aroma and taste perception due to physicohemical or cognitive interactions. Physicochemical factors include the physical or chemical binding of aroma components to the food matrix influencing the release of volatile aromas from the food matrix. Cognitive factors include congruency between various stimuli and expectation as well as attention given to a stimulus [237]. In chapter 9 it has been described, to the best of my knowledge for the first time, that aromas can be used to influence texture perception in cheese and dairy model gels. The firmness of dairy model gels decreased and creaminess increased with increasing butter aroma concentration. This study confirms results obtained in previous experiments showing a decrease of perceived firmness with increasing buttery notes in cheese (chapter 9). A decrease of firmness with increasing buttery notes strongly depended on the pH of the product. While at pH 4.7 a clear decrease of firmness with buttery notes was

obtained, this was not the case at pH 4.2. At pH 4.7 samples were described as cream cheese like and therefore congruent to butter aroma, while the yoghurt like samples at pH 4.2 are not associated with butter aroma. In this thesis congruency between stimuli in food perception was identified as a crucial factor for all sensory modalities. This applies to liquid as well as semi-solid products (chapter 2, 3 and 9). This should be taken into account when trying to apply strategies involving multimodal perception into industrial food production.

These findings could be applied to industrial food production of low fat cheeses in order to overcome undesired textural changes that often lead to rejection of those products by the consumer. The aroma profile of a cheese is determined during ripening by the microorganisms which are added at the beginning of the production process. The flavour profile of a cheese can be modulated by changing the strain composition or processing conditions. This possibly allows to generate more odours which enhance saltiness and to generate more buttery odours. This could allow salt and fat reduction, while maintaining good taste as well as creaminess and a soft texture. The second study presented in chapter 9 focused on dairy model gels of less complexity than real cheese. In study one the effect of the aroma phase on cheese perception was tested. Out of the six cheeses tested just two showed a correlation of an increase in buttery notes and a decrease in perceived firmness. The two cheeses were young Gouda cheese and Leerdammer, both immature cheeses of less strong aroma notes. Older cheeses or very aromatic cheeses did not show such a correlation. Whether butter aroma affects firmness perception therefore seems to be strongly depending on the cheese. Likewise to the use of aromas to enhance taste perception it currently seems to be necessary to test aroma induced texture modification for each aroma/texture pair individually.

The mere addition of butter aroma to cheese is not possible in various countries, due to legal restraints in cheese production. A direct application to low-fat products would be possible in cheese containing formulations, such as bread spreads or savoury creams. It is not known yet, to which extent the texture of a product can be modified by aromas. Likewise to taste enhancement by aromas it is assumed, that aroma induced texture modification can contribute to desired textures in low-fat products, however combination with other strategies to reduce fat, such as fat replacers will be needed in order to achieve the desired effect to its full extent.

Conclusions

It can be concluded that odour induced taste enhancement is a valuable tool to compensate for flavour loss when reducing sugar, salt and fat in industrial produced food. It was shown that ethylhexanoate can compensate a sugar reduction of up to 15% in apple juice. For optimal use of odours to enhance taste it is necessary to optimize aroma concentration and delivery. Also the perceived texture of food can be modified by aromas, which offers a wide range of application in low-fat products.

No general rules can be established for aromas that are capable of taste and texture modification. Although congruency, co-occurence and familiarity of odour, taste and texture are important factors in odour induced taste enhancement, the effect of aromas on taste and texture are more complex. The odour presentation time with respect to swallowing is an important factor when optimizing odour induced taste enhancement, with asynchronous presentation being most effective.

Currently it is necessary to identify possible aromas to induce certain effects on taste and texture for each product individually. Furthermore, aromas are only one tool to affect taste and texture in industrial produced food. It is necessary to combine aroma induced taste and texture modification with other strategies of sugar, salt and fat reductions in order to obtain nutritional sufficient reduction of those compounds.

Summary

In the light of increasing rates of nutrition related diseases, such as obesity, diabetes, high blood pressure and stroke it is necessary to reduce sugar, salt and fat contents of industrial produced food. Reduction of those components generally leads to changes of the sensory properties of the products and rejection by the consumer. Traditional compensation strategies like the use of taste enhancers, artificial sweeteners or fat replacers often lead to off-flavours and consequently to consumer rejection. This thesis describes cross-modal interactions as an alternative strategy to reduce sugar, salt and fat. In the past cross-modal interactions have been describe to influence taste and texture perception, however up to now research focused on simple model systems lacking in perceptual, as well as chemical complexity. This thesis aimed on the application of cross modal interactions involving aroma modification to complex food systems, such as apple juice and cheese.

At first, the question whether individual aroma components can be identified that have the capacity to enhance taste perception in a complex beverage was addressed [chapter 2]. Apple juice was choosen to represent a complex matrix where interactions between taste and aroma are a natural product characteristic. Ethylhexanoate was identified to significantly enhance sweetness in apple juice, while three other esters selected on the same basis did not show sweetness enhancement. Ethylhexanoate induced sweetness enhancement appeared to be concentration dependent. Concentrations of 5 ppm were found to be most effective to enhance sweetness in this specific system. However, next to sweetness undesired attributes such as flowery and synthetic were also increased significantly. As a conclusion it has to be noticed that flavour balance in complex food is fragile and has to be carefully altered in order to use odour induced taste enhancement as a tool in sugar, salt and fat reduction.

Chapter 3 describes the masking of off-flavours induced by ethylhexanoate as described in chapter 2. It was hypothesised that ethylhexanoate induced off-flavours can be masked by restoring flavour balance by equally adding combinations of all four esters. A combination of ethylhexanoate, ethylbutanoate and ethyl-2-methylbutanoate was found to be most effective to restore flavour quality while maintaining ethylhexanoate induced sweetness enhancement. It was concluded that all components that are naturally part of an aroma are needed to achieve a balanced product.

Optimisation of odour presentation time in order to achieve maximum taste enhancement was subject to the study described in chapter 4. Swallowing is the key to aroma release during food consumption. It was demonstrated that aroma is most presented most effectively either 1.7 – 2.5 seconds before or 2.6 – 3.6 seconds after the moment of swallow. Aroma presented directly at the moment of swallow was found to be least effective to enhance taste. It is assumed that olfactory receptor neurons (ORN) do not transmit the activation signal to the brain at the exact moment of swallow in order to spare energy and function most effective, as during normal food consumption the aroma is released shortly after swallowing. This hypothesis was further tested in an fMRI study. Preliminary results support this theory, however at the moment of completion of this thesis data evaluation was still in progress.

In chapter 5 further optimisation of odour/taste interactions was studied, by investigating the influence of temperature on odour induced taste enhancement. Consumption temperatures differ strongly among products. As aroma release strongly depends on the temperature of the food, it was hypothesised that the magnitude of aroma effects on taste perception changes over consumption temperature. Subjects consumed a sweet and a savoury system at four different temperatures (7, 25, 37 and 50°C). Stimuli temperature and odour presentation were fully controlled by temperature optimised gustometry and olfactometry. Both aroma/taste systems were known to have induced taste enhancement in the past (sweet: apple flavoured tea/ethylhexanoate; savoury: broth/sotolon). No significant effect of temperature was found for either of the stimulus pairs. Results indicate that the complexity of the experiment led to confusion by the panelists under fully randomised stimuli delivery conditions. It was therefore concluded, that temperature effects on the magnitude of odour induced taste enhancement need to be studied in a reduced experimental design in the future.

Temporal contrast as a strategy to enhance salty taste was studied in the experiments described in chapter 6. Salty solutions of different NaCl concentrations were presented in alternating sequence by a gustometer, creating a sensory contrast of low-in salt and high-in salt pulses. It was demonstrated that the sensory contrast induced by the pulsed delivery led to significant taste enhancement. It was shown that high concentrations of NaCl delivered in short pulses were most effective to enhance salty

taste. It was concluded that pulsed stimulus delivery can be an additional tool to reduce salt and sugar concentrations in industrial produced food.

Chapter 7 combines both cross-modal strategies to enhance taste in this thesis so far, odour induced taste enhancement and temporal contrast of stimulus delivery. Subjects were presented with taste and aroma pulses timed via a gustometer. The aroma was either presented in-phase or out-of-phase with the taste stimulus. A cumulative effect of aroma/taste interactions and temporal contrast of tastant delivery was found, resulting in higher taste enhancement than each of the strategies alone. Overall highest sweetness enhancement was observed when aroma and taste pulses were presented out-of-phase.

Texture modification is a third cross-modal strategy to enhance taste perception. Combinatory effects of texture modification and odour induced taste enhancement are subject to the study reported in chapter 8. Apple juice containing gels were engineered differing in textural properties, aroma and sugar concentration. In contrast to the results presented in chapter 7, only an additive but no cumulative effect was found for those strategies. Texture modification was found to be more effective than aroma modification.

Chapter 9 describes studies investigating effects of aroma on taste and texture of cheese and dairy model gels. In the first study subjects consumed different types of cheese with and without a nose-clip. This way the contribution of the aroma phase on the flavour and texture perception of cheese was studied. Saltiness was influenced significantly by the aroma. It was concluded that the aroma of cheese strongly contributes to a cheeses salty taste. Furthermore it was observed that a decrease in cheese firmness strongly correlated with an increase in buttery aroma notes. This was further studied in fully controlled dairy model gels. For gels tasting of cream cheese a significant decrease in firmness was found upon increasing aroma concentration. No effect of butter aroma on firmness was found for yoghurt-like gels. This once more demonstrates the importance of congruency between stimuli. Only congruent sensory impressions can influence each other. In addition, an increase in creaminess was observed with increasing aroma concentration. The results described in chapter 9 clearly show that aromas cannot only modify taste perception, but also are a valid tool for texture modification.

This thesis demonstrates that reduce sugar, salt and (possibly also) fat reduction in cross modal interactions involving aromas can be applied to complex food matrices. It further shows that combinations of different strategies are most effective in order to achieve healthier products reduced in sugar, salt and fat.

Samenvatting

Gezien de toename in voedings-gerelateerde ziektes, zoals obesitas (vetzucht), diabetes, hoge bloeddruk en beroertes, is het noodzakelijk om het suiker-, zout- en vetgehalte van industrieel geproduceerde voedingsmiddelen te verlagen. Echter een verlaging van deze ingrediënten leidt doorgaans tot een verandering van de sensorische eigenschappen van de producten die door consumenten minder gewaardeerd wordt. Traditioneel wordt daarom gebruik gemaakt van smaakversterkers, kunstmatige zoetstoffen of vetvervangers, maar toevoeging van deze ingrediënten leidt vaak tot bijsmaken die de consument afstraft. Dit proefschrift richt zich op de toepassingsmogelijkheden van cross-modale interacties (interacties tussen de verschillende zintuigen) als een alternatieve aanpak om suiker, zout en vet te verlagen in voedingsmiddelen. Eerder is reeds aangetoond dat interacties tussen de zintuigen de smaak en textuur perceptie kunnen beïnvloeden. Echter deze onderzoekingen waren gericht op simpele model systemen die zich moeilijk laten vergelijken met de complexiteit die gevonden wordt in echte voedingsmiddelen. Dit proefschrift is daarom gericht op het onderzoeken van de principes en mogelijkheden om cross-modale interacties toe te passen in complexe voedingsmiddelen zoals appelsap en kaas. Daarbij is in het bijzonder aandacht geschonken aan de rol van aroma's (geurstoffen).

Allereerst is onderzocht of het mogelijk is om individuele aroma componenten te identificeren die de smaakperceptie (zoetheid) in complexe dranken kunnen versterken [Hoofdstuk 2]. Appelsap is daarbij als drank gekozen, omdat deze drank van nature zowel suiker als aroma's bevat. Ethylhexanoaat werd geïdentificeerd als aromacomponent die significant de zoetheid in appelsap kan versterken, terwijl 3 andere esters (die ook van nature voorkomen in appelsap) dit niet vertoonden. De smaakversterking door ethylhexanoaat bleek afhankelijk van de hoeveelheid die toegevoegd werd, met een optimum bij 5 ppm. Echter, de toevoeging van ethylhexanoaat resulteerde tevens in ongewenste smaakattributen zoals 'bloemig' en 'synthetisch'. Daarom kan geconcludeerd worden dat de smaakbalans in complexe voedingsmiddelen delicaat is en om die reden met zorg aangepast moet worden om aroma's in te kunnen zetten voor de verlaging van suikergehaltes (en mogelijk geldt hetzelfde bij de verlaging van zout- of vetverlaging).

In hoofdstuk 3 is beschreven dat ongewenste smaakattributen veroorzaakt door de toevoeging van ethylhexanoaat (zoals beschreven in Hoofdstuk 2) gemaskeerd kunnen worden door het herstellen van de balans van alle 4 de esters die gebruikt zijn

in hoofdstuk 2 en die allen voorkomen in natuurlijk appelsap. Het blijkt dat een combinatie van ethylhexanoaat, ethylbutanoaat en ethyl-2-methylbutanoaat het meest effectief was om de afwijkende smaakattributen te onderdrukken in combinatie met het versterken van de zoetheid. Daarom werd geconcludeerd dat al deze componenten die ook van nature deel vormen van het appelaroma nodig zijn om een gebalanceerde smaak te krijgen in dit product.

Het onderzoek naar het optimum in de tijd bij het aanbieden van de combinatie geur- en smaakstoffen is beschreven in hoofdstuk 4. Het moment van slikken blijkt cruciaal te zijn voor het vrijkomen van de aromacomponenten tijdens voedselconsumptie. Door gebruik te maken van gespecialiseerde apparatuur kon aangetoond worden dat aromacomponenten die aanwezig zijn ofwel 1,7-2,5 seconden voor of 2,6 seconden na het slikmoment het meest effectief zijn in het versterken van de zoetheidsperceptie. Dit terwijl het presenteren van aromacomponenten tijdens het slikken juist minder effectief zijn in het versterken van de smaakperceptie. Mogelijk dat de neuronen van de geurreceptoren gedurende het moment van slikken geen signalen naar de hersenen sturen om zodoende energie te besparen. Deze hypothese dient verder onderzocht te worden in fMRI studies. En de eerste voorlopige onderzoeksresultaten duiden ook in die richting.

In hoofdstuk 5 is dieper ingegaan op de interactie tussen aroma- en smaakperceptie door te onderzoeken in hoeverre de temperatuur van het voedingsmiddel van invloed is op de smaakversterking. De temperatuur waarbij een voedingsmiddel wordt genuttigd, verschilt van product tot product. Aangezien ook het vrijkomen van aroma componenten toeneemt bij hogere temperaturen was de hypothese dat hogere temperaturen mogelijk een sterker effect zouden geven op de smaakperceptie. Proefpersonen kregen vloeibare zoete en hartige (zout) producten gepresenteerd onder volledig gecontroleerde condities bij 4 temperaturen (7, 25, 37 en 50°C), door gebruik te maken van een voor dit doel aangepaste gustometer en olfactometer. Beide aroma-smaak systemen waren eerder getest op smaakversterking (voor zoet: thee met appelsmaak en ethylhexanoaat als aroma en daarnaast voor hartig/zout: vleesbouillon met sotolon als aroma). Echter in deze experimenten werd geen significant effect gevonden van de temperatuur op de mate van smaakversterking. Mogelijk leidden de sterke wisselingen in temperatuur (de experimenten werden volledig gerandomiseerd uitgevoerd m.b.t. de temperatuur) de panellisten teveel af.

Om die reden zouden toekomstige experimenten anders opgezet moeten worden, om zodoende dit effect uit te kunnen sluiten.

Temporele contrasten als strategie om zoutperceptie te versterken is beschreven in hoofdstuk 6. Zoute oplossingen met verschillende zoutconcentraties worden aan panellisten aangeboden via een gustometer en wel op zodanige wijze dat er een contrast werd gecreëerd in momenten van hoge en lage zoutconcentraties. Aangetoond kon worden dat deze pulsering in sensorisch contrast al leidde tot een significante smaakversterking, waarbij korte momenten met een hoge concentratie NaCl (afgewisseld met langere momenten lage concentratie NaCl) het meest effectief waren. Daarmee kon geconcludeerd worden dat een variërende aanvoer van NaCl (zelfde totale hoeveelheid) een extra mogelijkheid is om zout (en suiker) in producten te verlagen in voedingsmiddelen.

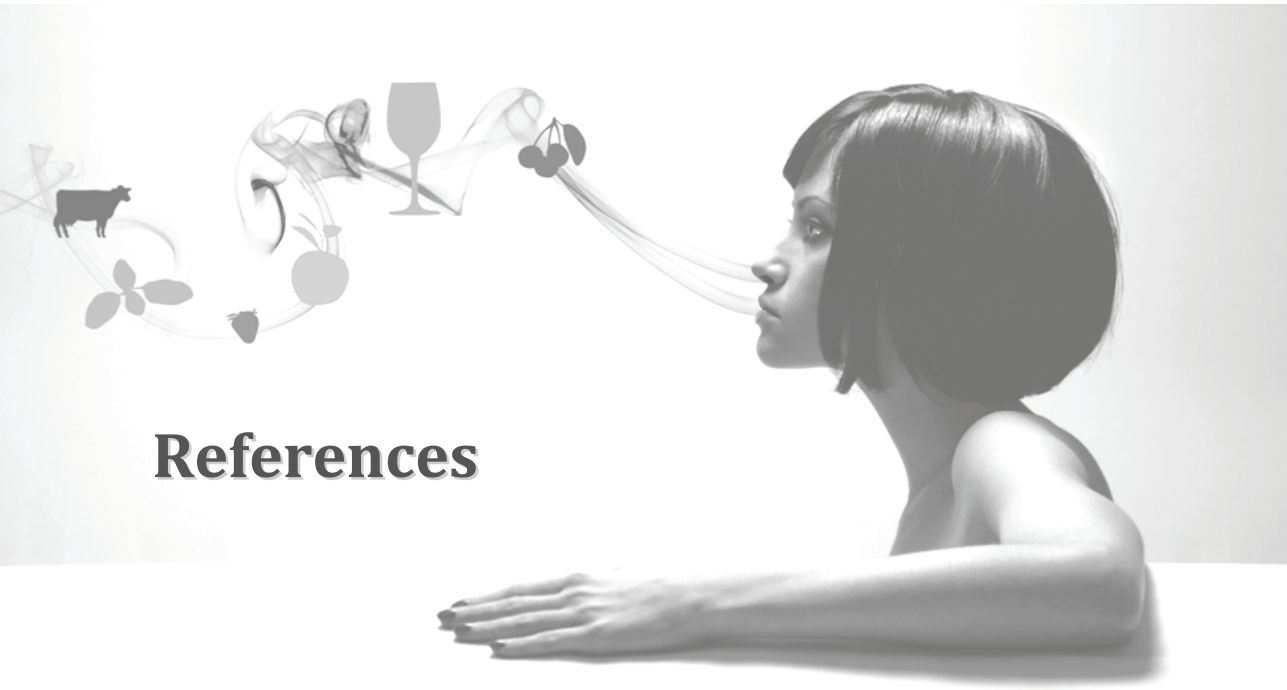
Hoofdstuk 7 combineert de cross-modale interactie strategieën om smaak te versterken samen met temporeel contrast beschreven in het vorige hoofdstuk. Panelleden kregen smaak en aroma gepulseerd aangeboden middels de gustometer. Het aroma werd ofwel samen met de smaakstoffen aangeboden (in fase) of juist daarmee afgewisseld (uit fase). Een cumulatief effect van aroma-smaak interacties en temporeel contrast van de smaakcomponent toevoer werd gevonden, die resulteerde in een grotere mate van smaakversterking dan ieder van de strategieën op zichzelf. Al met al werd de sterkste smaakversterking geobserveerd wanneer aroma en smaak pulsen uit fase werden gepresenteerd.

Het modificeren van de textuur van producten is een derde cross-modale strategie om smaakversterking te bewerkstelligen. De combinatie van textuurveranderingen en aroma-geïnduceerde smaakversterking is beschreven in hoofdstuk 8. Gelen met verschillende texturele eigenschappen werden samengesteld, waarbij allen appelsap bevatten, en met verschillende concentraties suiker en aroma's. In tegenstelling tot de resultaten beschreven in hoofdstuk 7, werd wel een additief effect gevonden in deze combinaties van aanpakken, maar geen cumulatief effect. De impact van de verandering van de textuur bleek bovendien een groter effect te hebben op de smaakperceptie van veranderingen in de aroma concentratie.

Hoofdstuk 9 beschrijft het onderzoek naar de effecten van aroma op smaak- en textuurperceptie in kaas en in zuivel-modelsystemen. In een deel van dit onderzoek consumeerde panelleden verschillende soorten kaas, en ze deden dit in aan of

afwezigheid van een neus clip. Op die manier kon de bijdrage van de geur op de algehele smaak- en textuurperceptie bepaald worden. De waargenomen zoutheid werd significant beïnvloed door het aroma van de kaas. Geconcludeerd kon worden dat het aroma van kaas een sterke bijdrage geeft aan de waargenomen zoutheid van de kaas. Bovendien werd waargenomen dat een verlaging in de stevigheid van de kaas sterk correleerde met het attribuut 'boteraroma'. Deze waarnemingen werden verder bestudeerd in een volledig gecontroleerd zuivel modelsysteem dat gebaseerd is op gelen. In gelen die smaken naar verse roomkaas bleek dat de waargenomen stevigheid van de gelen significant te verminderen bij een toename van de aroma concentratie (boter aroma). Een dergelijk effect werd niet waargenomen voor op yoghurt gelijkende gelen. Deze waarnemingen laten opnieuw het belang van congruentie zien tussen de cross-modale stimuli. Alleen congruente sensorische stimuli beïnvloeden elkaar. Daarnaast werd in deze onderzoeken gevonden dat de mate van romigheid toenam bij een toename van de aroma concentratie. De resultaten beschreven in hoofdstuk 9 tonen duidelijk aan dat aroma's niet alleen de smaakperceptie kunnen beïnvloeden, maar ook de waargenomen textuur.

Dit proefschrift toont dat inzichten in cross-modale interacties, en in het bijzonder de rol van aroma componenten daarbij, toegepast kunnen worden om suiker, zout en (mogelijk ook) vet te reduceren in complexe voedingsmatrices. Bovendien laat het zien dat combinaties van verschillende strategieën het meest effectief zijn in het realiseren van gezondere voedingsmiddelen gereduceerd in suiker, zout en vet.



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About the author

Curriculum Vitae

Janine Editha Knoop was born on May 5th, 1976 in Hamburg, Germany. After receiving her 'Abitur' from Emil-Krause Gymnasium, Hamburg, Germany, she entered the University of Hamburg. She received her Vordiplom (Bachelor's degree) in Food Chemistry in 2003 and went on studying for an advanced degree in Food Chemistry. In 2006 she received a first degree state exam (Master's degree) in Food Chemistry, as well as her Diploma in Food Chemistry for her Thesis entitled '*In vivo* metabolism of soy isoflavones – Evaluation of a human invention study using LC-MS and SPE-GC-MS'. To further educate herself, Janine decided to enroll the second degree state exam Food Chemistry program at the Office of Health and Environmental Affairs, Hamburg, Germany, focusing on the European Food Legislation. In May 2006 Janine started an Internship at Unilever R&D, Vlaardingen, The Netherlands, where she investigated aroma-taste and taste-taste interactions via gustometer delivery. From November 2006 onwards Janine continued her studies at the Hygieneinstitut, Hamburg, Germany, where she was trained on food monitoring with respect to the needs of the regulations of the European food legislation. She received the second degree state exam in April 2007. Afterwards she worked as an analytical research assistant at the 'Gesellschaft für Bioanalytik', Pinneberg. Janine joined Top Institute Food and Nutrition in September 2007 as a PhD-fellow. She was appointed to the project 'Texture-Taste-Interactions'. Her research focused on the investigation of interactions of sensory modalities in complex food matrices and their underlying mechanisms. During her PhD project Janine joined the educational program of the graduate school VLAG. At Wageningen University she was involved in teaching. She attended several international conferences and gave oral, as well as poster presentations on several occasions. In September 2011 Janine was awarded 2nd place at the J.T.M. Wouters Young Scientist Award at the 7th NIZO Dairy Conference.

Publications in peer-reviewed journals

Busch, J.L.H.C.; Tournier, C.; Knoop, J.E.; Kooyman, G.; Smit, G.; Effect of salt delivery in mouth on salt perception: approach with continuous delivery via Dynataste, Chemical Senses, 2009, 34 (4), 341 – 348

Burseg, K.M.M.; Camacho, S.; Knoop, J.E.; Bult, J.H.F.; Sweet taste intensity is enhanced by temporal fluctuation of aroma and taste and depends on taste shift; Physiology and Behavior; 2010; 101; 726 – 730

Knoop, J.E.; Bult, J.H.F.; Stieger, M.; Smit, G; Sweetness enhancement in apple juice by natural apple aroma components, submitted

Knoop, J.E.; Bult, J.H.F.; Stieger, M.; Smit, G; Masking off-flavours in real apple juice induced by sweetness enhancing aroma components, submitted

Knoop, J.E.; Sala, G.; Stieger, M.; Bult, J.H.F.; Smit, G; Texture modification by butter aroma in cheese and dairy model gels; submitted

Knoop, J.E.; Sala, G.; Stieger, M.; Smit, G.; Combinatory effects of odour induced taste enhancement and texture modification enable sugar reduction in gelled food systems; submitted

Knoop, J.E.; Stieger, M.; Bult, J.H.F.; Boek, W.; Smit, G; Asynchrony of odour presentation with swallowing: Effects on taste perception; submitted

Other Publications

Janine Knoop; Gerrit Smit; Harold Bult; Markus Stieger; Weinig suiker, veel smaak – geur versterkt de zoete smaak van appelsap; VMT, 2009,14/15, 26 - 28

Abstracts in scientific journals and proceedings

Busch, Johanneke; Knoop, Janine; Tournier, Carole; Smit, Gerrit; Effect of high-in taste pulses on taste perception; Proceedings of the 12th Weurman Symposium, Zuercher Hochschule fuer Angewandte Wissenschaften, Winterthur, 2010, 47 – 50

Knoop, J.E.*, Bult, J.H.F.; Smit, G.; Masking undesirable effects of Ethylhexanoate on apple flavour, In: Proceedings of the XVIIIth Congress of the European Chemoreception Research Organisation (ECRO), Portoroz, Slovenia, 5th-9th of September 2008; (Oral presentation)

Knoop, J.E.*; Bult, J.H.F.; Polet, I.; Smit, G.; Effects of Esters appearing in natural apple aroma on the sweetness impression of apple juice; In: Abstract book 3rd European Conference on sensory and consumer sciences, Hamburg, Germany, 9th-11th of September 2008 (Oral presentation)

Knoop, J.E.*; Bult J.H.F.; Scholtens, L.; van Beeck-Calkoen, E.; Boek, W.; Smit, G; Asynchrony of odour presentation with swallowing: Effects on taste perception; In Abstract book: 8th Pangborn Sensory Science Symposium; Florence, Italy, 25th-28th of July 2009 (Oral presentation)

Knoop, J.E.*; Sala, G.; Bult, J.H.F.; Smit, G.; Butter aroma influences texture perception in dairy model gels at higher pH;In: Abstract book 4th European Conference on sensory and consumer sciences, Vittoria-Gasteiz, Spain, 4th -7th of September 2010 (Oral presentation)

Knoop, J.E.; Bult, J.H.F; Stieger, M; Smit G.; Aroma induced taste enhancement in cheese and its relation to tastant concentration, 6th NIZO Dairy Conference, 30th – 2nd of September 2009, Arnhem, The Netherlands (Poster presentation)

Knoop, J.E.; Sala, G; Bult, J.H.F; Smit G.; Stieger, M; Texture modification by butter aroma in cheeses and dairy model gels, In: Abstract book; 6th NIZO Dairy Conference, 21st – 23rd of September 2011, Arnhem, The Netherlands (Oral and poster presentation)

Overview of completed training activities

Discipline specific activities

Courses

Food Perception and Food Preference 4, VLAG, 2007

Smell and Taste, Dresden, Germany, 2008

Summer School Human Olfaction, Dresden, Germany, 2009

Hands on Sensory Statistics, Hal Mac Fie Training, Paris, France, 2009

Toolkit for Cognitive Neuroscience, Donders Institute, Nijmegen, The Netherlands, 2009

Analysis of Sensory Data with SensoMiner and Factominer, Utrecht, The Netherlands, 2010

Study Period fMRI, University Hospital Dresden, Dresden, Germany, 2010

TIFN fMRI meeting Group, Utrecht, The Netherlands, 2009

Meetings

17th Conference of the European Chemoreception Organization, Portoroz, Slovenija, 2008

3rd European Conference on Sensory and Consumer Research, Hamburg, Germany, 2008

Food Summit: Taste of Sense, Oosterbeek, The Netherlands, 2008

6th NIZO Dairy Conference, Het Papendal, The Netherlands, 2009

8th Pangborn Sensory Science Symposium, Florence, Italy, 2009

4th European Conference on Sensory and Consumer Science, Vittoria-Gasteiz, Spain, 2010

7th NIZO Dairy Conference, Het Papendal, The Netherlands, 2011

General courses

Team Management and Development, Leeuwendal Training,
Ede, The Netherlands, 2008

Information Literacy including Endnote Introduction,
Wageningen, The Netherlands, 2008

Organising and supervising MSc thesis projects, Wageningen University, 2008

Annual TIFN Conference

Annual FND Day

Career Assesment, Wageningen, The Netherlands, 2011

Optionals

Preparation PhD research proposal

Annual Programm 2 Meeting

PhD Trip FCh, Switzerland and North Italy, 2010

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